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ANÁLISE EVOLUTIVA, METABÓLICA E PANGENÔMICA DE *RALSTONIA SOLANACEARUM*

BELO HORIZONTE – MG
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ATA DA DEFESA DE DISSERTAÇÃO

JUAN CARLOS ARIUTE OLIVEIRA

Às quatorze horas do dia **20 de dezembro de 2022**, reuniu-se, no aplicativo Zoom, a Comissão Examinadora de Dissertação, indicada pelo Colegiado do Programa, para julgar, em exame final, o trabalho intitulado: **"Análise Evolutiva, Metabólica e Pangenômica de Ralstonia Solanacearum"**, requisito para obtenção do grau de Mestre em **Bioinformática.** Abrindo a sessão, a Presidente da Comissão, **Dra. Ana Maria Benko Iseppon**, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra ao candidato, para apresentação de seu trabalho. Seguiu-se a arguição pelos Examinadores, com a respectiva defesa do candidato. Logo após, a Comissão se reuniu, sem a presença do candidato e do público, para julgamento e expedição de resultado final. Foram atribuídas as seguintes indicações:

Prof./Pesq.	**Instituição**	**Indicação**
Dra. Ana Maria Benko Iseppon	UFPE	**Aprovado**
Dra. Flavia Figueira Aburjaile	UFMG	**Aprovado**
Dr. José Miguel Ortega	UFMG	**Aprovado**
Dr. José Ribamar Ferreira Neto	UFPE	**Aprovado**

Pelas indicações, o candidato foi considerado: **Aprovado**

O resultado final foi comunicado publicamente ao candidato pela Presidente da Comissão. Nada mais havendo a tratar, a Presidente encerrou a reunião e lavrou a presente ATA, que será assinada por todos os membros participantes da Comissão Examinadora.

Belo Horizonte, 20 de dezembro de 2022.

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RESUMO

Bactérias do complexo de espécies *Ralstonia solanacearum* (RSSC) causam diversas fitobacterioses em culturas economicamente importantes no mundo, especialmente nos trópicos. No Brasil, os filotipos I e II são agentes causais de murcha bacteriana. Porém, não é possível distingui-los macroscopicamente. A doença do Moko, por outro lado, é causada apenas por linhagens do filotipo II. Análises com os genomas de linhagens do RSSC sugeriram que fatores de virulência e sistemas de secreção estavam envolvidos no sucesso da patogenicidade e adaptação bacteriana. Posteriormente, estudos focados no secretoma de *R. solanacearum* revelaram uma grande diversidade, composta majoritariamente por efetores do tipo III (T3Es) ou proteínas injetadas de *Ralstonia* (Rips). O secretoma desempenha funções essenciais no reconhecimento bacteriano, subversão dos mecanismos de defesa do hospedeiro, modulação metabólica, degradação da parede celular, especificidade de hospedeiros, e outras funções ainda desconhecidas. Neste trabalho, foram sequenciados e caracterizados 14 novos isolados de RSSC, das regiões norte e nordeste do Brasil, pertencentes aos ecótipos de murcha bacteriana (BW) e Moko. Os mecanismos de virulência e resistência foram anotados e o repertório de Rips foi predito para cada um dos isolados. Corroborando com estudos anteriores, o pangenoma de RSCC é aberto, devido à baixa quantidade de genomas representativos sequenciados. As informações genômicas de tamanho esperado e quantidade de sequências codificantes também corroboram com as descritas para *R. solanacearum* disponíveis no NCBI. Todos os 14 novos genomas sequenciados se agrupam no filotipo II, com similaridade acima de 96%, sendo cinco isolados do filotipo IIB e nove do filotipo IIA. Ademais, quase todos os genomas classificados como *R. solanacearum* no NCBI pertencem a outras espécies do complexo, como revelado pelas novas análises filogenômicas. No total, 43 Rips foram compartilhadas entre todos os 14 isolados. O repertório de Rips dos isolados Moko IIB foi mais homogêneo, exceto pelo isolado B4, que apresentou 10 Rips não compartilhadas entre os outros quatro isolados. O repertório de Rips dos isolados IIA foi mais diverso, tanto para os isolados BW quanto os de Moko. Curiosamente, os novos isolados BW compartilharam mais Rips com isolados de Moko IIA e Moko IIB do que com outros genomas públicos de isolados brasileiros de BW. As Rips que não foram compartilhadas dentre todos os isolados podem contribuir para a virulência individual, enquanto Rips comumente compartilhadas podem ser bons candidatos de avirulência. O alto número de Rips compartilhadas entre os novos isolados de Moko e BW sugerem que talvez estes isolados sejam capazes de infectar hospedeiros vegetais da família Solanaceae. Análises de enriquecimento funcional dos genomas dos 14 isolados revelaram a presença de genes para proteínas estruturais do sistema de secreção tipo III, genes *hrc/hrp* e reguladores AraC. A análise de interação proteína-proteína com Rips, genes estruturais do T3SS e reguladores transcricionais conseguiu identificar 70 de 89 candidatos no genoma de B4 com 203 interações. A delimitação de repertórios gênicos específicos para cada filotipo do complexo via análises pangenômicas oferece alvos potenciais para a prospecção de kits diagnósticos para isolados dos respectivos complexos. Por fim, este estudo abre novas perspectivas para ensaios de infecção e expressão gênica de Rips para melhor elucidar a associação entre o repertório de efetores e a especificidade de hospedeiro, além de auxiliar na compreensão de regulação gênica durante a patogênese.

Palavras-chave: Fitopatógeno; secretômica; sistemas de secreção bacterianos; T3Es; especificidade de hospedeiro; taxogenômica.

ABSTRACT

Strains of *Ralstonia solanacearum* species complex (RSSC) cause several phytobacteriosis in many economically important crops worldwide, especially in the tropics. In Brazil, phylotypes I and II are causal agents of bacterial wilt, preventing their distinction. In turn, Moko disease is caused only by phylotype II strains. Analyses with the genomes of RSSC strains suggested that many virulence factors and secretion systems were involved in successful pathogenicity. Subsequently, studies focused on the suite of secreted molecules of *R. solanacearum* revealed an extremely diverse secretome composed mostly of type III effectors (T3Es), or *Ralstonia* injected proteins (Rips). The secretome plays essential roles in bacterial recognition, subversion of host defense mechanisms, metabolic modulation, cell wall degradation, host specificity, and other yet unknown functions. In this work, we sequenced and characterized 14 new RSSC isolates from northern and northeastern regions of Brazil belonging to bacterial wilt (BW) and Moko ecotypes. The virulence and resistance mechanisms were annotated and the Rips repertoire was predicted for each isolate. Corroborating with past studies, the RSCC pangenome is open, as $\alpha \cong 0.77$. The genomic information of expected size and amount of coding sequences (CDSs) also corroborates with those described for *R. solanacearum* at NCBI. All the 14 new genomes sequenced cluster in phylotype II with similarity above 96%, with five isolates in phylotype IIB and nine in phylotype IIA. Furthermore, almost all genomes classified as *R. solanacearum* in NCBI actually belong to other species of the complex. In total, 43 Rips were shared between all 14 isolates. The Rips repertoire of the Moko IIB isolates was more homogeneous, except for isolate B4, which had 10 Rips not shared among the other four isolates. The Rips repertoire of the IIA isolates was more diverse in both BW and Moko isolates. Interestingly, the new BW isolates shared more Rips with Moko IIA and Moko IIB isolates than with other public genomes of Brazilian BW isolates. Rips that were not shared among all isolates may contribute to individual virulence, while commonly shared Rips may be good candidates for avirulence. The high number of shared Rips among the new isolates of Moko and BW suggests that these isolates may be capable of infecting the Solanaceae plants. Functional enrichment analyses of the genomes of the 14 isolates revealed the presence of genes for structural proteins of the type III secretion system and hrc/hrp genes and AraC regulators, however, failed to identify most of the effectors predicted for the repertoire. The protein-protein interaction with Rips, T3SS structural genes, and transcriptional regulators analysis was able to identify 70 of 89 candidates in the B4 genome, 203 interactions and an average degree of 6.03 per node. The delimitation of specific gene repertoires of each phylotype in the complex via pangenomic analyses offers potential targets for diagnostic kits to detect isolates of the respective complexes. Finally, infection and Rip gene expression assays in different hosts are needed to better elucidate the association between the effector repertoire and host specificity, to aid in the understanding of gene regulation during pathogenesis.

Keywords: Phytopathogen; secretomics; bacterial secretion systems; T3Es; host specificity; taxogenomics.

LISTA DE SIGLAS

BW – Bacterial Wilt; Murcha bacteriana

KEGG – Kyoto Encyclopedia of Genes and Genomes

NCBI – National Center for Biotechnology Information

PAI(s) – Pathogenicity Island(s) – Ilha(s) de Patogenicidade

Rips – *Ralstonia* Injected Proteins; Proteínas Injetadas de *Ralstonia*

Rsc – *Ralstonia solanacearum*

RSSC – *R. solanacearum* Species Complex; Complexo de espécies *R. solanacearum*

SILAC - Stable-Isotope Labeling By Aminoacids in Cell Culture; Marcação Isotópica Estável por Aminoácidos em Cultivo Celular

T3Es – Type III Effectors; Efetores do tipo III

T1SS - Type I Secretion System; Sistema de Secreção do Tipo I

T2SS – Type II Secretion System; Sistema de Secreção do Tipo II

T3SS – Type III Secretion System; Sistema de Secreção do Tipo III

T4SS – Type IV Secretion System; Sistema de Secreção do Tipo IV

T5SS – Type V Secretion System; Sistema de Secreção do Tipo V

T6SS – Type VI Secretion System; Sistema de Secreção do Tipo VI

SUMÁRIO

1. INTRODUÇÃO

A murcha bacteriana, fitobacteriose cosmopolita de difícil manejo e controle no campo, causada pelo complexo de espécies *Ralstonia solanacearum* (RSSC), é responsável por perdas significativas em culturas importantes, tais como banana, batata, eucalipto e tomate (LOPES; ROSSATO, 2018). No Brasil, os primeiros relatos da doença datam do século passado, quando foram relatados casos de murcha bacteriana em tabaco, batata e tomate nos estados do Rio Grande do Sul e Minas Gerais (LOPES; ROSSATO, 2018; MAGALHÃES, 1932; VON PARSEVAL, 1922). No estado de Pernambuco, este fitopatógeno já foi detectado nas mesorregiões do Agreste, Sertão, Zona da Mata, Região Metropolitana e Vale do São Francisco, sendo responsável por perdas significativas nas lavouras em que a doença foi identificada (COSTA et al., 2019; SANTIAGO et al., 2017).

R. solanacearum é uma espécie saprófita de beta-proteobactéria, encontrada no solo, sendo também patogênica a mais de 200 plantas no mundo todo, com distribuição marcante nos ambientes de clima quente, principalmente nos trópicos (GENIN; BOUCHER, 2004). Esta bactéria inicia seu processo infeccioso pela penetração nas raízes e posterior colonização nos vasos do xilema, até atingir as partes aéreas das plantas por meio do sistema vascular, induzindo a característica murcha dos órgãos vegetais aéreos (GENIN; BOUCHER, 2004). Por ter como um de seus habitats o solo, o emprego de cultivares resistentes ao fitopatógeno é a medida mais eficaz de evitar o contágio e dispersão no campo, além de menor custo e fácil adaptabilidade do produtor, o que torna esta prática mais difundida (COSTA et al., 2019).

O genoma de *R. solanacearum* compreende dois replicons circulares — um cromossomo e um megaplasmídeo — que abrigam fatores de virulência diversos (GENIN; BOUCHER, 2004). Para penetrarem, colonizarem e se reproduzirem com sucesso no organismo vegetal, as bactérias fitopatogênicas dispõem de uma série de mecanismos moleculares. Envolvem o reconhecimento de sinais extracelulares e também expressão dos genes que possibilitam a manifestação da doença no hospedeiro (GAMA *et al*., 2016). Dentre os mais relevantes, destacam-se os seus variados sistemas de secreção e efetores de virulência, e também os de hipersensibilidade.

Para *R. solanacearum*, os genes de adesão – codificadores de adesina – são uma peça chave para manifestar a patogenicidade, visto que tais proteínas são essenciais para a colonização do interior dos vasos condutores dos hospedeiros (GAMA *et al*., 2016; PRASANNATH, 2013). Além disso, esta espécie também apresenta genes de resistência a compostos tóxicos variados, incluindo metálicos, que permite sua sobrevivência a solos muito

degradados e dificulta os métodos de combate convencionais (GENIN; BOUCHER, 2004; SALANOUBAT *et al.*, 2002).

O reconhecimento da bactéria pela planta também é um ponto de extrema relevância, visto que este tipo de interação determina se o hospedeiro será suscetível, ou não, à infecção pela bactéria. Em fitopatógenos, essa interação se dá por meio de duas famílias de proteínas: Hrp/Hrc e Avr. Quando um hospedeiro é suscetível à infecção, ele não é capaz de reconhecer as proteínas Avr presentes na membrana plasmática externa da bactéria. Por sua vez, estas proteínas vão mudar a fisiologia da célula vegetal degradando-a lentamente, alterando o fluxo de íons H+/K+, favorecendo o efluxo de nutrientes para o meio intercelular e suprimindo a defesa basal do hospedeiro (DANGL, 1994; GAMA *et al.*, 2016). No entanto, quando um hospedeiro é capaz de reconhecer as proteínas Avr (indicando que é resistente à infecção), uma reação de hipersensibilidade é induzida na célula bacteriana por meio de suas proteínas Hrp para degradar a parede celular vegetal e ocasionar a morte celular (LINDGREN, 1997). Este processo é tão abrupto que acaba rompendo vacúolos com compostos tóxicos no meio intercelular, ocasionando a morte também das colônias de bactéria circundantes. As proteínas Hrp também estão envolvidas na detecção de sinais bioquímicos que estimulam o flagelo para levar a bactéria até a célula vegetal adequada para a infecção (GAMA *et al.*, 2016).

A virulência desta bactéria depende também de sistemas de secreção específicos, que realizam o transporte ativo de fatores de virulência para além da membrana plasmática, muitas vezes penetrando a maquinaria celular do hospedeiro, sem gerar acúmulo destas no periplasma. Para *R. solanacearum*, é importante destacar os sistemas de secreção tipo 2 e 3 (GAMA *et al.*, 2016). O sistema de secreção tipo 2 (T2SS), descrito como um sistema Sac/Tat-dependente ou de secreção geral, é composto geralmente por 12 genes e transporta proteínas com um peptídeo sinal amino-terminal através da membrana plasmática interna, realiza sua modificação com auxílio de uma proteína ATPase e então a transporta pela membrana plasmática externa por meio de um pili (CIANCIOTTO, 2005; COLLMER; BAUER, 1994). Essa é a via que muitos fitopatógenos utilizam para secreção de enzimas líticas como celulases, pectinases, proteases, exotoxina A, dentre outras moléculas (COLLMER; BAUER, 1994).

O sistema de secreção tipo 3 (T3SS) talvez seja o sistema mais importante para a virulência desta bactéria. Ele consiste de um complexo aparato molecular que envolve até 40 proteínas estruturais e 20 genes codificantes, responsáveis por formar um poro e um pili afiado que perfura a parede e a membrana plasmática da célula vegetal, transportando os fatores de virulência diretamente para dentro do citoplasma da célula hospedeira (GAMA *et al.*, 2016). Esta via de secreção é resultante da expressão do conjunto de genes *hrp*/*hrc*, também

responsáveis pela reação de hipersensibilidade ao patógeno no hospedeiro, comumente divididos entre tipos I, III e III. Supõe-se que são regulados por sinais do ambiente, como variação de temperatura, e também moléculas produzidas pelo hospedeiro, como as que levam à queda de pH no meio intercelular, liberação de flavonoides ou alteração na concentração dos íons Ca+ e Mg+ (GAMA *et al.*, 2016; TANG; XIAO; ZHOU, 2006). Os efetores de virulência do sistema de secreção tipo III são amplamente abordados no contexto das RSSC. Tanto a regulação transcricional do aparato de secreção quanto dos seus efetores de virulência é *hrp*-dependente, sendo HrpB o regulador transcricional responsável em *R. solanacearum*, do tipo AraC (GENIN *et al.*, 1992). Ademais, Estudos que investigaram a patogenômica de *R. solanacearum* revelaram que muitas sequências importantes para adaptação à fitopatogenicidade, como sistemas de secreção, aparato flagelar e afins, estão localizados no megaplasmídeo e não no cromossomo principal (GENIN; DENNY, 2012).

Ao longo da última década, estudos filogenéticos diversos utilizando marcadores moleculares desta bactéria, como as regiões gênicas e intergênicas do rRNA 16S-23S, CDS parcial para a endoglucanase (*egs*), e também estudos de hibridização DNA-DNA, revelaram que *R. solanacearum* na verdade compreendia um complexo de geno-espécies, e, portanto, não seria um grupo monofilético (SAFNI et al., 2014). Os isolados foram reorganizados em quatro filotipos de acordo com seus prováveis centros de origem geográficos, que abrangiam três espécies, distribuídas ao longo de oito clados (Figura 1): o filotipo I, originado na Ásia e o filotipo III, da África, formam a espécie *R. pseudosolanacearum* sp. nov.; os isolados do filotipo IV, da Indonésia, foram reclassificados em *R. syzygii* subsp. *indonesiensis*, *R. syzygii* subsp. *syzygii* e *R. syzygii* subsp. *celebensis* (ZHANG; QIU, 2016); e o filotipo II, das Américas, apresentou maior similaridade para a cepa-tipo de *R. solanacearum*. Dentre os grupos mencionados, no Brasil ocorrem os filotipos I e II, sendo o filotipo II amplamente distribuído por todo o país, tendo a bacia Amazônica como seu provável o centro de origem, e o filotipo I mais restrito às regiões Norte e Nordeste (SAFNI *et al.*, 2014; SANTIAGO; LOPES; MIZUBUTI, 2016).

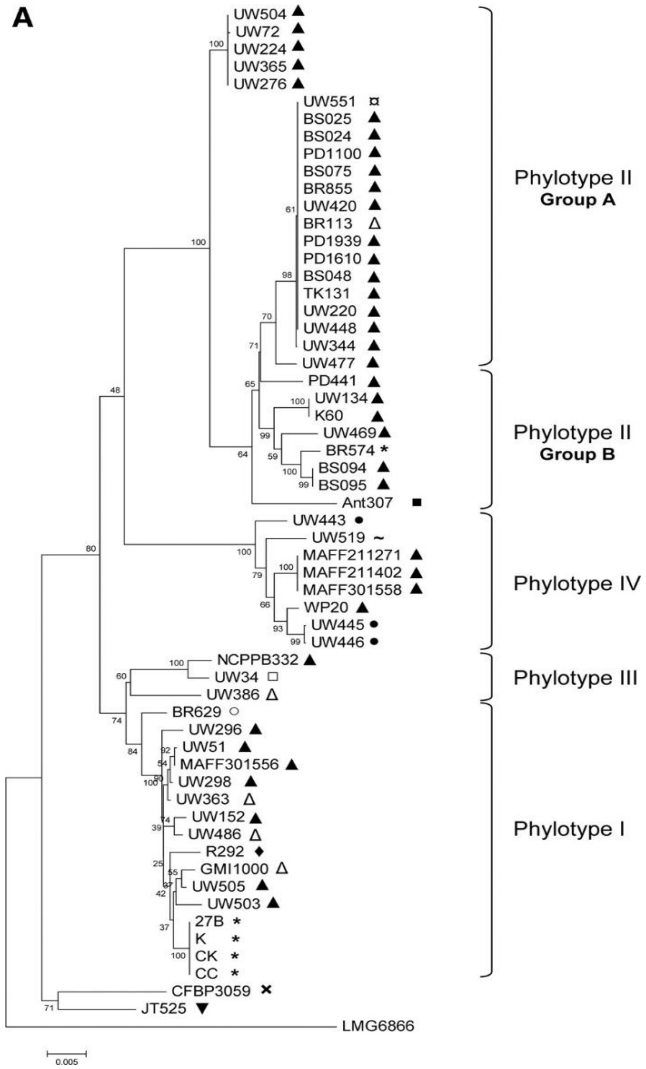

Figura 1. Árvore gerada pelo método de *neighbor-joining* a partir de dados concatenados do cromossomo bacteriano de isolados famosos do antigo complexo de espécies *R. solanacearum*. Fonte: Castillo e Greenberg (2007).

Para enriquecer os estudos com fitobacterioses, os filotipos tem sido subdivididos em ecótipos, de acordo com seus respectivos hospedeiros, as doenças que causam e suas características patogenômicas. Os filotipos I e III, de *R. pseudosolanacearum*, são responsáveis por causar murcha bacteriana em Solanaceae e não apresentam ecótipos definidos; o filotipo IV, de *R. syzygii*, apresenta os ecótipos para *blood disease bacteria* (IV7) e também doença-do-Sumatra-do-Cravo (IV8). Por sua vez, o filotipo II, brasileiro, apresenta ecótipos para murcha bacteriana em Solanaceae (isolados nos Estados Unidos da América e no Caribe) e também em banana (IIA), bem como para moko-da-bananeira, murcha bacteriana em gerânio e murcha bacteriana em Solanaceae (tomate, batata) (IIB) (GENIN; DENNY, 2012). Dentre os filotipos, os cientistas também utilizam análises filogenéticas para classificar os isolados de *R.*

solanacearum em sequevares (grupos infra-subespecíficos baseados em sequenciamento), ou seja, agrupamentos de isolados cujas sequências parciais para o gene *egl* tem divergência inferior ou igual a 1% (WICKER et al., 2012).

Por ser um dos centros de origem da espécie, a diversidade genética populacional de *R. solanacearum* no Brasil é alta. Os primeiros estudos que tentaram relacionar filotipo, sequevar e distribuição geográfica revelaram que o filotipo I, predominante na região Norte e Nordeste do país. Por sua vez, o filotipo IIB distribui-se ao logo de todo o país, e o IIA infecta hospedeiros em regiões mais isoladas, principalmente de baixa altitude, totalizando 22 sequevares identificados (SANTIAGO et al., 2017). Especificamente para a região Nordeste, estudos recentes revelaram que o filotipo I era predominante na mesorregião do Agreste e o filotipo IIA era predominante nas mesorregiões do Vale do São Francisco e Sertão, sendo os sequevares introduzidos de *R. pseudosolanacearum* mais virulentos que os sequevares nativos da região de *R. solanacearum* (ALBUQUERQUE et al., 2021).

A alta diversidade genética de *R. solanacearum* também traz um outro problema: a dificuldade em identificar em hospedeiros resistentes. No Brasil, os isolados apresentam características evolutivas bem distintas: enquanto o filotipo IIB têm uma população praticamente clonal, o filotipo IIA possui maior variabilidade genética e aparenta ainda estar em grande expansão, segundo técnicas de análise de sequências multilocus (MLSA) e BOX-PCR, por exemplo (CASTILLO; GREENBERG, 2007; SANTIAGO; LOPES; MIZUBUTI, 2016). Isso reforça indícios da capacidade desta espécie de suplantar a resistência dos hospedeiros com o passar do tempo, favorecidos principalmente pela migração de isolados via culturas contaminadas, recombinações genéticas e afins.

A genômica comparativa, primeiramente aplicada em genomas bacterianos de patógenos humanos de alta relevância clínica, como *Streptococcus agalactiae* e *Haemophilus influenziae*, visa avaliar a diversidade intraespecífica das linhagens a partir dos genomas inteiros (TETTELIN *et al.*, 2008). A principal vantagem das análises de genômica comparativa é a associação entre diversas características genômicas encontradas com o fenótipo apresentado por um espécime. Com o aperfeiçoamento das técnicas de bioinformática e observação dos resultados relevantes obtidos, a área se expandiu para outros microrganismos e cunhou em novas áreas, como a pangenômica. Esta, por sua vez, consiste em avaliar o repertório gênico de uma amostra de linhagens proximamente relacionadas evolutivamente para determinar o que é central ou comum, e o que é acessório, além de avaliar o processo evolutivo dos genomas com base na probabilidade ganhar novas sequências gênicas (COSTA *et al*., 2020). Nesta vertente, o pan-efetoma consiste no conjunto total de efetores que a amostra de linhagens possui. Este

conceito vem sido empregado para outros fitopatógenos de grande relevância na fitopologia e cujos efetores conhecidamente influenciam na patogenicidade, como *Xanthomonas* (ROUX et al., 2015).

Abordagens de genômica comparativa em linhagens permitiriam, por exemplo, estabelecer um pangenoma para o complexo de espécies *R. solanacearum*, determinando quais sequências fazem parte do genoma central e quais sequências são específicas de algumas linhagens ou ainda associadas a um determinado filotipo, ou ainda avaliar como o efetoma está distribuído ao longo do complexo de espécies. Análises anteriores que empregaram genômica comparativa, como a de Cho e colaboradores (2019), evidenciaram o potencial da abordagem no alvo em questão ao associar um sistema secretor específico a hospedeiros distintos em linhagens coreanas de *R. solanacearum,* através de pangenômica. Contudo, tais abordagens ainda não foram exploradas em linhagens brasileiras para esta finalidade, nem no tocante a outros mecanismos de virulência e resistência do patógeno, tratando-se do foco do presente estudo.

2. REFERENCIAL TEÓRICO

Nesta seção, será apresentada uma revisão bibliográfica a qual foi publicada na forma de capítulo de livro, com foco em secretômica e, em especial, como essa abordagem tem auxiliado na compreensão dos mecanismos de virulência e patogenicidade de *R. solanacearum*. O capítulo foi submetido ao volume especial "Microbial Genetics", da editora CRC Press – Taylor & Francis Group, em 2022 e foi publicado com distribuição online sob o código ISBN 978-1-03-235841-3. Permeia métodos utilizados para obtenção de dados de secretoma, sistemas de secreção bacterianos, efetores secretados mais bem estudados para *Ralstonia* e suas implicações na resistência e susceptibilidade de hospedeiros vegetais.

The Secretome Landscape of *Ralstonia*

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Abstract

Ralstonia is a late discovered genus comprising Gram-negative bacteria capable of surviving in the environment, although they are especially pathogenic to plants, including important crops of economic importance. Their ability to infect a broad range of hosts has always caught attention in the academic field, and the development of many omics science approaches (genomics, transcriptomics, and proteomics) has tried to answer how these bacteria cause pathogenesis, focusing on the RSSC molecular features. Genomic analysis of *R. solanacearum*' strains gave the first clues on pathogenesis success, as many different types of secretion systems and virulence factors were identified. Further studies focused on the set of molecules secreted by *R. solanacearum* during infection revealed a diverse secretome composed mainly type of III effectors (T3Es), or so-called *Ralstonia* injected proteins (Rips). Moreover, the *hrp/hrc* gene cluster was found to be a key factor for virulence, as they're related to type III secretion system

(T3SS) assembly and Rips secretion. The secretome plays important roles in bacterial recognition, defense mechanisms subversion on the host, metabolic modulation, plant cell wall degradation, host specificity, and still unknown functions. In this chapter, we will synthesize important virulence features of the *Ralstonia* genus, what are their secretion systems, and how they work. Finally, we will deepen into the importance and function of *Ralstonia* secretome, how it is estimated, and perspectives of future phytopathogen studies.

Keywords: Phytopathogen, secretomics, bacterial secretion systems, T3Es, host adaptation.

Introduction

The *Ralstonia* genus comprises rod-shaped, aerobic, non-fermenting, Gram-negative pathogen bacteria capable of surviving in environments such as soil and water (RYAN; ADLEY, 2014; YABUUCHI et al., 1995). The genus was finally settled when former species of the *Burkholderia* and *Alcaligenes* genera, such as *R. eutropha*, *R. solanacearum*, and the type species, *R. picketti*, were reclassified in 1995, based on phylogenetic analyses of 16S rRNA nucleotide sequences and rRNA-DNA hybridization, besides other phenotypic characteristics (YABUUCHI et al., 1995; ZHANG; QIU, 2016). Nowadays, the genus comprises 13 representative species with the *Cupriavidus* genus (PARTE, 2014). However, not all receive the same attention in the research field. While *Ralstonia basilensis*, *Ralstonia gilardii, Ralstonia oxalatica,* and *Ralstonia paucula* have no genomes available on public databases. Moreover, not long ago, researchers classified *R. solanacearum* as a species complex with four phylotypes according to their center of diversification and origin. However, new phylogenetic analyses separated them into two other species: *R. pseudosolanacearum* and *R. syzygii* (SAFNI et al., 2014; SANTIAGO; LOPES; MIZUBUTI, 2016). Currently, over 400 genomes of varied *Ralstonia* species are available in public databases, with *R. solanacearum* as the most abundant (Table 1).

In terms of pathogenesis, *Ralstonia* species exhibit diverse ecological niches: some of them are emergent opportunistic pathogens of humans, such as *R. picketti*, *R. mannitolilytica*, and *R. insidiosa,* while others are well-known plant pathogens, such as *R. pseudosolanacearum, R. syzygii*, and *R. solanacearum* (RYAN; ADLEY, 2014; SANTIAGO; LOPES; MIZUBUTI, 2016). Since they share a broad range of hosts, the latter species infect important crops all around the globe, causing substantial economic losses every year in banana, potato, and tomato plantations, for instance (LOPES; ROSSATO, 2018).

Many genomic studies have been carried out on the *R. solanacearum* genome to elucidate how the pathogenesis occurs on the host, emphasizing the development of sequencing technologies throughout the last decades. It's been announced that *R. solanacearum* has a bipartite genome consisting of one chromosome and one megaplasmid of 3.7 and 2.1 megabases, respectively, with a wide array of virulence factors, especially those secreted by types II, III, and IV secretion systems (GENIN; BOUCHER, 2004; SALANOUBAT et al., 2002). These virulence factors have also been severely studied in secretomics, i.e., proteomics focusing on secreted proteins by either eukaryotic or prokaryotic cells. This approach aims to elucidate the cellular context at a given time and condition (HATHOUT, 2007; TJALSMA et al., 2000), accessing essential molecular features for virulence and host defense. The secretome can be estimated with tools combining comprehensive genome analyses, genes annotation, and proteomics approaches such as mass spectrometry (HATHOUT, 2007). The secretome of *R. solanacearum* sheds light on type III effectors (T3Es), confirming that T3Es from other Gram-negative pathogenic bacteria play a critical role in host-pathogen relationships to cause pathogenesis, including *Yersinia, Shigella,* and *Pseudomonas*, for instance (CORNELIS; VAN GIJSEGEM, 2000).

Ralstonia's type III effectors are often called "Rips" – *Ralstonia* injected proteins, primarily dependent on *hrp* genes (MUKAIHARA; TAMURA; IWABUCHI, 2010). Besides them, proteins secreted through the type II secretion system have been associated with success for colonization and multiplication inside the host, achieving adaptation success, as in the case of *R. solanacearum*, which occurs in different environments (GENIN; BOUCHER, 2004; KANG, 1994). This chapter will focus on how *Ralstonia*'s secretome influences its pathogenicity through the effectome, focusing mainly on T3SS and T2SS, their peculiarities, bringing insights on host specificity, challenges, and perspectives of this field.

TABLE 1. Several genome assemblies for each species of *Ralstonia* are available in the NCBI and PATRIC databases.

Species Scientific Name	Number of Genomes Assembled	Host/Source	Country
Ralstonia eutropha (Davis 1969) Yabuuchi *et al.* 1996	2	-	-
Ralstonia insidiosa Coenye *et al.* 2003	20	Medical sample, food processing center	USA, China, Russia
Ralstonia mannitolilytica cor rig. De Baere *et al.* 2001	13	Medical sample and *Cupriavidus*	USA, China, Turkey,
Ralstonia pickettii (Ralston *et al.* 1973) Yabuuchi *et al.* 1996	87	Rice, medical samples	USA, China, Japan, New Zealand
Ralstonia pseudosolanacearum Safni *et al.* 2014	34	Soil-borne, tomato, tobacco, rose, *Cupriavidus*	Brazil, China, and the Netherlands
Ralstonia solanacearum (Smith 1896) Yabuuchi *et al.* 1996	297	Soil-borne, banana, platano, tobacco, eggplant, potato, eucalyptol, bell pepper, etc.	North America, South America, Asia, and Africa
Ralstonia syzygii (Roberts *et al.* 1990) Vaneechoutte *et al.* 2004	6	Banana, tobacco, and *Cupriavidus*.	China and Sumatra

Ralstonia's **Secretion Systems Make-Up a Powerful Secretome**

To fulfill many functions in the bacteria cell, proteins are usually translocated to cellular compartments other than the ones they are synthesized in, as soon as the cell machinery recognizes their signal peptides (TJALSMA et al., 2000). Secretion systems are usually responsible for protein translocations in and outside the cell. Secretome studies value secreted proteins because they are intimately related to many roles in the cell, such as homeostatic maintenance in normal physiologic and pathogenic conditions (HATHOUT, 2007). In this context, secretion systems of different types play a significant role in bacterial interaction with

the environment or other competitor bacteria, host recognition, and colonization. All these aspects receive equal attention in secretome analysis.

Bacterial cells display three basic types of secretion systems: the Tat (twin-arginine translocation) and Sec (general secretion) pathways and ABC (ATP-binding cassette) transporters, which are the most used machinery for protein translocation between plasmatic membranes (GREEN; MECSAS, 2016; NATALE; BRÜSER; DRIESSEN, 2008). Secretion systems may cross from one to three phospholipidic membranes: the inner plasmatic membrane, both plasmatic membranes or both the pathogen and the host's membranes (GREEN; MECSAS, 2016). However, for the effective transport of essential proteins, such as virulence factors, more complex secretion systems are commonly associated with the basic ones, especially in Gram-negative bacteria. The main difference between Gram-positive and Gram-negative secretion systems is that the latter can secrete proteins between and out of the cell plasmatic membrane, while the first may also secrete to the cell wall (TJALSMA et al., 2000).

To date, seven different types of specialized secretion systems have been identified in bacteria, and even though not every bacteria displays all of them, Gram-negative bacteria, like *Ralstonia*, usually have more types of secretion systems than Gram-positive bacteria (ABDALLAH et al., 2007; GAMA et al., 2016; GREEN; MECSAS, 2016; THANASSI; HULTGREN, 2000). Most genomes of the plant pathogen *R. solanacearum* count with secretion system type 1 (T1SS), T2SS, T3SS, T4SS, T5SS, and the recently discovered T6SS (GAMA et al., 2016; GENIN; DENNY, 2012) (Figure 1).

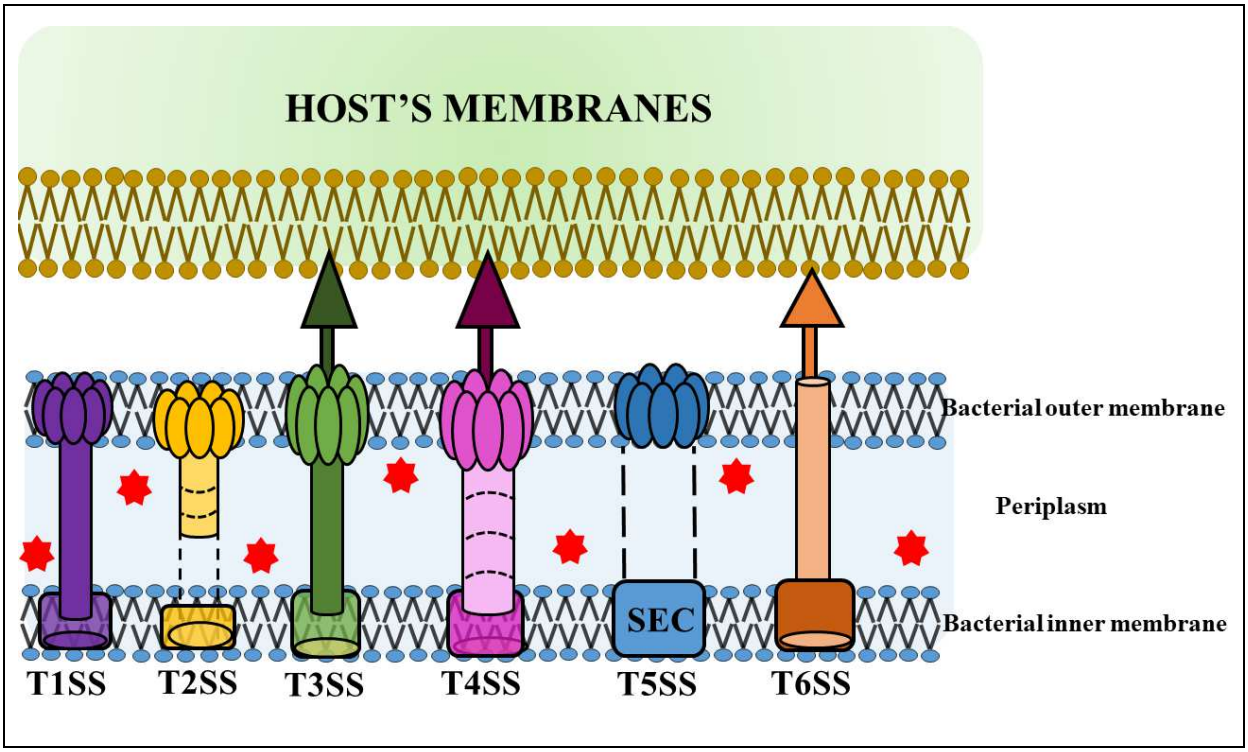

FIGURE 1. Hypothetical Gram-negative bacterial cell with the six mentioned types of secretion systems. Syringe-like secretion systems (T3SS, T4SS, and T6SS) can inject effectors (septagon-shape) directly into the host cell, while the reminiscent (T1SS, T2SS, and T5SS) only transport effectors from the cytoplasm and periplasm and out of the cell.

Multiple essential techniques used in genomics, transcriptomics, and proteomics may be optimized to investigate one organism's secretome. By far, the best method for secretomic approaches is mass spectrometry, which can be executed with a range of combined techniques, like liquid chromatography, 2D gel electrophoresis, and SILAC (stable-isotope labeling by amino acids in cell culture) (HATHOUT, 2007; LONJON et al., 2016; MANN, 2006). Genome-wide analysis has been crucial to refining secretome studies in the past years since they can identify sequences inside the genome, including predicted targets on a metabolic pathway especially when lacking *in vivo* and *in vitro* data (GAGIC et al., 2016). Tools for *in silico* protein prediction might work based on Bayesian inferences associated with Markov-chains, network-based inferences, and machine-learning algorithms, amongst other bioinformatics and statistic models (CACCIA et al., 2013; GAGIC et al., 2016; ZHOU et al., 2010). Transcriptomes can also be helpful for secretome insights. Still, it must be noted that not always the level of expression of one transcript corresponds to the exact amount of the respective protein,

demanding validation using other approaches under the same condition, such as mass spectrometry (HATHOUT, 2007).

As Gram-negative bacteria first need to transport proteins into the periplasm before translocation out of the cell or into the host, Tat and Sec pathways were the first targets in secretome studies involving *R. solanacearum*. Bioinformatics predictions of proteins containing Tat-associated motifs have indicated that approximately 70 *R. solanacearum* proteins are exported to the periplasm through the Tat pathway before potential secretion through T2SS (GONZÁLEZ et al., 2007; POUEYMIRO; GENIN, 2009). Subsequent studies focused on T2SS and T3SS mutants of this same species revealed many insights into how hundreds of effectors secreted by them were crucial for plant cell wall degradation, colonization, and multiplication inside the host, besides triggering host resistance mechanism, for instance (GENIN; BOUCHER, 2004; JONES; DANGL, 2006; LIU et al., 2005; POUEYMIRO; GENIN, 2009). Most recent data has revealed that the secretome of *R. solanacearum* comprises around 228 proteins involved in many cellular activities, and over one-third of them belong to Rips (LONJON et al., 2016).

Those cellular types of machinery collectively contribute to bacterial success in the environment and inside hosts. The following pages will go through the most relevant secretion systems for *Ralstonia* species: T2SS and T3SS. We will go over their structure, main functions, and why they are essential for these bacteria. Firstly, the role of the reminiscent secretion systems will be discussed.

***Ralstonia*'s Arsenal of Secretion Systems**

Type I secretion system is the most spread one across diverse Gram-negative bacteria, including animal and plant pathogens, like *Ralstonia* species (GAMA et al., 2016; GREEN; MECSAS, 2016). It is an ABC transporter dependent system that secretes proteins across the cell membranes composed of three essential components: an ABC transporter, which captures the proteins on the inner membrane; an MFP (membrane fusion protein), which transports molecules across the periplasm into the outer membrane; and an OMF (outer membrane factor), a porin-like protein that finally secretes the molecules (MORGAN; ACHESON; ZIMMER, 2017; THOMAS; HOLLAND; SCHMITT, 2014). One example of proteins secreted by T1SS is RTX-like toxin genes, important for host cell rupture, found in the *R. solanacearum* genome (GENIN; BOUCHER, 2004; GREEN; MECSAS, 2016).

Genes related to the type IV secretion system are also found in *R. solanacearum* genomes. This system is generally responsible for bacteria-bacteria interaction via conjugation but can also secrete protein-protein and protein-DNA complexes into a broad range of target cells, including eukaryotic host cells (CASCALES; CHRISTIE, 2003; GREEN; MECSAS, 2016). Usually, 12 proteins belonging to the VirB/D family assemble this system, comprising an ATP-power system on the inner membrane, a secretion channel through the periplasm and its associated proteins, besides an extracellular T pilus that recognizes and delivers molecules into the target cells (FRONZES; CHRISTIE; WAKSMAN, 2009; GREEN; MECSAS, 2016). This system serves different purposes depending on the species where they are found. Still, they are believed to uptake hosts' DNA sequences related to resistance and adaptation and deliver virulence effectors that numb the host immune responses, providing a better environment for pathogen colonization (GAMA et al., 2016; ISBERG; O'CONNOR; HEIDTMAN, 2009). Recently, the T4SS has been related to the acquisition of arsenite resistance genes through horizontal transference in gentamicin resistant strains of *R. pickettii* (FERRO; VAZ-MOREIRA; MANAIA, 2021).

The type V secretion system is unique as it is composed of autotransporter proteins that cross the outer membrane and secrete molecules themselves (GAMA et al., 2016; GREEN; MECSAS, 2016). The superfamily of autotransporter proteins found in T5SS usually exhibits one peptide with a C-terminal pore-forming β-barrel domain for substrate secretion and another peptide with an N-terminal exodomain, allowing this system to channel into the membrane and transport molecules which were delivered to the periplasm via the Sec pathway (GREEN; MECSAS, 2016; LEYTON; ROSSITER; HENDERSON, 2012). The T5SS is divided into three categories according to the number of proteins that participate in the secretion process: (i) type Va, the classical model; (ii) type Vb, the two-partner-secretion model, composed of two proteins (one with the exodomain and the pore-forming β-barrel domain) who are translated and translocated into the membranes separately; and (iii) type Vc, the trimeric AT adhesin, which is composed of polypeptides that are translated together and act together as a trimeric since they have short C-terminal β-barrel chains (JACOB-DUBUISSON; FERNANDEZ; COUTTE, 2004; LEYTON; ROSSITER; HENDERSON, 2012; MENG et al., 2006). The genome of *R. solanacearum* exhibits genes for at least two hemagglutinin-related proteins with autotransporter features, which may aid in the secretion of diverse virulence factors such as adhesins and toxins (GENIN; BOUCHER, 2004).

At last, the type VI secretion system is also a syringe-like apparatus responsible for the secretion of toxin substrates, which is crucial for bacterial interspecies competition and useful for pathogenesis success (RUSSELL; PETERSON; MOUGOUS, 2014). Despite the late identification and characterization of T6SS coding genes in some *R. solanacearum* genomes (ZHANG et al., 2012, 2014), their role in virulence contribution to host infection has only been recently proved in mutants essays infecting eggplants (ASOLKAR; RAMESH, 2020).

The Type II Secretion System: Wall-Breaker of Host's Cell

The translocation through T2SS is a two-step process requiring the protein first to be transported into the periplasmic environment through the Sec or Tat secretion pathways (GAMA et al., 2016; GREEN; MECSAS, 2016), and all of them are required for proper molecules delivery. It comprises a versatile system conserved in several Gram-negative bacteria, and has been shown to have a significant function in their pathogenesis. For example, *Vibrio cholerae* and *Vibrio vulnificus* mutants lacking PilD (which plays an essential role in pseudopili formation) are attenuated in animal models (FULLNER; MEKALANOS, 1999; PARANJPYE; STROM, 2005). Furthermore, the T2SS was one of the first described for *R. solanacearum* (KANG, 1994). While not considered as vital to pathogenesis as the type III secretion system, T2SS is essential for *R. solanacearum*'s virulence, responsible for the secretion of various cell wall degrading enzymes (KANG, 1994; LIU et al., 2005; POUEYMIRO; GENIN, 2009). Lastly, the T2SS counts with highly conserved 15 protein complexes that transport folded proteins from the periplasm to the extracellular environment.

The T2SS comprises four subunits: an ATPase, an inner-membrane platform, an outer-membrane complex, and a pseudopilus. The ATPase, located in the cytoplasm, provides energy to the system, allowing protein transport. The inner-membrane platform extends into the periplasm, facilitating signaling communication between the pilus, the secretin, and ATPase. The outer-membrane complex serves as a transmembrane channel composed of multimeric secretin. The pseudopilus, which is structurally similar to type IV pili found on the bacterial cell surface, theoretically pushes the substrate through the outer membrane complex (GAMA et al., 2016; GREEN; MECSAS, 2016). An analysis of the *Ralstonia solanacearum* genome shows that it contains the complete set of core T2SS genes located in the RSc3105–RSc3116 gene cluster.

The general secretion (Sec) pathway is responsible for transporting unfolded proteins from the cytoplasm to the periplasmic environment. Because the T2SS only secretes folded

substrates, proteins transported through the Sec pathway must be folded in the periplasm before secretion. The Sec system is composed of three distinct subunits: SecB, a protein targeting component that recognizes and binds to pre-secretory proteins, SecA, a motor protein and ATPase providing energy for transport, and SecYEG, a membrane-integrated translocase (GREEN; MECSAS, 2016; PAPANIKOU; KARAMANOU; ECONOMOU, 2007).

The twin-arginine protein translocation (Tat) pathway is vital to *R. solanacearum* virulence. Unlike the Sec pathway, the Tat pathway transports proteins that have already been folded. It is mainly related to the secretion of proteins that go through post-translational modifications or cannot be folded within the periplasm (GREEN; MECSAS, 2016; NATALE; BRÜSER; DRIESSEN, 2008). The Tat pathway for Gram-negative bacteria is composed of three proteins: TatA and TatB, which bind to the signal peptide of Tat-secreted proteins, and TatC, which is then recruited to form a transmembrane channel. The signal peptide in Tat-secreted proteins is recognized by the "twin" arginines at the N-terminus of the folded protein (GONZÁLEZ et al., 2007; GREEN; MECSAS, 2016; NATALE; BRÜSER; DRIESSEN, 2008). As for its importance in pathogenesis, a *R. solanacearum* strain with a mutated TatC gene lost much of its capability to cause bacterial wilt, proving that the Tat pathway participates in the secretion of vital virulence factors (GONZÁLEZ et al., 2007).

The most relevant studies on *R. solanacearum* revealed that the T2SS main contribution to pathogenesis is to secrete a variety of extracellular proteins with degrading features. Those proteins are often called cell-wall-degrading enzymes (CWDEs), important in damaging plant cells for nutrient uptake (GAMA et al., 2016). Among these, there are pectic enzymes (PehA, PehB, PehC, and Pme), and cellulolytic enzymes (Egl, CbhA), as well as extracellular nucleases (NucA, NucB), which serve to degrade and avoid host defenses. Mutant strains of *R. solanacearum* lacking these CWDEs have been shown to have diminished virulence (GONZÁLEZ et al., 2007; KANG, 1994; LIU et al., 2005; POUEYMIRO; GENIN, 2009). Furthermore, another *R. solanacearum* mutant strain with an inactivated T2SS secretin gene has been shown to significantly attenuate virulence, even with the mutant strains lacking all known cell-wall-degrading enzymes. This diminished virulence points to further unidentified virulence factors associated with T2SS in *Ralstonia solanacearum*.

The Type III Secretion System: An effectome powerplant

Of all secretion systems, the type III must be the critical player for host invasion and colonization success, as it secretes proteins capable of numbing the host basal immune

responses (GAMA et al., 2016; STASKAWICZ et al., 2001). Like T4SS and T6SS, the T3SS structure is described as a needle or syringe that allows it to secrete protein substrates directly into the target cell's cytoplasm (GREEN; MECSAS, 2016). This system comprises nine core proteins, but up to 20 accessory proteins can be associated with them, depending on the target species (GAMA et al., 2016; GREEN; MECSAS, 2016). The basic structure called the "injectisome" comprises two basal body rings, one in the inner and another on the outer membrane, making a hollow channel for protein translocation; one inner rod that guides the protein from inside the periplasm; and an export pilus-like apparatus, which carries a needle for protein secretion inside the host cell's cytoplasm (BURKINSHAW; STRYNADKA, 2014). Since this system is essential for pathogenesis, it requires a complex temporal and spatial regulation of the genes involved for its correct assembly.

The coding genes responsible for the T3SS are the *hrp*/*hrc* (hypersensitive response and pathogenicity) gene clusters. As they're located in few operons inside the genome, they are commonly horizontally transferred (i.e., via pathogenicity islands and plasmids) amongst pathogenic bacteria, so one bacterium may present different types of T3SS, even when compared to close related strains of the same species (GREEN; MECSAS, 2016; TROISFONTAINES; CORNELIS, 2005). The nine core Hrp proteins are homologous and highly conserved in both animal and plant Gram-negative pathogen bacteria, so their respective genes were renamed *hrc* (hypersensitive response conserved) (BOGDANOVE et al., 1996; DE PEDRO-JOVÉ et al., 2021; GAMA et al., 2016; VAN GIJSEGEM et al., 1998).

In *R. solanacearum*, this cluster is located in an operon region and organized on at least seven transcriptional units regulated by *hrr*G and *hrp*B genes. Interestingly, this cluster is located on the megaplasmid (instead of the main chromosome) and lacks pathogenicity islands (PAI) transference characteristics, such as the absence of nearby mobile elements and GC content similar to the core genome (TAMPAKAKI et al., 2010). Moreover, many studies on *R. solanacearum* and other phytopathogenic bacteria have revealed that HrpB is a crucial regulator to many effector proteins, including the ones secreted by different types of secretion systems, like T2SS (FURUTANI et al., 2004; JEONG et al., 2011; KANG et al., 2008; MUKAIHARA; TAMURA; IWABUCHI, 2010).

The first studies on T3SS involving *Ralstonia* species tried to understand the importance of this system in plant interactions, as the host signal during infection induces the *hrp/hrc* cluster expression and T3SS assembly. They proved that mutants with *hrp*-depleted genomes had significantly reduced capacity for both host colonization and ability to cause disease since they

could not assemble T3SS and other genomic regions regulated by the *hrp/hrc* cluster were prejudiced (KANDA et al., 2003; MUKAIHARA; TAMURA; IWABUCHI, 2010). In *R. solanacearum*, the *hrp*Y gene is responsible for the T3SS pilus assembly, involved in either protein and harpins secretion, and host signal receptor (KVITKO et al., 2007; STASKAWICZ et al., 2001). Finally, later studies have shown that the T3SS in *R. solanacearum* not only impacts the ability to cause pathogenicity but also influences how the host interacts with other microbes, such as mutualistic nodulating bacteria and the ability for nodulation itself (BENEZECH; LE SCORNET; GOURION, 2021; GUAN et al., 2013). The following section will go deeper into effectors secreted by the T3SS and their impact on *R. solanacearum* pathogenicity and regulation.

Hrp Regulators' Refined Roles in the Pathogenicity Scenario

Gene regulation analyses were crucial to explain how *R. solanacearum* sharply tunes its pathogenicity. The transcription of the *hrp* family genes - which encode the T3SS - and its related effectors were found to be managed by the HrpB transcriptional activator (DE PEDRO-JOVÉ et al., 2021). The first studies regarding this gene revealed that the *hrp*B gene is located downstream of a regulatory cascade induced by the contact of the bacterium with the plant cell wall (BRITO et al., 1999). In *R. solanacearum,* HrpB (an AraC-type regulator), and HrpG (an upstream OmpR-like response regulator), are responsible for controlling *hrp*/*hrc* family gene expression (PEETERS et al., 2013b). Moreover, HrpB is a direct trigger for the transcription of T3SS genes, likely by binding to the *hrpII* box found in their promoter regions, and its expression is governed by HrpG (PEETERS et al., 2013b). Both genes are genetically and functionally conserved/preserved in *Xanthomonas* spp. Still, a unique feature of the *R. solanacearum* genome is the presence of upstream regulators, which can specifically induce *hrp*G expression when the bacterium identifies a plant cell-wall component (ALDON et al., 2000). PrhA perceives the signal from detecting cell-wall components, an outer membrane receptor, which transmits the signal to PrhI and PrhR, membrane-associated proteins that trigger *hrp*/*hrc* expression through the transcriptional regulators PrhJ, HrpG, and HrpB (BRITO et al., 2002; PEETERS et al., 2013a)

Gene expression studies have shown that *R. solanacearum hrp* genes and T3SS effectors were transcribed *in planta* at late infection stages (MONTEIRO et al., 2012). Other studies of bacteria infecting tomato, banana, and potato roots have confirmed these findings (AILLOUD et al., 2016; JACOBS et al., 2012; PUIGVERT et al., 2017). Further, transcriptomics has

indicated that the *hrp* regulators may control additional functions other than the T3SS and its effectors. For instance, the HrpB regulator has previously been involved in chemotaxis and the biosynthesis of certain bacterial compounds, such as the Hrp-dependent factor (HDF), which possibly stimulates a cell density-dependent LuxR system (DELASPRE et al., 2007). In addition, the HrpG regulator has also been shown to be involved in a variety of genetic regulatory processes unrelated to bacterial secretion (PEETERS et al., 2013b; VALLS; GENIN; BOUCHER, 2006). Plener and collaborators (2012) have shown that HrpG is involved in methionine synthesis regulation. It has been proposed that it may be a promoter of MetE, which synthesizes methionine without vitamin B12. This process is vital for methionine biosynthesis in vitamin-poor environments in the plant. Thus, it can be said that HrpG is central to the regulation of *R. solanacearum* pathogenicity through the T3SS and controls a wide array of unrelated virulence genes (PLENER et al., 2012).

Ralstonia Injected Proteins (Rips): Diversity, Function, and Importance

As we just mentioned, the type III secretion system is vital to *Ralstonia solanacearum* pathogenesis and virulence due to its ability to inject effector proteins directly into the host cell (LANDRY et al., 2020). These type 3 effectors (T3Es) are thus referred to as *Ralstonia* injected proteins (Rips), a highly diverse repertoire of virulence factors that enable *R. solanacearum* to infect a wide array of host organisms (BOCSANCZY et al., 2022; LANDRY et al., 2020; LONJON et al., 2016). The bacterium's vast array of T3Es contributes to virulence and pathogen fitness *in planta*, with many roles in pathogenicity (GENIN, 2010; LONJON et al., 2016; POUEYMIRO; GENIN, 2009).

The first Rips were cloned and identified in the 1990s, discovered in 1990, and initially named AvrA, now referred to as RipAA (CARNEY; DENNY, 1990; LANDRY et al., 2020). Since then, various technologies and techniques have allowed for the identification and functional characterization of additional Rips. The advent of cheaper, more accessible sequencing technologies has allowed the development of sequence-based methods to identify Rips in the genome by searching for homology to known Rips and known molecular patterns in the sequence (LANDRY et al., 2020). In addition, bioinformatics-based tools capable of predicting and identifying Rip sequences have been developed, further improving our capacity to map virulence-associated T3Es (PEETERS et al., 2013c; SABBAGH et al., 2019). Additionally, the ongoing evolution of genetic engineering techniques has allowed the advent of regulation-based methods to identify Rips, as the hrp genes regulate the T3SS expression,

and their manipulation can be used in assays aimed at finding T3Es and, consequently, Rips (FURUTANI et al., 2004; JEONG et al., 2011; KANG et al., 2008; MUKAIHARA; TAMURA; IWABUCHI, 2010).

Rips have many functions in *Ralstonia*'s life cycle and pathogenicity, some identified and described over the last few decades (Table 2). Over 50 have already been characterized in some level of detail (LANDRY et al., 2020). Several Rips, such as RipP2, RipAY, and RipX (the first Rip characterized), are understood to interfere with plant immunity and trigger hypersensitive responses on hosts (ARLAT et al., 1994; FUJIWARA et al., 2016, 2020; LE ROUX et al., 2015). Others, such as RipTAL, act as a transcriptional activator-like effector, targetting and manipulating plant metabolism to boost the production of specific antimicrobial molecules, possibly inhibiting the growth of *R. solanacearum* competitors (WU et al., 2019). At last, some Rips suppress host recognition and response to other effectors, such as RipAY, RipAC, and RipAK (SANG et al., 2020; SUN et al., 2017; YU et al., 2020). A micro review published by Landry and colleagues (2020) has synthesized the many functions Rips fulfill for a successful infection.

Recent studies on the multitude of available *R. solanacearum* strains have sought to identify this bacterium and its subspecies' pan-effectome or the common effectors found within the species and its variants. The pan-effectome of *Ralstonia solanacearum* was reported to include 102 type III effector genes and 16 hypothetical T3E genes, with each strain having, on average, 64 T3E genes (SABBAGH et al., 2019). There is also a significant amount of variation between strains, as the core effectome (effectors that are shared by over 95% of the strains) of the bacterium had only 16 proteins, including RipG5, RipB, RipW, RipAC, RipAB, RipR, RipE1, RipAM, RipAN, RipAY, RipAJ, RipF1, and RipAI (AILLOUD et al., 2015; LANDRY et al., 2020). Both the core-effectome and the pan-effectome of *R. solanacearum* are very large and diverse compared with other phytopathogens, such as *Pseudomonas syringae* and *Xanthomonas campestris* (DILLON et al., 2019; LANDRY et al., 2020; ROUX et al., 2015). A couple of Rips families found for *R. solanacearum* to date is available at Table 2. This relatively large and diverse repertoire of Rips has been theorized to be related to this bacterium's ubiquity, although few host-specificity determinants have been found so far (Sabbagh, et al. 2019, Bocsanczy et al. 2022).

The pan-effectome of *R. solanacearum* is highly redundant, which may, in part, be related to its size (ANGOT et al., 2006; CHEN et al., 2014; SOLÉ et al., 2012). These redundant effectors point toward a robust virulence strategy: having many genes means having an additive

effect on their functions, making the bacterium less susceptible to the deleterious impacts that may arise from mutations in those genes (LANDRY et al., 2020). A remarkable feature of strains from the former *R. solanacearum* species complex is the presence of genes for at least one of these Rip families: RipG (GALA), RipS (SKWP), RipA (AWR), RipH (HLK), or RipP (PopP) (LANDRY et al., 2020), as discussed below.

The RipG (GALA) family is composed of seven genes with leucine-rich repeats on their sequences, initially identified by their homology with plant proteins with F-box domains, which participate in the ubiquitination of a broad range of proteins (ANGOT et al., 2006; KIRKPATRICK; DENISON; GYGI, 2005). These effectors are believed to associate with SKP-1-like proteins and promote the ubiquitination of chloroplast proteins, leading to plant cell death (LANDRY et al., 2020; WANG et al., 2016). The RipG family can be subdivided into two clades based on neofunctionalization: RipG1, 3, 4, 5, and RipG2, 6, 7, in which RipG1, 2, and RipG5 are ancestral genes, and other acquired new functions through diversification (REMIGI et al., 2011). In addition, RipG4 is responsible for callose deposition inhibition in *A. thaliana*, and RipG1 and 3 inhibit host immune responses dependent on salicylic acid (MEDINA-PUCHE et al., 2020; REMIGI et al., 2011). Besides, the core effector RipG7 is one of the most studied in this family and is attributed to promoting full virulence in tomato and *A. thaliana* infections and compatible interaction with other hosts (ANGOT et al., 2006; WANG et al., 2016).

When the RipS (SKWP) family was discovered, it was described as the family with the largest effectors of plant and animal pathogenic bacteria. It comprises six effectors (RipS1-6) characterized by a 42-long novel motif in 12-18 tandem repeats, called the SKWP repeats (MUKAIHARA; TAMURA, 2009; SABBAGH et al., 2019). *R. solanacearum* mutants with RipS deleted genes did not lose their capacity to infect tobacco roots and leaves; however, they were necessary to achieve full virulence on the host (PEETERS et al., 2013c). Effectors that portray long domains, like SKWP, usually interact with plant factors to fulfill virulence functions (MUKAIHARA; TAMURA, 2009). Encoded by initially five genes (*awr*1-5), the RipA (AWR) family proteins have triad-domains of alanine-tryptophan-arginine, and the core effectors RipA2, RipA3, and RipA5 are crucial for *R. solanacearum* virulence, multiplication inside the host, and cell death (SOLÉ et al., 2012).

The RipH (HLK) family comprises three effectors (RipH1-3) that are 700-800 amino acids long and have no particular known motifs or domains, being named after the histidine-leucine-lysine triad conserved on the sequence C-terminal region (CHEN et al., 2014;

POUEYMIRO; GENIN, 2009). Two of the coding genes (*hlk*2 and *hlk*3) are located on the megaplasmid, while the other remains on the main chromosome (CHEN et al., 2014). Collectively, these proteins have been associated with promoting full virulence in tomatoes, but mutant strains with *rip*H deleted genomes were as virulent as wild strains in tobacco and eggplant (CHEN et al., 2014). The RipP (PopP) family comprises two effectors, RipP1 and Rip2, that belong to the YopJ/AvrRxv superfamily of proteins related to the avirulence/immune responses on both animal and plant hosts. Once the bacteria are inside the host, RipP2 plays a crucial role in Avr hypersensitive reactions. It promotes virulence on hosts numbing the immune responses via acetylation of WRKY transcriptions factors while RipP1 is somehow related to avirulence responses in some petunia lines (DESLANDES et al., 2003; MACHO et al., 2010).

TABLE 2. List of type III effectors (T3Es) genes currently identified in the *R. solanacearum* species.

Type III effectors (T3Es)	Representative gene member	Other Name	References
RipA1	RSc2139	AWR1	SOLÉ et al., 2012
RipA2	RSp0099	RipA, Rip29, Hpx31, AWR2	CUNNAC et al., 2004; MUKAIHARA et al., 2010
RipA3	RSp0846	Rip44, Hpx32, AWR3	MUKAIHARA et al., 2010
RipA4	RSp0847	Rip45, Hpx4, AWR4	MUKAIHARA et al., 2010
RipA5	RSp1024	Rip56, Hpx10, AWR5	MUKAIHARA et al., 2010
RipAA	RSc0608	AvrA, Rip5, Brg46	MUKAIHARA et al., 2010
RipAB	RSp0876	PopB, Rip48	GUENERON et al., 2000; MUKAIHARA et al., 2010
RipAC	RSp0875	PopC, Rip47	GUENERON et al., 2000; MUKAIHARA et al., 2010
RipAD	RSp1601	Rip72	MUKAIHARA et al., 2010
RipAE	RSc0321	Rip4	MUKAIHARA et al., 2010
RipAF1	RSp0822	Rip40	MUKAIHARA et al., 2010
RipAF2	R. syzygii RALSY_20037	-	PEETERS et al., 2013a
RipAG	RSc0824	Rip6	MUKAIHARA et al., 2010
RipAH	RSc0895	Rip11	MUKAIHARA et al., 2010
RipAI	RSp0838	Rip41	MUKAIHARA et al., 2010
RipAJ	RSc2101	Rip21, Hpx18	MUKAIHARA et al., 2010
RipAK	RSc2359	Rip23, Hpx28, Brg36	MUKAIHARA et al., 2010
RipAL	UW551 RRSL_02221	Rip38	MUKAIHARA et al., 2010
RipAM	RSc3272	Brg40	PEETERS et al., 2013a
RipAN	RSp0845	Rip43, Hpx33, Brg33	MUKAIHARA et al., 2010
RipAO	RSp0879	Rip50, Hpx2, Brg34	MUKAIHARA et al., 2010
RipAP	UW551 RRSL_04655	Rip60	MUKAIHARA et al., 2010
RipAQ	RSp0885	Rip51, Brg35	MUKAIHARA et al., 2010
RipAR	RSp1236	Rip61	MUKAIHARA et al., 2010
RipAS	RSp1384	Rip66, Hpx9, Brg43	MUKAIHARA et al., 2010
RipAT	RSp1388	Rip67, Brg48	MUKAIHARA et al., 2010
RipAU	RSp1460	Rip68, Hpx8, Brg45	MUKAIHARA et al., 2010
RipAV	RSp0732	Rip39, Hpx27, Brg39	MUKAIHARA et al., 2010
RipAW	RSp1475	Rip69	MUKAIHARA et al., 2010; PEETERS et al., 2013a
RipAX2	RSp0572	Rip36, Brg14	MUKAIHARA et al., 2010
RipAY	RSp1022	Rip55, Hpx21, Brg37	MUKAIHARA et al., 2010

Type III effectors (T3Es)	Representative gene member	Other Name	References
RipAZ1	RSp1582	Rip71	MUKAIHARA et al., 2010
RipAZ2	*R. syzygii* RALSY_20407	-	PEETERS et al., 2013a
RipB	Rsc0245	RipB, Rip2, Hpx11	MUKAIHARA et al., 2010
RipBA	RSc0227, RSp0228 [pseudogene]	-	PEETERS et al., 2013a
RipBB	Psi07 RPSI07_mp0573	-	PEETERS et al., 2013a
RipBC	CFBP2957 RCFBP_mp30170	-	PEETERS et al., 2013a
RipBD	R. syzygii RALSY_20184	-	PEETERS et al., 2013a
RipBE	RS1000	Rip10	MUKAIHARA et al., 2010
RipBF	Psi07 RPSI07_2863	-	PEETERS et al., 2013a
RipBG	Molk2 RSMK00763	-	PEETERS et al., 2013a
RipBH	Psi07 RPSI07_mp1715	-	PEETERS et al., 2013a
RipBI	CFBP2957 RCFBP_mp30113	-	PEETERS et al., 2013a
RipC1	RSp1239	Rip62	CUNNAC et al., 2004; MUKAIHARA et al., 2010
RipC2	CFBP2957 RCFBP_mp20032	-	MUKAIHARA et al., 2010
RipD	RSp0304	Rip34, Hpx25, Brg8	MUKAIHARA et al., 2010
RipE1	RSc3369	Rip26, Brg9	MUKAIHARA et al., 2010
RipE2	CFBP2957 RCFBP_mp10565	-	MUKAIHARA et al., 2010
RipF1	RSp1555	PopF1, PopF2, Rip70	MEYER et al., 2006; MUKAIHARA et al., 2010
RipF2	CFBP2957 RCFBP_mp30453	-	MEYER et al., 2006; MUKAIHARA et al., 2010
RipG1	RSp0914	Gala1, Rip53	MUKAIHARA et al., 2010
RipG2	RSp0672	Gala2, Rip37, Hpx20	MUKAIHARA et al., 2010
RipG3	RSp0023	Gala3, Rip28	MUKAIHARA et al., 2010
RipG4	RSc1800	Gala4, Rip17, Hpx15	MUKAIHARA et al., 2010
RipG5	RSc1801	Gala5, Rip18, Hpx16	MUKAIHARA et al., 2010
RipG6	RSc1356	RipG, Gala6, Rip13, Hpx13	BALTRUS et al., 2011; CUNNAC et al., 2004
RipG7	RSc1357	Gala7, Rip14, Hpx14	MUKAIHARA et al., 2010
RipG8	CMR15 CMR15v4_10224	Gala8	MUKAIHARA et al., 2010
RipH1	RSc1386	HLK1, Rip15, Brg19	MUKAIHARA et al., 2010
RipH2	RSp0215	HLK2, Rip32	MUKAIHARA et al., 2010
RipH3	RSp0160	HLK3, Rip30, Brg18	MUKAIHARA et al., 2010
RipH4	Psi07 RPSI07_mp0161	HLK4	MUKAIHARA et al., 2010
RipI	RSc0041	Rip1	MUKAIHARA et al., 2010
RipJ	RSc2132	Rip22	MUKAIHARA et al., 2010
RipK	CFBP2957 RCFBP_mp10024	-	MUKAIHARA et al., 2010
RipL	RSp0193	Rip31, Brg22	MUKAIHARA et al., 2010
RipM	RSc1475	Rip16, Brg42	MUKAIHARA et al., 2010
RipN	RSp1130	Rip58, Hpx26, Brg44	MUKAIHARA et al., 2010
RipO1	RSp0323	Rip35, Brg12	MUKAIHARA et al., 2010
RipO2	R. syzygii RALSY_mp30159	-	PEETERS et al., 2013a
RipP1	RSc0826	PopP1, Rip7	LAVIE et al., 2002; MUKAIHARA et al., 2010
RipP2	RSc0868	PopP2, Rip8	CUNNAC et al., 2004; MUKAIHARA et al., 2010
RipP3	UW163 [GenBank accession : CAF32358.1]	PopP3	PEETERS et al., 2013a
RipQ	RSp1277 Rip63	Hpx23	MUKAIHARA et al., 2010
RipR	RSp1281	Rip64, Hpx24, Brg15, PopS	MUKAIHARA et al., 2010
RipS1	RSc3401	SKWP1, Rip27, Hpx37	MUKAIHARA et al., 2010
RipS2	RSp1374	SKWP2, Rip65, Hpx36	MUKAIHARA et al., 2010
RipS3	RSp0930	SKWP3, Rip54	MUKAIHARA et al., 2010
RipS4	RSc1839	SKWP4, Rip20, Hpx30	MUKAIHARA et al., 2010

Type III effectors (T3Es)	Representative gene member	Other Name	References
RipS5	RSp0296	SKWP5, Rip33, Hpx34	MUKAIHARA et al., 2010
RipS6	RSc2130	SKWP6	PEETERS et al., 2013a
RipS7	Molk2 RSMK02658	SKWP7	PEETERS et al., 2013a
RipS8	Psi07 RSPsi07_1850	SKWP8	PEETERS et al., 2013a
RipT	RSc3212	RipT, Rip25	CUNNAC et al., 2004; MUKAIHARA et al., 2010
RipTAL1	Rsc1815	Rip19, Hpx17, Brg11	MUKAIHARA et al., 2010
RipTPS	RSp0731	-	PEETERS et al., 2013a
RipU	RSp1212	Rip59	MUKAIHARA et al., 2010
RipV1	RSc1349	Rip12, Hpx29, Brg17	MUKAIHARA et al., 2010
RipV2	Psi07 RSPsi07_1895	-	MUKAIHARA et al., 2010
RipW	RSc2775	PopW, Rip24	LI et al., 2010; MUKAIHARA et al., 2010
RipX	RSp0877	PopA, Rip49	MUKAIHARA et al., 2010; YANG et al., 2000
RipY	RSc0257	Rip3, Brg23	MUKAIHARA et al., 2010
RipZ	RSp1031	Rip57, Brg38	MUKAIHARA et al., 2010

Ralstonia's T3Es and Host Specificity

When the former RSSC was grouped in phylotypes, the clades were also characterized based on the host range of the strains and the varied diseases they would cause, often referred to as ecotypes. Common ecotypes of RSSC are the classic bacterial wilt, Moko disease, brown rot of potato, banana blood disease, and the emergent NPB (non-pathogenic to banana) (GENIN; DENNY, 2012; SANTIAGO et al., 2017; SANTIAGO; LOPES; MIZUBUTI, 2016). This variability of hosts directly reflects the genetic variability of strains, which portrays different molecular tools to infect and cause disease on a given host, which in turn also responds in a specific manner. For example, when a pathogen invades a plant cell, a standard, robust defense mechanism is to induce the release of many chemical and biochemical compounds and alter ions effluxes, leading to cell death and thus stopping the pathogen from spreading across the host's systems (Figure 2). This mechanism is called a hypersensitive response, or HR.

The simplest model explains that an HR occurs due to an incompatible interaction between the products of a pathogen *avr* gene and a plant resistance gene (*R)*¸ with a compatible interaction leading to further disease on the host (BENT, 1996; MOREL; DANGL, 1997) (Figure 2). The *hrp* gene cluster on *Ralstonia's* genome is crucial for inducing and suppressing HRs since they also regulate *avr/rip* genes (GAMA et al., 2016; HE, 2006; LAM; KATO; LAWTON, 2001). The pathogen's virulence effectors will subvert metabolic responses and diminish the host's defense molecules on a susceptible host. In this context, *Ralstonia*'s T3Es injected inside the host cell may be recognized (or not) by plant R proteins, undergoing a selective pressure that occasionally causes loss of recognized effectors to infect a broader range

of hosts. That explains why some *Ralstonia* strains can infect some hosts while others cannot (KRAEPIEL; BARNY, 2016).

Phylogenetic and comparative assays are indispensable to understanding which groups of strains are able to infect a given host. One of the first studies focused on Japanese strains of RSSC revealed that despite most strains belonging to phylotype I, a specific genetically related group of strains would initiate HR on tobacco, and others would cause bacterial wilt (LIU et al., 2009). When looking deeper into the effectome, more correlations between Rips and host specificity are found. Comparative genomic studies on *R. solanacearum* strains of different ecotypes showed that the emergent NPB and brown rot ecotypes probably changed their host range due to losing a few (but essential) conserved genes in Moko disease strains including *ripAA* and *ripAU*, respectively (AILLOUD et al., 2015). Contributing to this correlation, comparative analysis of emergent strains of *R. pseudosolanacearum* pathogenic to new different hosts, like blueberry, tea rose, mandevilla, and osteospermum, has revealed that despite strains being phylogenetically close, their ability to infect specific hosts are related to absence and presence of specific assets of T3Es (BOCSANCZY et al., 2022).

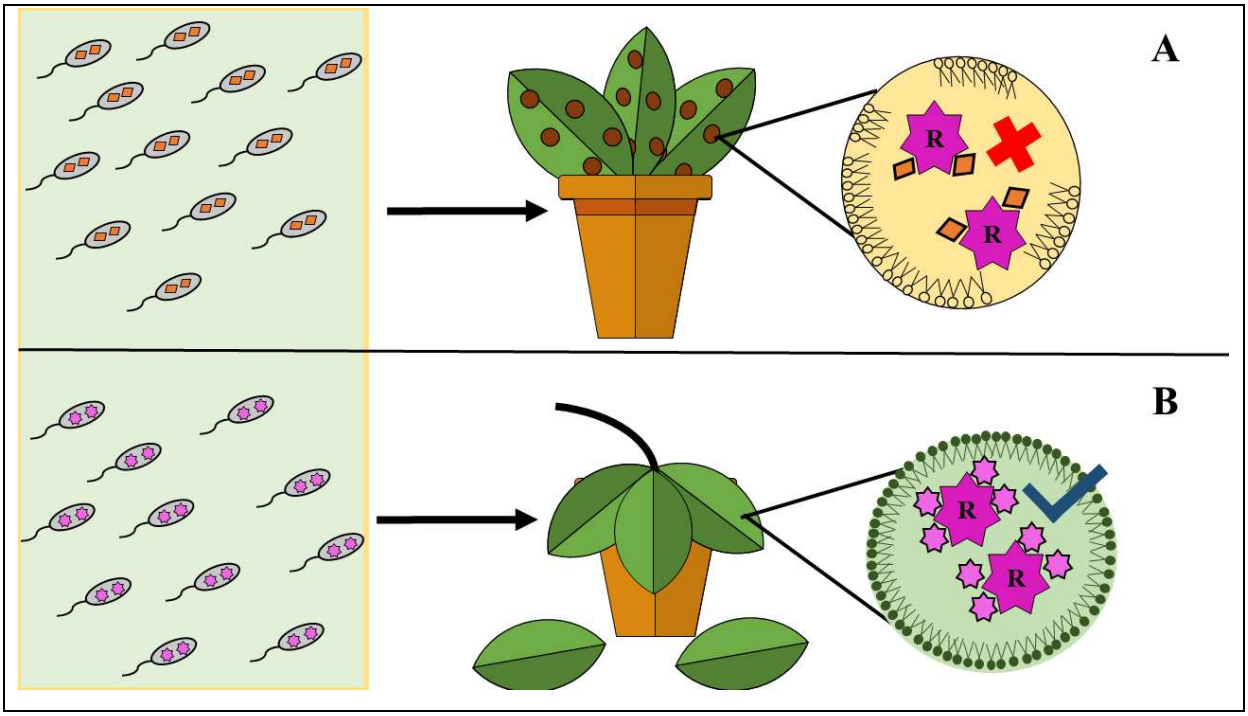

FIGURE 2. Scheme for pathogen adaptation for new hosts via a change in the Rips repertoire. One given Rip triggers a robust hypersensitive response protecting the host from infection (A), while the other has a compatible interaction with host defense R protein (B), leading to disease.

Conclusion

This chapter aimed to update the findings of the *Ralstonia* secretome, addressing the main secretion systems and the characterization of new discoveries about *Ralstonia* Injected Proteins. With the crescent advance on sequencing technologies and structural bioinformatics, future secretomics studies could focus on not only the discovery of new secreted molecules, but also on different isoforms of the same effector and how their structure interacts with key host molecules in infection response. Different omics data revealed that the existence of a significant genetic diversity of *Ralstonia* type III effectors might be a result from the coevolutionary host-pathogen response. That said, ancient effectors broadly distributed in *Ralstonia* are more prone to trigger HR responsens on hosts, while recent effectors or recent mutations in effectors may explain outbreak reports on field. Additionaly, comparative omics studies focused on broadly distributed effectors could lead to candidates strongly related host defense in further plant pathology studies thus offering alternative for genetic engineering projects in plant defense. Finally, with an in-depth understanding of this topic, we can encompass new perspectives of biotechnological applications, including developing diagnostic kits to detect and control emerging pathogens and the prospection of targets to combat *Ralstonia´*s phytopathogens of worldwide relevance.

References

ABDALLAH, A. M. et al. Type VII secretion--mycobacteria show the way. **Nature Reviews. Microbiology**, v. 5, n. 11, p. 883–891, nov. 2007.

AILLOUD, F. et al. Comparative genomic analysis of *Ralstonia solanacearum* reveals candidate genes for host specificity. **BMC Genomics**, v. 16, n. 1, p. 270, 8 abr. 2015.

AILLOUD, F. et al. *In planta* comparative transcriptomics of host-adapted strains of *Ralstonia solanacearum*. **PeerJ**, v. 4, p. e1549, 5 jan. 2016.

ALDON, D. et al. A bacterial sensor of plant cell contact controls the transcriptional induction of *Ralstonia solanacearum* pathogenicity genes. **The EMBO Journal**, v. 19, n. 10, p. 2304–2314, 15 maio 2000.

ANGOT, A. et al. *Ralstonia solanacearum* requires F-box-like domain-containing type III effectors to promote disease on several host plants. **Proceedings of the National Academy of Sciences of the United States of America**, v. 103, n. 39, p. 14620–14625, 26 set. 2006.

ARLAT, M. et al. PopA1, a protein which induces a hypersensitivity-like response on specific Petunia genotypes, is secreted via the Hrp pathway of *Pseudomonas solanacearum* . **The EMBO Journal**, v. 13, n. 3, p. 543–553, 1 fev. 1994.

ASOLKAR, T.; RAMESH, R. The involvement of the Type Six Secretion System (T6SS) in the virulence of *Ralstonia solanacearum* on brinjal. **3 Biotech**, v. 10, n. 7, p. 324, 29 jun. 2020.

BENEZECH, C.; LE SCORNET, A.; GOURION, B. Medicago-Sinorhizobium-Ralstonia: A Model System to Investigate Pathogen-Triggered Inhibition of Nodulation. **Molecular Plant-Microbe Interactions®**, v. 34, n. 5, p. 499–503, maio 2021.

BENT, A. Plant Disease Resistance Genes: Function Meets Structure. **The Plant Cell**, v. 8, n. 10, p. 1757–1771, out. 1996.

BOCSANCZY, A. M. et al. Identification of candidate type 3 effectors that determine host specificity associated with emerging *Ralstonia pseudosolanacearum* strains. **European Journal of Plant Pathology**, v. 163, n. 1, p. 35–50, 1 maio 2022.

BOGDANOVE, A. J. et al. Unified nomenclature for broadly conserved hrp genes of phytopathogenic bacteria. **Molecular Microbiology**, v. 20, n. 3, p. 681–683, maio 1996.

BRITO, B. et al. prhJ and hrpG, two new components of the plant signal-dependent regulatory cascade controlled by PrhA in *Ralstonia solanacearum*. **Molecular Microbiology**, v. 31, n. 1, p. 237–251, 1999.

BRITO, B. et al. A Signal Transfer System Through Three Compartments Transduces the Plant Cell Contact-Dependent Signal Controlling *Ralstonia solanacearum hrp* Genes. **Molecular Plant-Microbe Interactions®**, v. 15, n. 2, p. 109–119, fev. 2002.

BURKINSHAW, B. J.; STRYNADKA, N. C. J. Assembly and structure of the T3SS. **Biochimica et Biophysica Acta (BBA) - Molecular Cell Research**, Protein trafficking and secretion in bacteria. v. 1843, n. 8, p. 1649–1663, 1 ago. 2014.

CACCIA, D. et al. Bioinformatics tools for secretome analysis. **Biochimica Et Biophysica Acta**, v. 1834, n. 11, p. 2442–2453, nov. 2013.

CARNEY, B. F.; DENNY, T. P. A cloned avirulence gene from *Pseudomonas solanacearum* determines incompatibility on *Nicotiana tabacum* at the host species level. **Journal of Bacteriology**, v. 172, n. 9, p. 4836–4843, set. 1990.

CASCALES, E.; CHRISTIE, P. J. The versatile bacterial type IV secretion systems. **Nature Reviews. Microbiology**, v. 1, n. 2, p. 137–149, nov. 2003.

CHEN, L. et al. Involvement of HLK effectors in *Ralstonia solanacearum* disease development in tomato. **Journal of General Plant Pathology**, v. 80, 1 jan. 2014.

CORNELIS, G. R.; VAN GIJSEGEM, F. Assembly and function of type III secretory systems. **Annual Review of Microbiology**, v. 54, p. 735–774, 2000.

DE PEDRO-JOVÉ, R. et al. Dynamic expression of *Ralstonia solanacearum* virulence factors and metabolism-controlling genes during plant infection. **BMC Genomics**, v. 22, n. 1, p. 170, 9 mar. 2021.

DELASPRE, F. et al. The *Ralstonia solanacearum* pathogenicity regulator HrpB induces 3-hydroxy-oxindole synthesis. **Proceedings of the National Academy of Sciences**, v. 104, n. 40, p. 15870–15875, 2 out. 2007.

DESLANDES, L. et al. Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. **Proceedings of the National Academy of Sciences**, v. 100, n. 13, p. 8024–8029, 24 jun. 2003.

DILLON, M. M. et al. Molecular Evolution of *Pseudomonas syringae* Type III Secreted Effector Proteins. **Frontiers in Plant Science**, v. 10, 2019.

FERRO, P.; VAZ-MOREIRA, I.; MANAIA, C. M. Evolution of gentamicin and arsenite resistance acquisition in *Ralstonia pickettii* water isolates. **Research in Microbiology**, v. 172, n. 1, p. 103790, 1 jan. 2021.

FRONZES, R.; CHRISTIE, P. J.; WAKSMAN, G. The structural biology of type IV secretion systems. **Nature Reviews. Microbiology**, v. 7, n. 10, p. 703–714, out. 2009.

FUJIWARA, S. et al. RipAY, a Plant Pathogen Effector Protein, Exhibits Robust γ-Glutamyl Cyclotransferase Activity When Stimulated by Eukaryotic Thioredoxins*. **Journal of Biological Chemistry**, v. 291, n. 13, p. 6813–6830, 25 mar. 2016.

FUJIWARA, S. et al. Characterization of the mechanism of thioredoxin-dependent activation of γ-glutamylcyclotransferase, RipAY, from *Ralstonia solanacearum* . **Biochemical and Biophysical Research Communications**, v. 523, n. 3, p. 759–765, 12 mar. 2020.

FULLNER, K. J.; MEKALANOS, J. J. Genetic Characterization of a New Type IV-A Pilus Gene Cluster Found in Both Classical and El Tor Biotypes of *Vibrio cholerae* . **Infection and Immunity**, v. 67, n. 3, p. 1393–1404, mar. 1999.

FURUTANI, A. et al. Evidence for HrpXo-dependent expression of type II secretory proteins in *Xanthomonas oryzae* pv. *oryzae* . **Journal of Bacteriology**, v. 186, n. 5, p. 1374–1380, mar. 2004.

GAGIC, D. et al. Exploring the Secretomes of Microbes and Microbial Communities Using Filamentous Phage Display. **Frontiers in Microbiology**, v. 7, 2016.

GAMA, M. A. S. et al. Genética da interação bactéria-planta. Em: GUIMARÃES, L. M. P. et al. (Eds.). **Estado da Arte em Fitobacterioses Tropicais**. Recife, Pernambuco, Brasil: EDFRPE - Editora Universitária da UFRPE, 2016. p. 43–64.

GENIN, S. Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum* . **New Phytologist**, v. 187, n. 4, p. 920–928, 2010.

GENIN, S.; BOUCHER, C. LESSONS LEARNED FROM THE GENOME ANALYSIS OF *RALSTONIA SOLANACEARUM*. **Annual Review of Phytopathology**, v. 42, n. 1, p. 107–134, set. 2004.

GENIN, S.; DENNY, T. P. Pathogenomics of the *Ralstonia solanacearum* Species Complex. **Annual Review of Phytopathology**, v. 50, n. 1, p. 67–89, 8 set. 2012.

GONZÁLEZ, E. T. et al. Using the Ralstonia solanacearum Tat Secretome To Identify Bacterial Wilt Virulence Factors. **Applied and Environmental Microbiology**, v. 73, n. 12, p. 3779–3786, 15 jun. 2007.

GREEN, E. R.; MECSAS, J. Bacterial Secretion Systems: An Overview. **Microbiology Spectrum**, v. 4, n. 1, 29 jan. 2016.

GUAN, S. H. et al. Experimental evolution of nodule intracellular infection in legume symbionts. **The ISME Journal**, v. 7, n. 7, p. 1367–1377, jul. 2013.

HATHOUT, Y. Approaches to the study of the cell secretome. **Expert Review of Proteomics**, v. 4, n. 2, p. 239–248, 1 abr. 2007.

HE, S. Y. Role of the type III protein secretion system in bacterial infection of plants. Em: BROGDEN, K. A. et al. (Eds.). **Virulence mechanisms of bacterial pathogens**. 4. ed. Washington: ASM Press, 2006. p. 209–220.

ISBERG, R. R.; O'CONNOR, T. J.; HEIDTMAN, M. The *Legionella pneumophila* replication vacuole: making a cosy niche inside host cells. **Nature Reviews. Microbiology**, v. 7, n. 1, p. 13–24, jan. 2009.

JACOB-DUBUISSON, F.; FERNANDEZ, R.; COUTTE, L. Protein secretion through autotransporter and two-partner pathways. **Biochimica Et Biophysica Acta**, v. 1694, n. 1–3, p. 235–257, 11 nov. 2004.

JACOBS, J. M. et al. The *In Planta* Transcriptome of *Ralstonia solanacearum*: Conserved Physiological and Virulence Strategies during Bacterial Wilt of Tomato. **mBio**, v. 3, n. 4, p. e00114-12, 31 ago. 2012.

JEONG, Y. et al. An HrpB-dependent but type III-independent extracellular aspartic protease is a virulence factor of *Ralstonia solanacearum* . **Molecular Plant Pathology**, v. 12, n. 4, p. 373–380, 2011.

JONES, J. D. G.; DANGL, J. L. The plant immune system. **Nature**, v. 444, n. 7117, p. 323–329, nov. 2006.

KANDA, A. et al. Type III secretion machinery-deficient mutants of *Ralstonia solanacearum* lose their ability to colonize resulting in loss of pathogenicity. **Journal of General Plant Pathology**, v. 69, n. 4, p. 250–257, 1 ago. 2003.

KANG, Y. Dramatically Reduced Virulence of Mutants of *Pseudomonas solanacearum* Defective in Export of Extracellular Proteins Across the Outer Membrane. **Molecular Plant-Microbe Interactions**, v. 7, p. 370, 1 jan. 1994.

KANG, Y. et al. Proteomic analysis of the proteins regulated by HrpB from the plant pathogenic bacterium *Burkholderia glumae* . **PROTEOMICS**, v. 8, n. 1, p. 106–121, 2008.

KIRKPATRICK, D. S.; DENISON, C.; GYGI, S. P. Weighing in on Ubiquitin: The Expanding Role of Mass Spectrometry-based Proteomics. **Nature cell biology**, v. 7, n. 8, p. 750–757, ago. 2005.

KRAEPIEL, Y.; BARNY, M.-A. Gram-negative phytopathogenic bacteria, all hemibiotrophs after all? **Molecular Plant Pathology**, v. 17, n. 3, p. 313–316, 2016.

KVITKO, B. H. et al. Identification of harpins in *Pseudomonas syringae* pv. *tomato* DC3000, which are functionally similar to HrpK1 in promoting translocation of type III secretion system effectors. **Journal of Bacteriology**, v. 189, n. 22, p. 8059–8072, nov. 2007.

LAM, E.; KATO, N.; LAWTON, M. Programmed cell death, mitochondria and the plant hypersensitive response. **Nature**, v. 411, n. 6839, p. 848–853, jun. 2001.

LANDRY, D. et al. The large, diverse, and robust arsenal of *Ralstonia solanacearum* type III effectors and their in planta functions. **Molecular Plant Pathology**, v. 21, n. 10, p. 1377–1388, out. 2020.

LE ROUX, C. et al. A Receptor Pair with an Integrated Decoy Converts Pathogen Disabling of Transcription Factors to Immunity. **Cell**, v. 161, n. 5, p. 1074–1088, 21 maio 2015.

LEYTON, D. L.; ROSSITER, A. E.; HENDERSON, I. R. From self sufficiency to dependence: mechanisms and factors important for autotransporter biogenesis. **Nature Reviews Microbiology**, v. 10, n. 3, p. 213–225, mar. 2012.

LIU, H. et al. Pyramiding unmarked deletions in *Ralstonia solanacearum* shows that secreted proteins in addition to plant cell-wall-degrading enzymes contribute to virulence. **Molecular plant-microbe interactions: MPMI**, v. 18, n. 12, p. 1296–1305, dez. 2005.

LIU, Y. et al. Molecular typing of Japanese strains of *Ralstonia solanacearum* in relation to the ability to induce a hypersensitive reaction in tobacco. **Journal of General Plant Pathology**, v. 75, n. 5, p. 369–380, 1 out. 2009.

LONJON, F. et al. Comparative Secretome Analysis of *Ralstonia solanacearum* Type 3 Secretion-Associated Mutants Reveals a Fine Control of Effector Delivery, Essential for Bacterial Pathogenicity. **Molecular & Cellular Proteomics**, v. 15, n. 2, p. 598–613, fev. 2016.

LOPES, C. A.; ROSSATO, M. History and Status of Selected Hosts of the *Ralstonia solanacearum* Species Complex Causing Bacterial Wilt in Brazil. **Frontiers in Microbiology**, v. 9, p. 1228, 13 jun. 2018.

MACHO, A. P. et al. A Competitive Index Assay Identifies Several *Ralstonia solanacearum* Type III Effector Mutant Strains with Reduced Fitness in Host Plants. **Molecular Plant-Microbe Interactions®**, v. 23, n. 9, p. 1197–1205, set. 2010.

MANN, M. Functional and quantitative proteomics using SILAC. **Nature Reviews Molecular Cell Biology**, v. 7, n. 12, p. 952–958, dez. 2006.

MEDINA-PUCHE, L. et al. A Defense Pathway Linking Plasma Membrane and Chloroplasts and Co-opted by Pathogens. **Cell**, v. 182, n. 5, p. 1109- 1124.e25, 3 set. 2020.

MENG, G. et al. Structure of the outer membrane translocator domain of the *Haemophilus influenzae* Hia trimeric autotransporter. **The EMBO Journal**, v. 25, n. 11, p. 2297–2304, 7 jun. 2006.

MONTEIRO, F. et al. A luminescent reporter evidences active expression of *Ralstonia solanacearum* type III secretion system genes throughout plant infection. **Microbiology**, v. 158, n. 8, p. 2107–2116, 1 ago. 2012.

MOREL, J.-B.; DANGL, J. L. The hypersensitive response and the induction of cell death in plants. **Cell Death & Differentiation**, v. 4, n. 8, p. 671–683, dez. 1997.

MORGAN, J. L. W.; ACHESON, J. F.; ZIMMER, J. Structure of a Type-1 Secretion System ABC Transporter. **Structure**, v. 25, n. 3, p. 522–529, 7 mar. 2017.

MUKAIHARA, T.; TAMURA, N. 2009. Identification of novel *Ralstonia solanacearum* type III effector proteins through translocation analysis of hrpB-regulated gene products. **Microbiology**, v. 155, n. 7, p. 2235–2244, 1 jul. 2009.

MUKAIHARA, T.; TAMURA, N.; IWABUCHI, M. Genome-Wide Identification of a Large Repertoire of Ralstonia solanacearum Type III Effector Proteins by a New Functional Screen. **Molecular Plant-Microbe Interactions®**, v. 23, n. 3, p. 251–262, mar. 2010.

NATALE, P.; BRÜSER, T.; DRIESSEN, A. J. M. Sec- and Tat-mediated protein secretion across the bacterial cytoplasmic membrane--distinct translocases and mechanisms. **Biochimica Et Biophysica Acta**, v. 1778, n. 9, p. 1735–1756, set. 2008.

PAPANIKOU, E.; KARAMANOU, S.; ECONOMOU, A. Bacterial protein secretion through the translocase nanomachine. **Nature Reviews Microbiology**, v. 5, n. 11, p. 839–851, nov. 2007.

PARANJPYE, R. N.; STROM, M. S. A *Vibrio vulnificus* Type IV Pilin Contributes to Biofilm Formation, Adherence to Epithelial Cells, and Virulence. **Infection and Immunity**, v. 73, n. 3, p. 1411–1422, mar. 2005.

PARTE, A. C. LPSN—list of prokaryotic names with standing in nomenclature. **Nucleic Acids Research**, v. 42, n. Database issue, p. D613–D616, 1 jan. 2014.

PEETERS, N. et al. *Ralstonia solanacearum* , a widespread bacterial plant pathogen in the post-genomic era: *Ralstonia solanacearum* and bacterial wilt disease. **Molecular Plant Pathology**, v. 14, n. 7, p. 651–662, set. 2013a.

PEETERS, N. et al. Repertoire, unified nomenclature and evolution of the Type III effector gene set in the *Ralstonia solanacearum* species complex. **BMC Genomics**, v. 14, p. 859, 6 dez. 2013b.

PLENER, L. et al. Metabolic Adaptation of *Ralstonia solanacearum* during Plant Infection: A Methionine Biosynthesis Case Study. **PLoS ONE**, v. 7, n. 5, p. e36877, 16 maio 2012.

POUEYMIRO, M.; GENIN, S. Secreted proteins from *Ralstonia solanacearum* : a hundred tricks to kill a plant. **Current Opinion in Microbiology**, Host–Microbe Interactions: Bacteria. v. 12, n. 1, p. 44–52, 1 fev. 2009.

PUIGVERT, M. et al. Transcriptomes of *Ralstonia solanacearum* during Root Colonization of *Solanum commersonii*. **Frontiers in Plant Science**, v. 8, 20 mar. 2017.

REMIGI, P. et al. Functional diversification of the GALA type III effector family contributes to *Ralstonia solanacearum* adaptation on different plant hosts. **The New Phytologist**, v. 192, n. 4, p. 976–987, dez. 2011.

ROUX, B. et al. Genomics and transcriptomics of *Xanthomonas campestris* species challenge the concept of core type III effectome. **BMC Genomics**, v. 16, n. 1, p. 975, 18 nov. 2015.

RUSSELL, A. B.; PETERSON, S. B.; MOUGOUS, J. D. Type VI secretion system effectors: poisons with a purpose. **Nature Reviews. Microbiology**, v. 12, n. 2, p. 137–148, fev. 2014.

RYAN, M. P.; ADLEY, C. C. *Ralstonia* spp.: emerging global opportunistic pathogens. **European Journal of Clinical Microbiology & Infectious Diseases**, v. 33, n. 3, p. 291–304, mar. 2014.

SABBAGH, C. R. R. et al. Pangenomic type III effector database of the plant pathogenic *Ralstonia* spp. **PeerJ**, v. 7, p. e7346, 6 ago. 2019.

SAFNI, I. et al. 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. solanacearum* phylotype IV strains as *Ralstonia syzygii* subsp. *indonesiensis* subsp. nov., banana blood disease bacterium strains as *Ralstonia syzygii* subsp. *celebesensis* subsp. nov. and *R. solanacearum* phylotype I and III strains as *Ralstonia pseudosolanacearum* sp. nov. **International Journal of Systematic and Evolutionary Microbiology**, v. 64, n. Pt_9, p. 3087–3103, 1 set. 2014.

SALANOUBAT, M. et al. Genome sequence of the plant pathogen *Ralstonia solanacearum* . **Nature**, v. 415, n. 6871, p. 497–502, jan. 2002.

SANG, Y. et al. Intra-strain Elicitation and Suppression of Plant Immunity by *Ralstonia solanacearum* Type-III Effectors in *Nicotiana benthamiana* . **Plant Communications**, Special Issue on Plant-Pathogen Interactions (Organizing Editors: Paul Birch, Savithramma Dinesh-Kumar, Hui-Shan Guo, Ping He, Xin Li, Frank Takken, Yuanchao Wang). v. 1, n. 4, p. 100025, 13 jul. 2020.

SANTIAGO, T. R. et al. Phylotype and sequevar variability of *Ralstonia solanacearum* in Brazil, an ancient centre of diversity of the pathogen. **Plant Pathology**, v. 66, n. 3, p. 383–392, abr. 2017.

SANTIAGO, T. R.; LOPES, C. A.; MIZUBUTI, E. S. Diversidade e Variabilidade de *Ralstonia* spp. Em: GAMA, M. A. S. et al. (Eds.). **Estado da Arte em Fitobacterioses Tropicais**. Recife, Pernambuco, Brasil: EDFRPE - Editora Universitária da UFRPE, 2016. p. 243–256.

SOLÉ, M. et al. The awr Gene Family Encodes a Novel Class of *Ralstonia solanacearum* Type III Effectors Displaying Virulence and Avirulence Activities. **Molecular Plant-Microbe Interactions®**, v. 25, n. 7, p. 941–953, jul. 2012.

STASKAWICZ, B. J. et al. Common and Contrasting Themes of Plant and Animal Diseases. **Science**, v. 292, n. 5525, p. 2285–2289, 22 jun. 2001.

SUN, Y. et al. The *Ralstonia solanacearum* effector RipAK suppresses plant hypersensitive response by inhibiting the activity of host catalases. **Cellular Microbiology**, v. 19, n. 8, p. e12736, 2017.

TAMPAKAKI, A. P. et al. Playing the “Harp”: Evolution of Our Understanding of hrp/hrc Genes. **Annual Review of Phytopathology**, v. 48, n. 1, p. 347–370, 2010.

THANASSI, D. G.; HULTGREN, S. J. Multiple pathways allow protein secretion across the bacterial outer membrane. **Current Opinion in Cell Biology**, v. 12, n. 4, p. 420–430, ago. 2000.

THOMAS, S.; HOLLAND, I. B.; SCHMITT, L. The Type 1 secretion pathway — The hemolysin system and beyond. **Biochimica et Biophysica Acta (BBA) - Molecular Cell Research**, Protein trafficking and secretion in bacteria. v. 1843, n. 8, p. 1629–1641, 1 ago. 2014.

TJALSMA, H. et al. Signal Peptide-Dependent Protein Transport in *Bacillus subtilis*: a Genome-Based Survey of the Secretome. **Microbiology and Molecular Biology Reviews**, v. 64, n. 3, p. 515–547, set. 2000.

TROISFONTAINES, P.; CORNELIS, G. R. Type III Secretion: More Systems Than You Think. **Physiology**, v. 20, n. 5, p. 326–339, out. 2005.

VALLS, M.; GENIN, S.; BOUCHER, C. Integrated Regulation of the Type III Secretion System and Other Virulence Determinants in *Ralstonia solanacearum*. **PLoS Pathogens**, v. 2, n. 8, p. e82, 25 ago. 2006.

VAN GIJSEGEM, F. et al. The *Ralstonia solanacearum* hrp Gene Region: Role of the Encoded Proteins in Interactions with Plants and Regulation of Gene Expression. Em: PRIOR, P.; ALLEN, C.; ELPHINSTONE, J. (Eds.). **Bacterial Wilt Disease: Molecular and Ecological Aspects**. Berlin, Heidelberg: Springer, 1998. p. 178–183.

WANG, K. et al. Functional assignment to positively selected sites in the core type III effector RipG7 from *Ralstonia solanacearum* . **Molecular Plant Pathology**, v. 17, n. 4, p. 553–564, 2016.

WU, D. et al. A Plant Pathogen Type III Effector Protein Subverts Translational Regulation to Boost Host Polyamine Levels. **Cell Host & Microbe**, v. 26, n. 5, p. 638- 649.e5, 13 nov. 2019.

YABUUCHI, E. et al. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. Nov.: Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. Nov., *Ralstonia solanacearum* (Smith 1896) comb. Nov. and *Ralstonia eutropha* (Davis 1969) comb. Nov. **Microbiology and Immunology**, v. 39, n. 11, p. 897–904, 1995.

YU, G. et al. A bacterial effector protein prevents MAPK-mediated phosphorylation of SGT1 to suppress plant immunity. **PLOS Pathogens**, v. 16, n. 9, p. e1008933, 25 set. 2020.

ZHANG, L. et al. TssM is essential for virulence and required for type VI secretion in *Ralstonia solanacearum* . **Journal of Plant Diseases and Protection**, v. 119, n. 4, p. 125–134, 1 ago. 2012.

ZHANG, L. et al. TssB is essential for virulence and required for Type VI secretion system in *Ralstonia solanacearum* . **Microbial Pathogenesis**, v. 74, p. 1–7, 1 set. 2014.

ZHANG, Y.; QIU, S. Phylogenomic analysis of the genus *Ralstonia* based on 686 single-copy genes. **Antonie van Leeuwenhoek**, v. 109, n. 1, p. 71–82, jan. 2016.

ZHOU, M. et al. LAB-Secretome: a genome-scale comparative analysis of the predicted extracellular and surface-associated proteins of Lactic Acid Bacteria. **BMC Genomics**, v. 11, n. 1, p. 651, 23 nov. 2010.

3. JUSTIFICATIVA

A murcha bacteriana é uma fitobacteriose de difícil manejo e controle no Brasil e no mundo, afetando culturas variadas, incluindo tubérculos, frutas e verduras. O agente causador, *R. solanacearum*, por se tratar de uma bactéria saprófita e resistente aos bactericidas e agrotóxicos clássicos de combate, possui incidência recorrente em países de clima tropical, como o Brasil, tratando-se de importante peça no agronegócio mundial. Como consequência do amplo espectro de hospedeiros e condições ideiais, a murcha bacteriana causa grandes prejuízos econômicos.

Em paralelo, dentro do complexo de espécies *R. solanacearum*, os filotipos I e II são encontrados ao longo de todo o território brasileiro. O filotipo I, *R. pseudosolanacearum*, tem seu centro de dispersão atribuído ao continente asiático, enquanto os filotipos IIA e IIB são considerados nativos di Brasil, tendo a bacia Amazônica como centro de dispersão. Sabe-se que os efetores de virulência são reflexo direto do conteúdo genômico, compreendendo um dos mecanismos de virulência e patogenicidade mais importantes destas bactérias. Finalmente, por se tratar de um complexo de espécies, a identificação de isolados que acometem lavouras pode ser equivocada, especialmente quando métodos mais refinados de bancada, como PCR-Multiplex, não são envolvidos. Porém, é importante ressaltar que tais métodos são custosos e demandam tempo.

Considerando-se a dificuldade de classificação dos isolados e sabendo-se que as estratégias de infectividade dessas bactérias são pautadas, principalmente, em inovações a nível molecular, análises de bioinformática podem fornecer resultados robustos especialmente quando aplicadas a comparações em grande escala abordando um espectro maior de acessos bacterianos. Como consequência, estudos deste tipo permitem uma maior rastreabilidade das regiões brasileiras afetadas por doenças causadas pelo RSSC a partir da classificação taxonômica correta considerando o genoma total. Ademais, o conhecimento específico do repertório genômico de cada espécie existente dentro do complexo enriquece o conhecimento acerca dos isolados em nível individual. No que tange especificamente às doenças, a bioinformática ainda auxilia na prospecção de culturas resistentes correlacionando repertórios gênicos de virulência aos hospedeiros onde foram infectados.

4. OBJETIVOS

4.1. Objetivo Geral

O presente projeto visa caracterizar e comparar os genomascompletos públicos e 14 novas linhagens brasileiras do complexo de espécies *Ralstonia solanacearum* através de abordagens *in silico*, incluindo análises pangenômicas e metabólicas, a fim de avaliar suas características evolutivas, associação com hospedeiros e distribuição geográfica.

4.2. Objetivos específicos

a) Classificar 14 novos isolados brasileiros de RSSC causadores de Moko-da-bananeira e murcha bacteriana (BW) em tomateiro, a partir pangenômica e taxogenômica;
b) Predizer o repertório de Rips para os 14 novos isolados utilizando um banco de dados específico para RSSC;
c) Correlacionar repertórios de Rips específicos de cada ecótipo e compará-los com repertórios anteriores;
d) Predizer as interações entre proteínas de grande relevância para a patogenicidade de *R. solanacearum*, a fim de minerar efetores de virulência que atuam em conjunto num contexto de infecção.

5. RESULTADOS

Capítulo I - Characterization and association of Rips repertoire to host range of novel *Ralstonia solanacearum* strains by *in silico* approaches

Para melhor compreensão dos resultados obtidos, esta seção será apresentada em dois capítulos. O capítulo I faz referência a um artigo submetido à revista "Microorganisms – ISSN 2076-2607", do MDPI, edição especial "Plant Pathogen Bacteria", informando todos os resultados referentes à confirmação da espécie por métodos de taxogenômica, avaliação do pangenoma do complexo de espécies e também predição de efetores do tipo III dos 14 isolados sequenciados. Este artigo está disponível em: https://doi.org/10.3390/microorganisms11040954. O capítulo II é focado no enriquecimento de anotação dos genomas, especialmente nos genes que sintetizam o aparato do T3SS e a relação existente entre essas proteínas e os efetores do tipo III preditos, segundo as bases de dados existentes.

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Characterization and Association of Rips Repertoire to Host Range of Novel *Ralstonia solanacearum* Strains by In Silico Approaches

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Abstract: *Ralstonia solanacearum* species complex (RSSC) cause several phytobacteriosis in many economically important crops around the globe, especially in the tropics. In Brazil, phylotypes I and II cause bacterial wilt (BW) and are indistinguishable by classical microbiological and phytopathological methods, while Moko disease is caused only by phylotype II strains. Type III effectors of RSSC (Rips) are key molecular actors regarding pathogenesis and are associated with specificity to some hosts.In this study, we sequenced and characterized 14 newly RSSC isolates from Brazil's Northern and Northeastern regions, including BW and Moko ecotypes. Virulence and resistance sequences were annotated, and the Rips repertoire was predicted. Confirming previous studies, RSSC pangenome is open as $\alpha \cong 0.77$. Genomic information regarding these isolates matches those for *R. solanacearum* in NCBI. All of them fit in phylotype II with a similarity above 96%, with five isolates in phylotype IIB and nine in phylotype IIA. Almost all *R. solanacearum* genomes in NCBI are actually from other species in RSSC. Rips repertoire of Moko IIB was more homogeneous, except for isolate B4, which presented ten non-shared Rips. Rips repertoire of phylotype IIA was more diverse in both Moko and BW, with43 common shared Rips among all 14 isolates. New BW isolates shared more Rips with Moko IIA and Moko IIB than with other public BW genome isolates from Brazil. Rips not shared with other isolates might contribute to individual virulence, but commonly shared Rips are good avirulence candidates. The high number of Rips shared by new Moko and BW isolates suggests they are actually Moko isolates infecting solanaceous hosts. Finally, infection assays and Rips expression on different hosts are needed to better elucidate the association between Rips repertoire and host specificities.

Keywords: bioinformatics; agronomy; phythopathogens; genomic taxonomy; T3Es; host specificity

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1. Introduction

Per year, around 20% of yield losses are due to infection by soil borne microbes [1,2]. Bacterial wilt, caused by *Ralstonia solanacearum* species complex (RSSC), is a cosmopolitan phytobacteriosis of difficult management and control in the field. It is responsible for significant yield losses in many crops in tropical regions and worldwide, affecting potato, tomato, eggplant, peppers, banana, eucalyptus, and ginger, among others [1,3–5].

In the state of Pernambuco, Brazil, this bacterium was detected in all mesoregions, being responsible for total loss in crops where the disease was identified [6,7]. In the last decade, many phylogenetic studies proposed the reclassification of RSSC into three distinct species according to their phylotype position and center of origins: *R. pseudosolanacearum* (phylotypes I and III, from Asia and Africa), *R. solanacearum* (phylotypes IIA and IIB, from America), and *R. syzygii* (phylotype IV, from Indonesia) [8–10]. Due to the broad range of hosts of RSSC, they're commonly described in ecotypes according to the infected host and disease caused. All species in RSSC cause bacterial wilt (BW). Moko disease of Musa, brown rot of potato, and non-pathogenic to banana (NPB) are caused only by *R. solanacearum*,with brown rot and NPB being positioned in IIB as recent *R. solanacearum* strains derived from Moko. In turn, Sumatra disease of clove and blood disease bacterium (BDB) are caused by *R. syzygii* [8,11]. In Brazil, no occurrences of *R. syzygii* have been reported, whereas *R. pseudosolanacearum* and *R. solanacearum* are pointed out as being responsiblefor all BW cases [11]. Furthermore, Moko disease is highly prevalent in Latin America, considered an A2-level quarantine disease in the Northern (Amazonas, Pará, Rondônia, and Roraima) and Northeastern (Pernambuco and Sergipe) regions [12]. However, no phenotypic characteristics or symptoms displayed by infected plants enable distinguishing RSSC in phylotypes.

A relevant molecular mechanism in RSSC related to pathogenicity and virulence is their type III effectors (T3Es), commonly referred to as *Ralstonia* injected proteins (Rips). Those proteins are essential in pathogenicity success because they interfere with plant basal immunity and act on specific targets within cascade reactions in the cell, eliciting or attenuating hypersensitive responses [13]. Effectors triggering hypersensitive responses are related to avirulence traits, while effectors eliciting immune responses are related to virulence traits. Moreover, a series of studies has been carried out to identify the role of those effectors in host infection success. For instance, the absence of RipAA and RipP1 are linked to infection success in tobacco [14], RipS1 is linked to virulence contribution in African daisy and eggplant [15,16], and RipAZ1 is linked to avirulence in black nightshade plants [17]. Only 16% of the currently known Rips subfamilies compose the core effectome of RSSC [13,18]. Therefore, Rips repertoire tends to vary significantly with isolates' phylotype, ecotype, and local area of occurrence.

In this study, we aimed to apply *in silico* approaches to predict the pangenome and identify the exact taxonomy of RSSC isolates causing Moko disease and BW in Brazil's Northern and Northeastern regions, as well as investigate resistance and virulence genes, predict their Rips repertoire, and compare with previously identified Rip candidates related to host specificity.

2. Materials and Methods

2.1. Genomes Database

In total, 120 complete genomes were used for this study, 118 of *R. solanacearum* and 2 genomes of *R. pseudosolanacearum*; 106 of them were retrieved from the public genome repository of the National Center for Biotechnology (NCBI) and 14 unpublished private genomes were isolated from the Northern and Northeastern regions of Brazil, from which 12 cause Moko disease and 2 cause BW in tomato. These 14 isolates were previously sequenced on the Illumina Hi-Seq 2500 platform in a paired-end library of 2×150 bp at the University of Göttingen (Germany).

2.2. Quality Control and Assembly

The sequencing quality was estimated using the FastQC (v0.11.8) metrics report [19]. Subsequently, we used SPAdes (v3.14) [20] for genome assembly with default parameters. For genome completeness verification and assembly parameters, assembled genomes underwent evaluation through BUSCO (v4.1.2) (Benchmarking Universal Single-Copy Orthologs) [21] against the bacteria_odb10 database. Assemblies with completeness below 90% were discarded. In parallel, we also used QUAST (v5.2.0) (Quality Assessment Tool for

Genome Assemblies) [22] with default parameters. The information regarding the strain name, collection site, host, and disease caused for all genomes used is available in Table A1.

2.3. Genome Annotation and T3Es Recovery

All genomes underwent automatic annotation through Prokka (v1.13.4) [23], a specific annotation tool for prokaryotes, to identify coding sequences (CDS) and non-coding RNAs using default parameters and databases. Further, we used PanViTa (https://github.com/dlnrodrigues/panvita, accessed on 15 December 2022) [24] to predict virulence and metal resistance genes for all 120 genomes, using VFDB [25] and BacMet [26] databases. Finally, Rips were predicted for the new 14 isolates plus 2 public genomes of Brazilian *R. solanacearum* BW isolates using the RalstoT3E's database (https://iant.toulouse.inra.fr/bacteria/annotation/site/prj/T3Ev3/, accessed on 16 February 2022) [27] with default parameters. In this step, we only considered Rips with at least one copy in one of the isolates. From the Rips repertoire prediction, we tried to find candidate Rips for host specificity in each ecotype and compared them to previously found candidates for Moko disease [28]: RipAA, RipAB, RipAC, RipAD, RipAE, RipAI, RipAN, RipAO, RipAP, RipAU, RipAY, RipB, RipC1, RipD, RipE2, RipF1, RipG2, RipG3, RipG6, RipH1, RipH2, RipP, RipV1, and RipW. For visualization and comparison, both the absence–presence heatmaps and Venn diagrams were plotted using R standard packages.

2.4. Prediction of the RSSC Pangenome

To identify clusters of similar genomes, the RSSC pangenome was estimated with Roary (v3.13.0) [29] with subsequent visualization of the matrix and phylogenetic tree on Phandango (https://jameshadfield.github.io/phandango/#/main, accessed on 6 June 2022) [30] and Roary's built-in R script. A phylogenomic tree inferred on single-copy orthologs was plotted using OrthoFinder (v2.5.4) and iTOL (v6.0) [31,32]. The pangenome's alpha value was calculated with an *in-house* script using OrthoFinder's outputs with a formula based on the Heap's Law model, in which $\alpha < 1$ indicates an open pangenome [33,34]:

$$n = k \times N_{\gamma} \quad (1)$$

In which: n = number of genes, N = number of genomes, and k and γ are constants defined to fit the specific curve. Following, γ can be calculated as:

$$\alpha = 1 - \gamma \quad (2)$$

By that, $\alpha < 1$ indicates an open pangenome, in which the more genomes are sequenced and added to the analysis, the more genes will be discovered. On the other hand, $\alpha > 1$ indicates a closed pangenome, meaning despite more genomes being added, no significant increase in new genes would be discovered. Additionally, we also used the Least Squares Fit Principle to predict the number of singletons added to each genome and a probable number of genes for core genome stabilization, following:

$$n = k \times e^{(x-\tau)} + tg\theta \quad (3)$$

In which: n = number of genes, x = number of genomes, e is the Euler number, and k, $\tau$and $tg\Theta$ are constants.

2.5. Whole-Genome Methods for Taxonomy Insights

Furthermore, all genomes underwent two distinct approaches for species classification: first, an Average Nucleotide Identity (ANI) analysis was conducted through the MUMmer alignment method using pyANI (v3.0) [35], considering a 96% $\geq$similarity criteria for different genomes belonging to the same species. Afterward, an in silico DDH (DNA– DNA hybridization) analysis was performed in the GGDC web server (v3.0) [36,37] (https://ggdc.dsmz.de/ggdc.php#, accessed on 30 June 2022), with subsequent visualization in

Morpheus (https://software.broadinstitute.org/morpheus/, accessed on 8 January 2022). Due to the limitation in the number of genomes allowed in GGDC web server, we only used 77 of 120 genomes from the database, including the 14 newly sequenced ones. A ≥70% similarity criterion was considered for different genomes belonging to the same species, and ≥79–80% similarity criterion for subspecies classification [38].

3. Results

3.1. Genome Sequencing and Characterization of New Brazilian RSSC Isolates

The QUAST report revealed that the isolates B4 and CCRMRs121 had the largest and smallest genomes, with 5,858,492 and 5,364,378 base pairs, respectively. All genome sizes were similar to the average genome size for *R. solanacearum* at NCBI (5,059,182 bp). Other quality metrics for all isolates are available in Table 1. Furthermore, the BUSCO report revealed all genomes were complete considering single-copy orthologous genes. None of the assembled genomes had missing genes from the database. However, most had at least one fragmented or duplicated gene, which did not interfere with further analyses (Figure 1).

Table 1. Quality metrics for Northern and Northeastern sequenced RSSC isolates.

Isolate	Size (Mb)	Contigs	N50	L50
B106	5.50	46	399,454	5
B4	5.85	50	574,994	5
B75	5.42	77	333,179	6
CCRMRs121	5.36	35	504,573	4
CCRMRs223	5.57	53	296,540	5
CCRMRs279	5.70	441	46,716	34
CCRMRs283	5.46	81	204,913	8
CCRMRs286	5.46	81	185,753	9
CCRMRs294	5.47	81	204,912	8
CCRMRs302	5.64	380	37,222	42
CCRMRs314	5.69	381	37,222	42
CCRMRs317	5.50	80	205,138	7
CCRMRs339	5.50	249	238,614	8
CCRMRs91	5.46	81	105,718	18

Genomes presented between 5034 and 4592 coding sequences and 61 to 66 non-coding RNAs (including tRNAs, rRNAs, tmRNAs, and others). According to NCBI, the average count of coding sequences in *R. solanacearum* is 4774. No direct correlation was found between CDS-ncRNA amounts and the type of disease caused by each isolate. Overall, 19 virulence genes (*ade*G, *che*A, *che*B, *che*D, *che*W, *che*Y, *cya*B, *flg*G, *fli*A, *fli*M, *fli*P, *htp*B, *icl*, *kat*G, *mot*A, *pil*T, *sod*B, *tsr*, *tuf* A, and *mot*A) and 29 metal resistance genes (*ade*B, *ade*G, *bcr*C, *chr*A1, *chr*B1, *chr*F, *cnr*A, *cnr*T, *cop*A, *cop*R, *cue*A, *czc*A, *dps*A, *mdt*B, *mdt*B/*yeg*N, *mer*A, *mer*P, *mer*R, *mer*T, *mex*K, *opr*J, *pbr*A, *pst*A, *pst*B, *pst*C, *rcn*A/*yoh*M, *ruv*B, *sil*A, and *smr*A) were predicted for the isolates. All predicted genes had a similarity of ≥60%. The most abundant genes were *ade*G, *che*Y, *htp*B, and *pil*T, present in 118 of 120 genomes. Similarly, for metal resistance genes, the most abundant were *ade*G, *bcr*C, *czc*A, *dps*A, *pst*B, and *ruv*B. Strains UW386, T110, and SL3022, isolated from soil and infected potato, exhibited the most quantity of metal resistance genes, with 17 and 16, respectively. In parallel, strains T110 and SL3730, exhibited the highest amounts of virulence genes, although all genomes presented 10 to 7 virulence genes. The newly sequenced genomes presented 9 to 7 virulence genes and 10 to 8 metal resistance genes. Neither host/source nor isolate origins seemed to have a correlation to genetic virulence of resistance. The clustermaps for VFDB and BacMet are available in Figures 2 and 3.

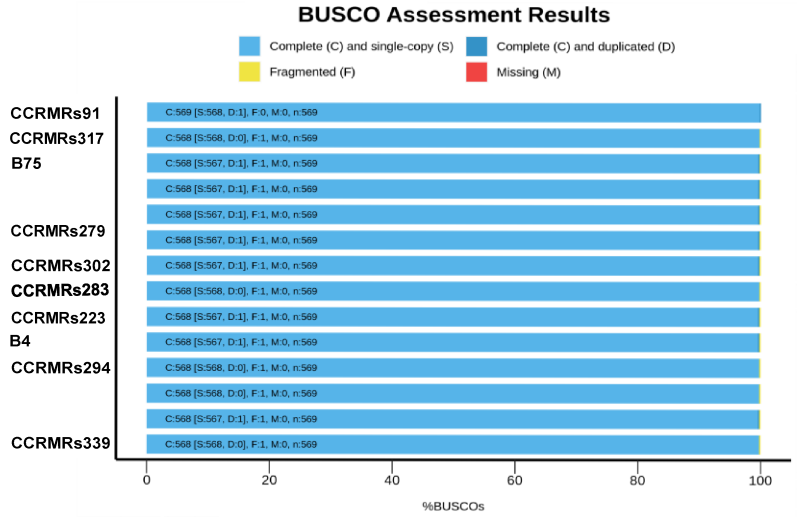

Figure 1. Genome completeness for Northern and Northeastern sequenced RSSC isolates.

3.2. RSSC Pangenome and Genomic Taxonomy of Newly Sequenced R. solanacearum Isolates

A total of 29,507 genes were predicted for the RSSC pangenome, of which 22,002 are unique genes, 6040 are in the accessory genome, and 1465 are in the core pangenome. The predicted pangenome clusterized the 120 genomes in at least four different groups, which match the phylotype classification of RSSC based on their centers of origin and phylotypes (Figure 4). Applying the OrthoFinder results to Heap's Law, $n \cong 4517.340 \times n^{(0.223)}$ resulted in 12,094 ortholog families in the pangenome, with $\alpha \cong 0.77$ indicating an open pangenome. Moreover, the value of tgΘ in the Least Squares Fit Principle revealed that 2883 ortholog families compose the RSSC core genome (as $n = 1663.212 \times exp[-x/444.977] + 1561.917$), and 28 ortholog families are strain-specific (as $n = 90.024 \times exp[-x/101.874] + 0.1905$). By that, we predict that at each new genome added to the RSSC pangenome, ≅0.1905 new ortholog genes would be discovered, and the core genome would stabilize in around 1562 genes. Overall, the RSSC pangenome tends to stabilize once a steady low number of conserved genes was maintained through all genomes (Figure 5).

In ANI analysis, three groups of genomes were formed, separated by similarity below 90% (Figure 6). First, *R. solanacearum* genomes from public databases isolated in China and Japan clustered with *R. pseudosolanacearum* genomes isolated in Brazil, indicating they all belong to phylotype I. Next, in phylotype III, three *R. solanacearum* genomes isolated in Africa composed a much smaller cluster relatively similar to the previous one, indicating they're also *R. pseudosolanacearum* genomes. The following cluster was composed of *R. solanacearum* genomes isolated in South Korea, but since they were not similar to the previous or next cluster, they make up phylotype IV or *R. syzygii*. None of the new isolates clustered within the clusters mentioned up to now. The reminiscent cluster was composed of American isolates, except for CFBP strains from Iran. Among this last cluster, there was a clear division of more similar genomes: isolates CCRMRs283, CCRMRs286, CCRMRs294, and CCRMRs317 formed a smaller cluster (Cluster 1), and B75, B106, CCRMRs91, CCRMRs121, CCRMRs223, CCRMRs279, CCRMRs302, CCRMRs314, and CCRMRs339 formed a more significant cluster (Cluster 2). The genome from isolate B4 was the most distinct of all 14 but still more similar to Cluster 1 than Cluster 2 genomes. The new isolates present in Cluster 1 caused only Moko disease, while public genomes in it were obtained from isolates of other ecotypes, including NPB and brown rot. New isolates present in Cluster 2 caused Moko disease or BW on tomato only, indicating they are, respectively, phylotypes IIB and IIA, and part of the actual *R. solanacearum* species. This distribution of strains was also true for the phylogenomic tree based on gene family conservation found on OrthoFinder (Figure 7); however, it is possible to notice that isolate B4 was more distant from other IIB isolates than in ANI profile, clustering a clade with CFBP8695, CFBP8697, RS488, UY031, and UW163. That being the case, all sequenced isolates causing BW in this study are phylotype II strains.

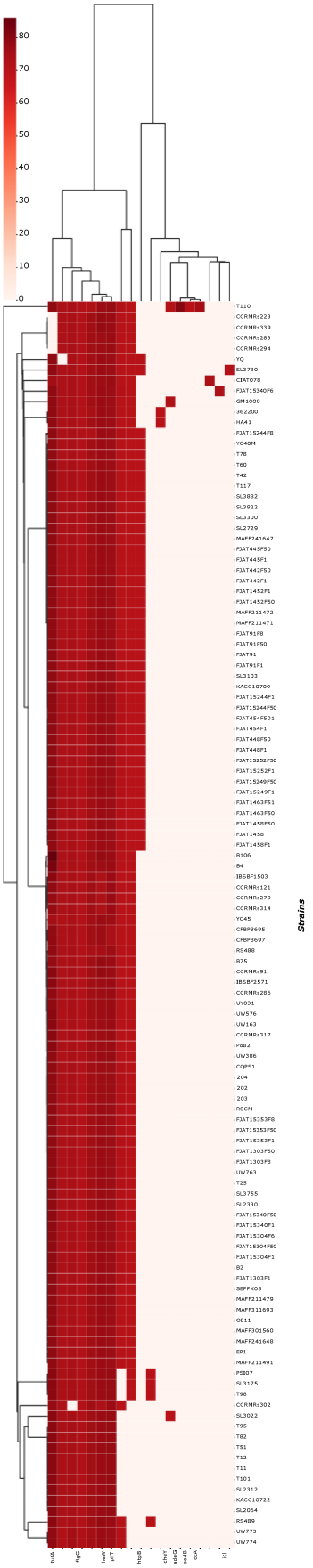

Figure 2. Clustermap for virulence genes presence and similarity against VFDB.

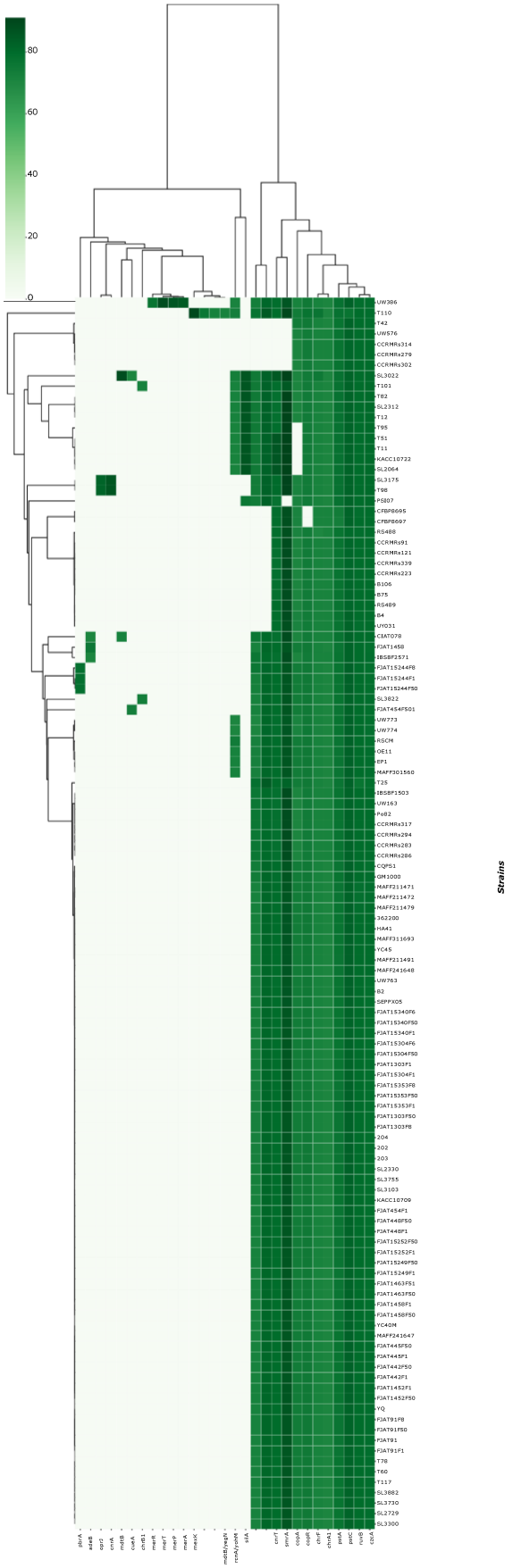

Figure 3. Clustermap for metal resistance genes presence and similarity against BacMet database.

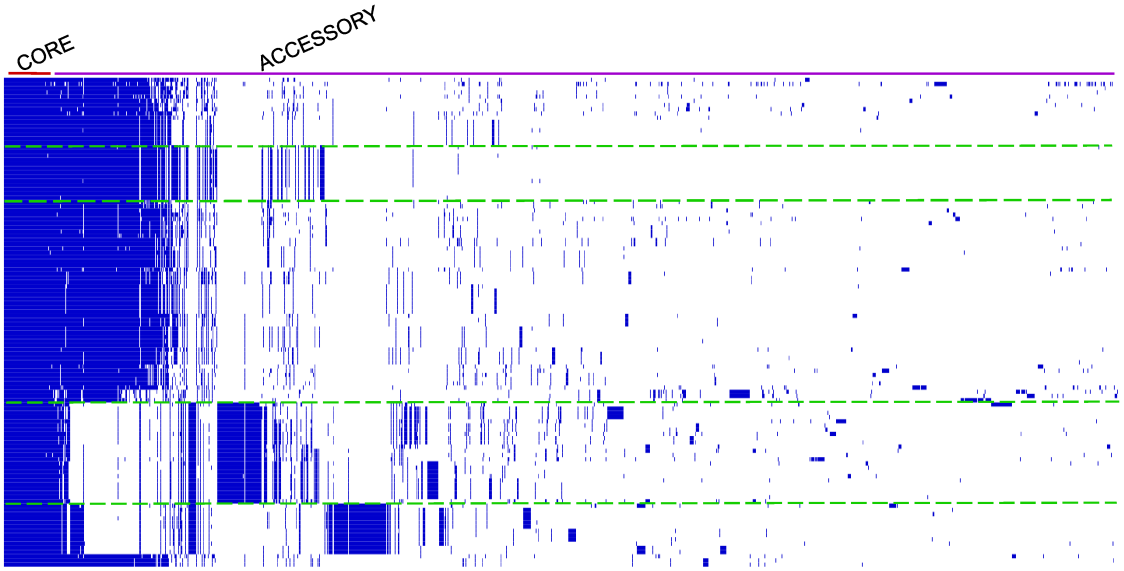

Figure 4. *R. solanacearum* species complex (RSSC) pangenome profile. From left to right, blue regions homogeneously distributed represent the core genome, while blue spots represent the unique genome. Blocks underneath red line make up the core genome, whilst blocks underneath purple line make up the accessory genome. From the presence–absence profile, it is possible to identify 4 major patterns in the pangenome profile, delimited by the green dotted lines.

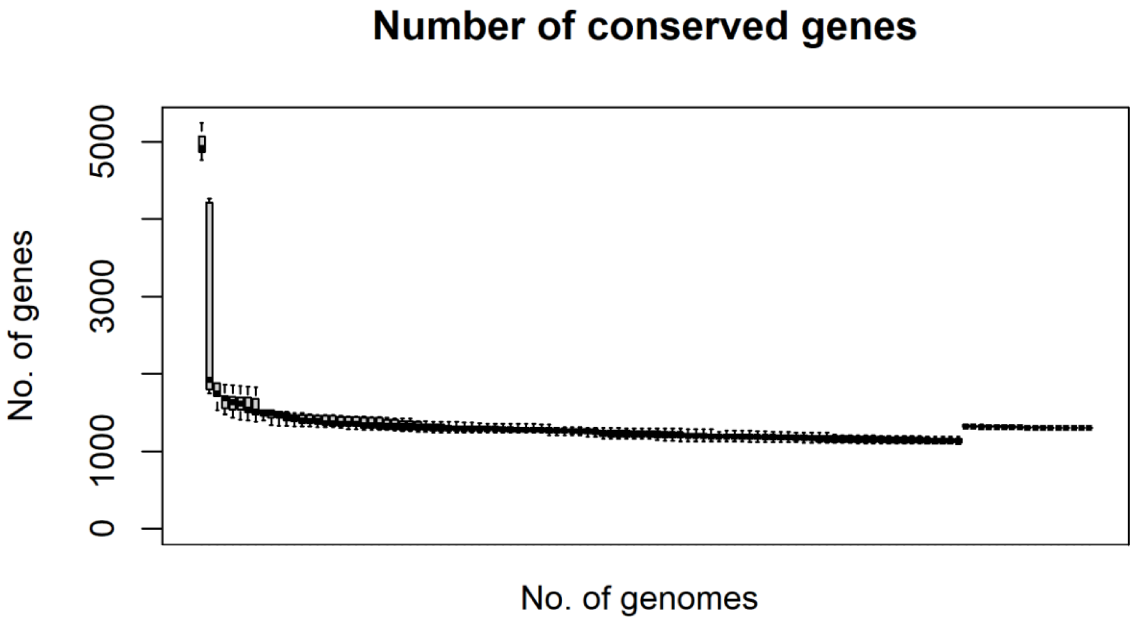

Figure 5. Pangenome development considering conserved genes throughout the 120 genomes.

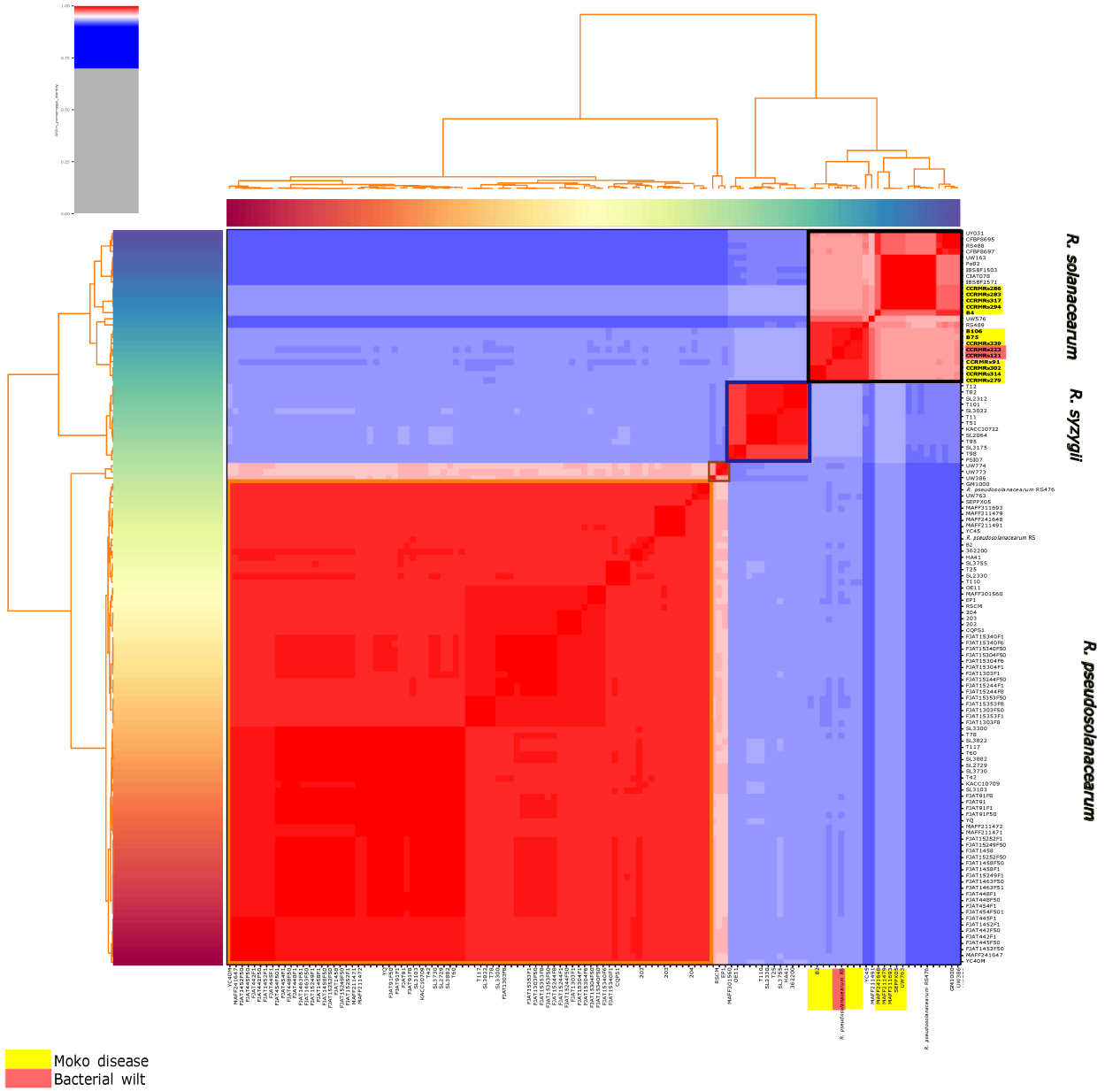

Figure 6. ANI analysis clustermap of public *R. solanacearum* genomes on NCBI and 14 new Brazilian *R. solanacearum* genomes. Upwards, there are phylotypes I (orange box), III (brown box), IV (blue box), IIA, and IIB clusters (black box), composing *R. pseudosolanacearum*, *R. syzygii*, and *R. solanacearum*, respectively. Newly sequenced genomes are in bold and highlighted in red and yellow according to their respective ecotypes.

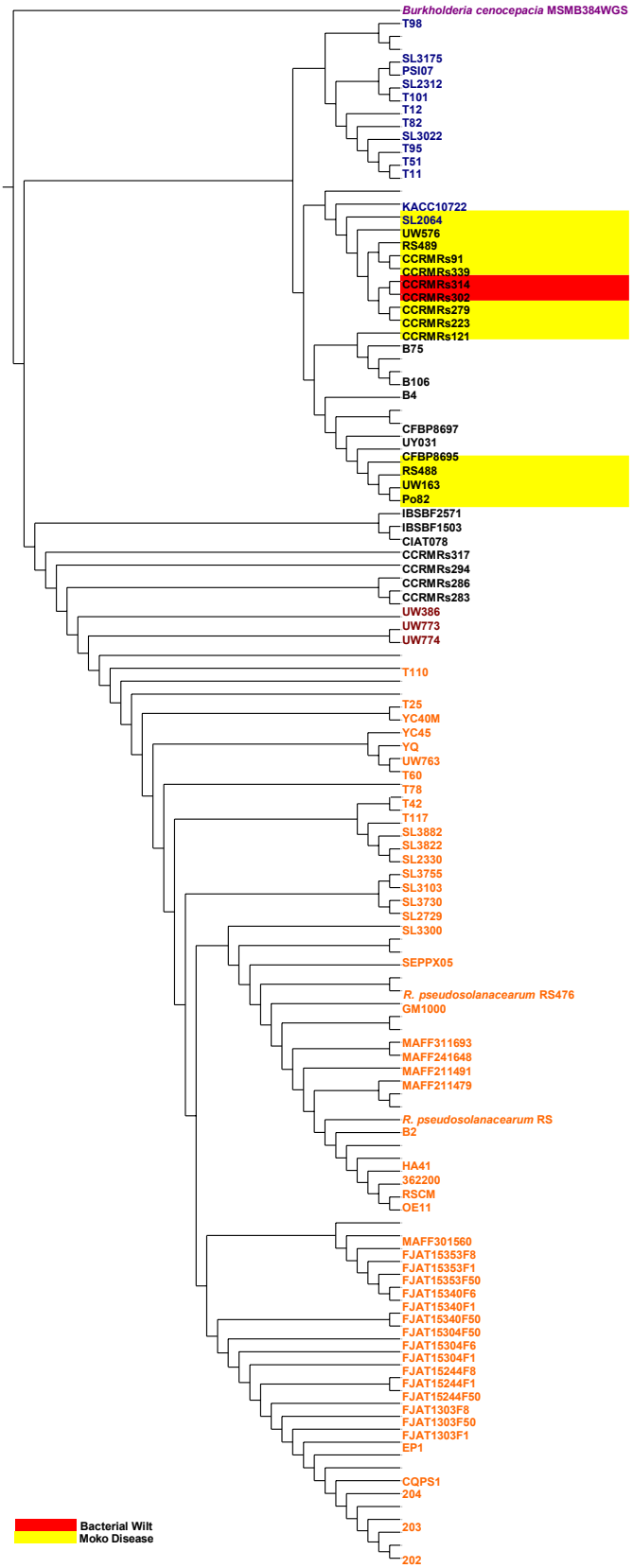

Figure 7. Phylogenomic tree of *R. solanacearum* species complex (RSSC) strains used in this work. Strains' names are colored according to Figure 6 pattern: phylotypes I in orange, phylotypes III in brown, phylotypes IV in blue, and phylotypes II in black. Newly sequenced genomes are also highlighted according to their respective ecotypes.

Even though the ANI analysis and phylogenomic tree evidence two distinct clades within the newly sequenced isolates, the in silico DDH values varied significantly from pairwise comparison, and it was not possible to find a consensus that separated IIA and IIB isolates in subspecies (Figure 8).

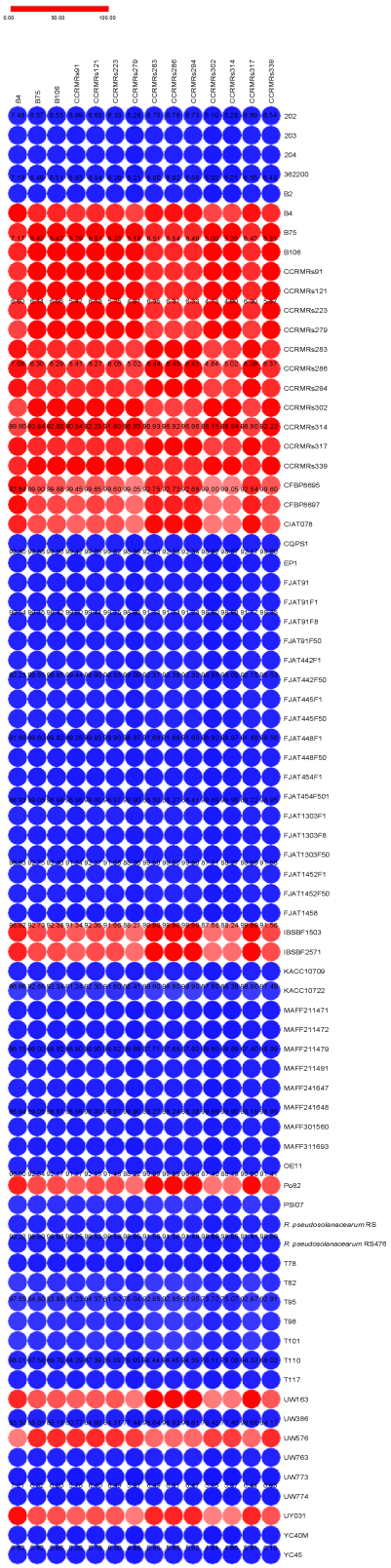

Figure 8. Heatmap representing the in silico DNA–DNA hybridization (*is*DDH) of genomes from *R. solanacearum* species complex (RSSC). The value for each DDH is available inside each dot.

3.3. Prediction of Rips Repertoire of *R. solanacearum* Strains and Ecotype Correlation

In total, 88 subfamilies of Rips were present in the new 14 isolates. Overall, B4 and B76 had the lowest and the highest number of predicted Rips, with 67 and 76 out of 88, respectively. The Rips repertoire of each isolate is available in Figure 9.

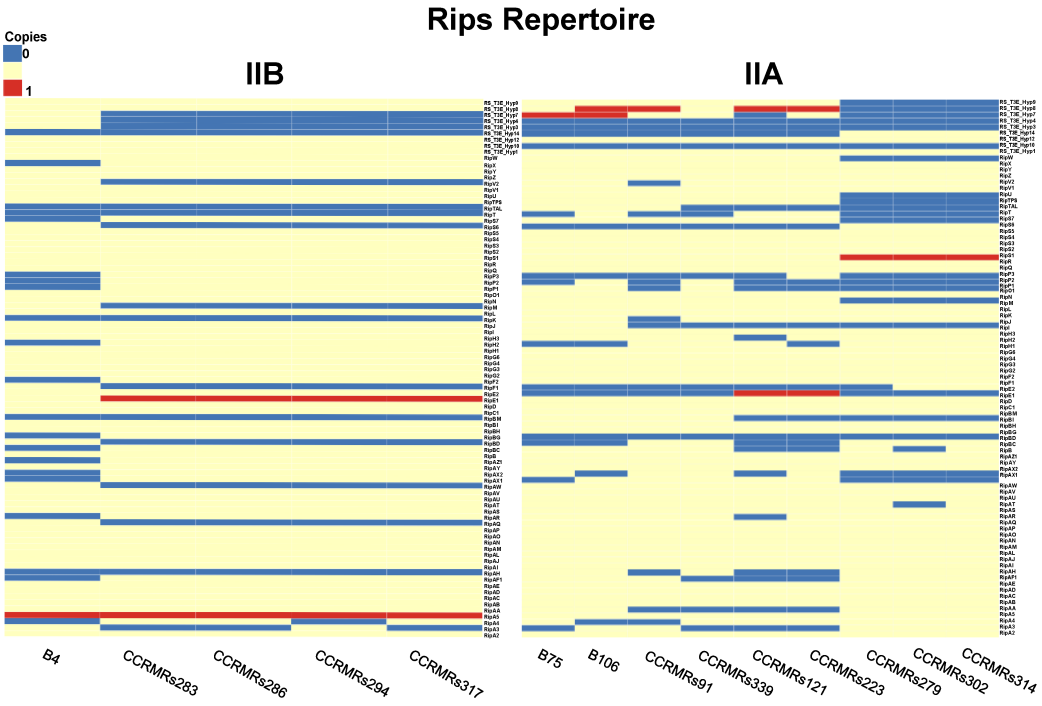

Figure 9. Heatmap of Rips repertoire of each new Brazilian *R. solanacearum* isolates IIB (**left**) and IIA (**right**).

Cluster IIB Rips repertoire was more homogeneous than Cluster IIA's, despite a higher number of isolates causing Moko in the latter. Considering both clusters, only six events of Rip duplication occurred: RipA5, RipEI1, RipE2, RipS1, RS_T3E_Hyp7, and RS_T3E_Hyp8. There was no duplication event in common for both Clusters, and there was no Rip absent in all 14 isolates besides the hypothetical ones. Congruently with the profile observed in ANI, B4 also exhibited the most distinct pattern of Rips presence–absence–duplication compared to the other 13 isolates. Starting with Cluster IIB, they commonly shared 55 Rips, and B4 presented ten exclusive Rips: RipAQ, RipAW, RipBD, RipF1, RipM, RipS6, RipV2, RS_T3E_Hyp3, RS_T3E_Hyp4, and RS_T3E_Hyp7. All isolates shared a duplication of RipA5; however, only B4 did not share a duplication of RipE1, and B4 and CCRMRs294 shared an absence of RipA4. In Cluster IIA, isolates causing Moko disease commonly shared 51 Rips. The isolates CCRMRs279, CCRMRs302, and CCRMRs314 had remarkably similar repertoires, except for the absence of RipAT and RipBC in CCRMRs302, and the absence of RipF1 in CCRMRs279. Finally, the new BW isolates commonly shared 63 Rips. Both isolates have almost the same repertoire, except for the absence of RipAR, RipAX2, RipH3, RipP3, and RS_T3E_Hyp7 in CCRMRs121, and the absence of RipH2 in CCRMRs223. Moko IIA, Moko IIB, and BW isolates commonly shared 43 Rips. These comparisons also revealed that very few Rips were ecotype-specific; the 12 isolates causing Moko disease only commonly shared RipH3. Moko IIA isolates exclusively shared RipAR, while Moko IIB isolates exclusively shared four Rips (RipAA, RipJ, and RS_T3E_Hyp10), and BW isolates exclusively shared six Rips (RipA4, RipAX1, RipK, RipS7, RipT, and RipV2). More Rips were exclusively shared among Moko IIB and BW isolates (RipAT, RipE2, RipN, RipTPS, RipU, RipZ, RS_T3E_Hyp8, and RS_T3E_Hyp9) than among Moko IIA and BW (RipAQ, RipAW, RipAZ1, RipF2, RipM, and RipY) (Figure 10). Of the 22 candidate Rips

for Moko disease suggested by Ailloud et al.[28], only 4 were not commonly shared by the 12 Moko isolates: RipAA, RipE2, and RipF1 in Moko IIA and RipF1 and RipH2 in Moko IIB. However, it is important to highlight that only three of those Moko candidate Rips were not commonly shared by BW isolates: RipF1, RipH2, and RipAA. In contrast to public genomes of other Brazilian *R. solanacearum* infecting tomato, RS488 and RS489 (BW2) shared 44 Rips with CCRMRs121 and CCRMRs223 (BW1), with 16 Rips exclusively shared by BW1. Interestingly, isolates in BW1 shared more Rips with Moko IIA and IIB isolates than BW2, resting only 29 Rips shared by all four groups from the 43 early found (Figure 11). The Rips repertoire comparison of BW1 and BW2 is available in Figure A1.

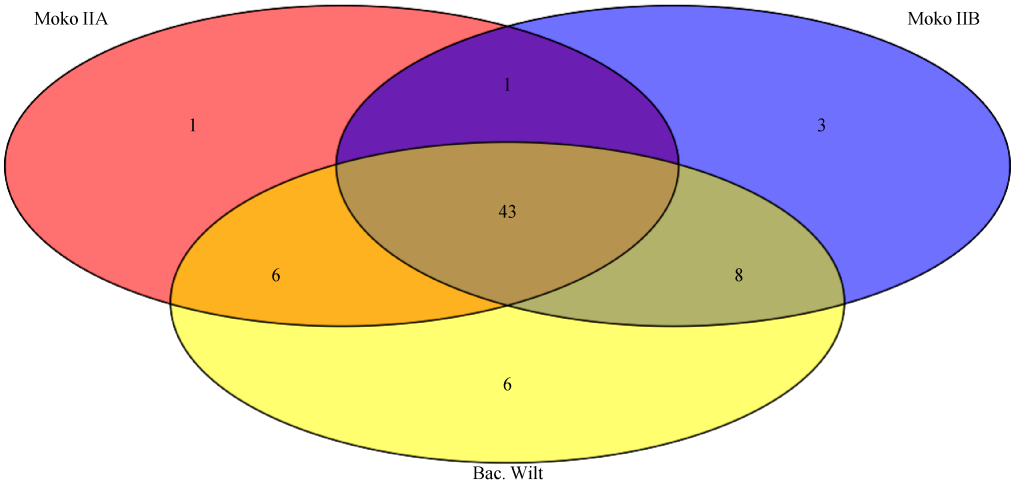

Figure 10. Rips shared among the sequenced isolates used in this study only.

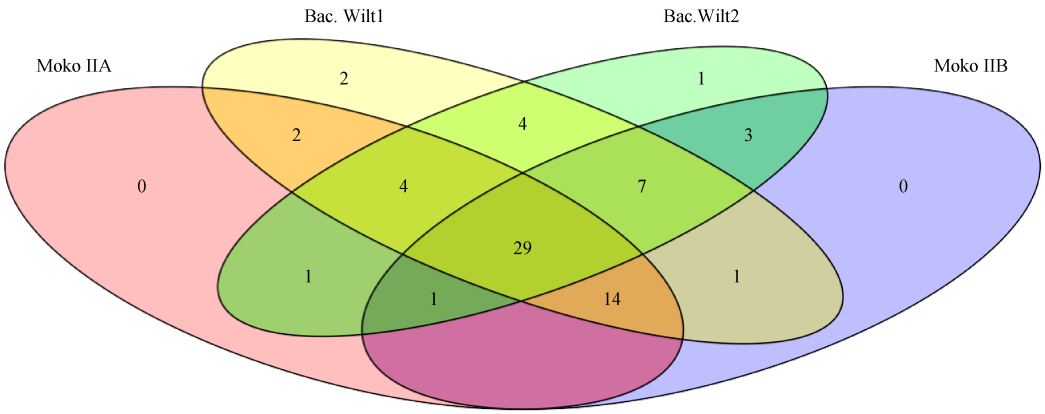

Figure 11. Rips shared among newly sequenced isolates (Moko IIA, Moko IIB, and BW1) plus public Brazilian BW isolates, RS488 and RS489 (BW2).

4. Discussion

4.1. Pangenome and Nucleotide Identity Analysis Reveal Global Misclassification of RSSC Isolates in Public Databases and Genetic Diversity of New Brazilian Isolates

Our study used a large dataset of high-quality publicly available *R. solanacearum* complete genomes, elucidating their taxonomy via robust in silico whole-genome approaches confirming many previous findings [9,10,35,39,40]. The misclassification of older *R. solanacearum* genomes has recently been addressed by Sharma et al. [41], who also pointed out the discrepancy in representative genomes from African and South Asian isolates available at NCBI. As the sequevar/biovar classification has been shown to fail at represent the diversity of highly recombinogenic isolates [41], whole genome methods, such as ANI, *is*DDH and phylogenomic inferences are more prone to accurately provide

the genetic diversity on RSSC and other bacterial phytopathogens with controversial taxonomy [35]. The open pangenome profile observed through our analysis corroborates up-to-date studies, with similar values found for core, accessory, and unique genomes [42] (Geng et al., 2022). As soil borne microbes, the resistance of *Ralstonia* strains to heavy metals was described a long time ago [5,43,44], but no recent analyses have included Brazilian isolates. Considering that pesticides and fertilizers commonly used in high-production crops typically comprise heavy metals in their composition [45,46], these findings raise an alert for small and big producers in countries such as Brazil that struggle with *R. solanacearum* infestation. As for the new RSSC isolates from Brazil's Northern and Northeastern regions, they fit in *R. solanacearum*, but are from separate phylotypes. The fact that 12 isolates infecting *Musa* in close geographic spots still differ in phylotype sublevel only shows how diverse Brazilian RSSC isolates are, which corroborates with the hypothesis of the Amazon region being the diversity center of phylotypes IIA and IIB [7,47]. At first look, most of the newly sequenced isolates fitting in phylotype IIA might seem a surprise, as most of the Brazilian isolates are actually included in phylotype IIB, followed by phylotypes IIA and I. However, it has also been reported that phylotype IIA isolates have a higher propor-tional presence in Brazil's North and Northeastern regions. In contrast, phylotype IIB has a higher abundance in Central, Southeastern and South regions [7,48]. Since phylotype IIA isolates have been characterized as more genetically diverse and recombinant than IIB [47], a less diverse repertoire of Rips was expected for the latter, and also because atleast two different ecotypes were suspected for IIA isolates. Analyzing the Rips repertoire is important because each subfamily of Rip plays distinct roles throughout the infection process, depending on the environment, tissue, and signals recognized within the host [49]. On that thought, we suggest here that Rips that were not commonly shared by all isolates of their respective ecotype might contribute to their individual virulence when infecting the host. In this sense, a good indicator is that B4 was isolated from a banana plant with more severe symptoms on roots, while all other Moko isolates were isolated from banana plants with wilted leaves and healthy roots. The presence of more than one copy of Rips and paralog subfamilies in RSSC is largely documented. Even though it has been described as genetic redundancy, it is also seen as a general strategy for giving bacterial virulence robustness via acting on similar targets, participating in the same molecular functions and biological processes [18,50].

4.2. Rips Repertoire of Brazilian Isolates Are More Correlated to Genomic Similarity Rather Than Ecotype

As for the duplicated Rips, RipA5 (AWR5), and RipE act as typical avirulence factors eliciting hypersensitive responses on *Arabidopsis thaliana* and *Nicotiana benthamiana* and suppressing the expression of jasmonic-acid-dependent genes and salicylic acid synthesis [51,52]; however, RipAC and RipAY inhibit RipE1-mediated HR [18]. In contrast, RipS1 acts as a virulence factor that inhibits key targets on reactive oxygen species (ROS) path-ways [51], considering that alone, CCRMRs279, CCRMRs302, and CCRMRs314 would have a higher potential for more virulent behavior.

A few Rips have been correlated with host specificity in South Asian RSSC strains infecting solanaceous hosts, with RipAS3 and RipH3 linked to pathogenicity in tomato and RipAC linked to pathogenicity in eggplants [53]. However, in RS488 no copy for RipS3 was predicted (see Figure A1), even though it caused BW in tomato. Indeed, this reinforces the hypothesis that a repertoire of Rips is keener to the success of pathogenicity in some hosts than in a few groups of Rips. We suggest that the presence of previous Moko candidates was more accurate for IIB isolates because it compared different ecotypes present only in phylotype IIB, such as NPB and brown rot, and also due to their clonal behavior. Therefore, the greater difference observed in Moko IIA isolates might indicatedifferent selective pressure on those strains derived from the higher genetic diversity observed in this phylotype, making Rips gain or loss more probable. The broad presence of Moko candidates in the new BW isolates and their broadly shared Rips repertoire can be

explained by two main arguments: the first one is that the most common recent ancestor of *Ralstonia* isolates was already capable of infecting banana and host-adapted polymorphisms (HAP) would be present in the Rips derived from it, making them functionally specialized either for solanaceous and musaceous hosts respective defense mechanisms [28].

Moreover, it has been shown that NPB and Moko disease strains have minimal genomic differences and still have high gene expression differences when infecting their respective hosts [54]. Thus, even if Moko and BW isolates have no significant differences in their Rip sequences, their gene expression would still differ when infecting different hosts. The second argument is that these BW isolates are actually from Moko ecotype infecting solanaceous hosts due to the optimal environmental conditions found in Brazil's Northeastern region, as it has been previously reported in environments with high temperatures and humidity conditions [55,56]. This argument gains strength when considering that the pan-effectome of *R. solanacearum* is clearly diverse with a small core effectome of 16 Rips [13,18] contrasting with the 43 Rips present in all 14 isolates of two different ecotypes. Moreover, their Rips repertoire was more similar with Moko isolates than with RS488 and RS489. Hence, based on what we found and considering the second argument, only 14 Rips would be eligible candidates for Moko disease: RipA2, RipAS, RipAU, RipG3, RipG4, RipG6, RipH1, RipL, RipS1, RipS2, RipS3, RipS4, and RS_T3E_Hyp12.

5. Conclusions

The present study is the first to include Brazilian isolates of *Ralstonia* and use a robust effector database to characterize the effectome of Brazilian isolates. The commonly shared Rips by isolates in different ecotypes might aid in further phytopathology studies by providing target avirulence proteins in hosts when searching for breeds resistant to bacterial wilt, Moko, and so on. It is important to note that further research efforts, preferably with in vitro and in vivo data on gene expression and infection essays on different hosts are required to determine whether the Rips identified here are essential candidates for ecotype specificity. In addition, more phylotype IIA isolates causing bacterial wilt in Solanaceae to identify commonly shared Rips in this ecotype are needed. Finally, even though Rips presence/absence is an excellent indication for host specificity association, it is not the final determinant. The whole-genome approaches were essential in correctly identifying these isolates' taxonomy, proving their potential for solving complicated bacterial species complexes, such as RSSC. Efforts to characterize hypothetical and redundant Rips are essential to elucidate missing roles on molecular pathways linked to triggered and innate immunity in plants.

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Data Availability Statement: All 14 newly announced sequenced genomes from Brazilian Northern and Northeastern regions are available on GenBank/NCBI under BioProject PRJNA763940 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA763940, accessed on 30 January 2022). All other publicly available genomes are listed in Table A1.

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Abbreviations

The following abbreviations are used in this manuscript:

BW	Bacterial Wilt
Rips	*Ralstonia* Injected Proteins
T3Es	Type III Effectors
MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
TLA	Three letter acronym
LD	Linear dichroism

Appendix A

Table A1. General information regarding genomes used in this work.

Strains	GenBank	Bioproject	Origin	Host	Disease
R. pseudosolanacearum RS	NZ_CP046674	PRJNA594457	China (YN)	Tobacco	Bacterial wilt
R. pseudosolanacearum RS476	NZ_CP021762	PRJNA388859	Brazil (MA)	Tomato	Bacterial wilt
R. solanacearum B106	JAIVFC000000000	PRJNA763940	Benjamin Constant, AM, BR	Banana	Moko disease
R. solanacearum B4	JAIVEX000000000	PRJNA763940	Anamã, AM, BR	Banana	Moko disease
R. solanacearum B75	JAIVFE000000000	PRJNA763940	Tefé, AM, BR	Banana	Moko disease
R. solanacearum CCRMRs121	JAIVEU000000000	PRJNA763940	Belém de São Francisco, PE, BR	Tomato	Bacterial wilt
R. solanacearum CCRMRs223	JAIVEY000000000	PRJNA763940	Bezerros, PE, BR	Tomato	Bacterial wilt
R. solanacearum CCRMRs279	JAIVFD000000000	PRJNA763940	Manicoré, AM, BR	Banana	Moko disease
R. solanacearum CCRMRs283	JAIVEZ000000000	PRJNA763940	Benjamin Constant, AM, BR	Banana	Moko disease
R. solanacearum CCRMRs286	JAIVEV000000000	PRJNA763940	Benjamin Constant, AM, BR	Banana	Moko disease
R. solanacearum CCRMRs294	JAIVEW000000000	PRJNA763940	Benjamin Constant, AM, BR	Banana	Moko disease
R. solanacearum CCRMRs302	JAIVFA000000000	PRJNA763940	Fonte Boa, AM, BR	Banana	Moko disease
R. solanacearum CCRMRs314	JAIVFB000000000	PRJNA763940	Tefé, AM, BR	Banana	Moko disease
R. solanacearum CCRMRs317	JAIVFF000000000	PRJNA763940	Tefé, AM, BR	Banana	Moko disease
R. solanacearum CCRMRs339	JAIVET000000000	PRJNA763940	Coari, AM, BR	Banana	Moko disease
R. solanacearum CCRMRs91	JAIVFG000000000	PRJNA763940	Igreja Nova, AL, BR	Banana	Moko disease
R. solanacearum 202	NZ_CP049789	PRJNA609910	China (GD)	Tobacco	Bacterial wilt
R. solanacearum 203	NZ_CP049791	PRJNA609906	China (GD)	Tobacco	Bacterial wilt
R. solanacearum 204	NZ_CP049793	PRJNA609905	China (GD)	Tobacco	Bacterial wilt
R. solanacearum 362200	NZ_CP065531	PRJNA668065	China (FJ)	Peanut	Bacterial wilt
R. solanacearum B2	NZ_CP049787	PRJNA609907	China (GD)	Tobacco	Bacterial wilt
R. solanacearum CFBP8695	CP047138	PRJNA596809	Iran	Potato	Bacterial wilt
R. solanacearum CFBP8697	CP047136	PRJNA596668	Iran	Potato	Bacterial wilt
R. solanacearum CIAT078	NZ_CP051295	PRJNA608676	Colombia	Plaintain	Moko disease
R. solanacearum CQPS1	NZ_CP016914	PRJNA331070	China (SD)	Tobacco	Bacterial wilt
R. solanacearum EP1	NZ_CP015115	PRJNA288736	China (GD)	Eggplant	Bacterial wilt
R. solanacearum FJAT1303F1	NZ_CP052128	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT1303F50	NZ_CP052126	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT1303F8	NZ_CP052130	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT1452F1	NZ_CP052124	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT1452F50	NZ_CP052122	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT1458	NZ_CP016554	PRJNA329182	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT1458F1	NZ_CP052120	PRJNA622642	China (FJ)	Tomato	Bacterial wilt

Table A1. *Cont.*

Strains	GenBank	Bioproject	Origin	Host	Disease
R. solanacearum FJAT1458F50	NZ_CP052118	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT1463F50	NZ_CP052114	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT1463F1	NZ_CP052116	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15244F1	NZ_CP052112	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15244F50	NZ_CP052110	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15244F8	NZ_CP059376	PRJNA647244	China (FJ)	-	-
R. solanacearum FJAT15249F1	NZ_CP052108	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15249F50	NZ_CP052106	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15252F1	NZ_CP052104	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15252F50	NZ_CP052102	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15304F1	NZ_CP052100	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15304F50	NZ_CP052098	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15304F6	NZ_CP052096	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15340F1	NZ_CP052094	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15340F50	NZ_CP052092	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15340F6	NZ_CP052090	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15353F1	NZ_CP052088	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15353F50	NZ_CP052086	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT15353F8	NZ_CP052084	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT442F1	NZ_CP052082	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT442F50	NZ_CP052080	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT445F1	NZ_CP052078	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT445F50	NZ_CP052076	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT448F1	NZ_CP052074	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT448F50	NZ_CP052072	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT454F1	NZ_CP052070	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT454F501	NZ_CP060701	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT91	NZ_CP016612	PRJNA329188	China (FJ)	Tomato (healthy)	Bacterial wilt
R. solanacearum FJAT91F1	NZ_CP056083	PRJNA640736	China (FJ)	-	-
R. solanacearum FJAT91F50	NZ_CP052068	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum FJAT91F8	NZ_CP056085	PRJNA622642	China (FJ)	Tomato	Bacterial wilt
R. solanacearum GM1000	NC_003295 AL646057-AL646075	PRJNA13	-	*Arabdopsis thaliana*	Bacterial wilt
R. solanacearum HA41	NZ_CP022481	PRJNA392775	China (HB)	Peanut	Bacterial wilt
R. solanacearum IBSBF1503	NZ_CP012943	PRJNA297402	Brazil	Pepino	NPB (non-pathogenic to banana)

Table A1. *Cont.*

Strains	GenBank	Bioproject	Origin	Host	Disease
R. solanacearum IBSBF2571	NZ_CP026307	PRJNA431203	Brazil (SE)	Plaintain	Moko disease
R. solanacearum KACC10709	NZ_CP016904	PRJNA314721	South Korea (GY)	Potato	Bacterial wilt
R. solanacearum KACC10722	NZ_CP014702	PRJNA314571	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum MAFF211471	NZ_AP024097	PRJDB10588	Japan (Kochi)	Ginger	Bacterial wilt
R. solanacearum MAFF211472	NZ_AP024157	PRJDB9507	Japan (Kyushu)	Ginger	Bacterial wilt
R. solanacearum MAFF211479	NZ_AP024099	PRJDB10588	Japan (Kochi)	Ginger	Bacterial wilt
R. solanacearum MAFF211491	NZ_AP024101	PRJDB10588	Japan (Kochi)	Ginger	Bacterial wilt
R. solanacearum MAFF241647	NZ_AP024105	PRJDB10588	Japan (Kochi)	Ginger	Bacterial wilt
R. solanacearum MAFF241648	NZ_AP024107	PRJDB10588	Japan (Kochi)	Ginger	Bacterial wilt
R. solanacearum MAFF301560	NZ_AP024103	PRJDB10588	Japan (Kochi)	Ginger	Bacterial wilt
R. solanacearum MAFF311693	NZ_AP024161	PRJDB9507	Japan (Kyushu)	Wild turmeric	Bacterial wilt
R. solanacearum OE11	NZ_CP009763	PRJDB4012	Japan (Kochi)	Eggplant	Bacterial wilt
R. solanacearum Po82	NC_017574	PRJNA66837	Mexico	Potato	Bacterial wilt/Moko disease
R. solanacearum PSI07	NC_014310	PRJEA50683	-	Tomato	Bacterial wilt
R. solanacearum RS488	NZ_CP021652	PRJNA388430	Brazil (PR)	Tomato	Bacterial wilt
R. solanacearum RS489	NZ_CP021766	PRJNA388980	Brazil (PR)	Tomato	Bacterial wilt
R. solanacearum RSCM	NZ_CP025985	PRJNA422474	China (GD)	Pumpkin	Bacterial wilt
R. solanacearum SEPPX05	NZ_CP021448	PRJNA379485	China (JX)	Sesame	Bacterial wilt
R. solanacearum SL2064	NZ_CP022798	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum SL2312	NZ_CP022796	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum SL2330	NZ_CP022794	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum SL2729	NZ_CP022792	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum SL3022	CP023016	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum SL3103	NZ_CP022790	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum SL3175	NZ_CP022788	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum SL3300	NZ_CP022786	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum SL3730	NZ_CP022784	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum SL3755	NZ_CP022782	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum SL3822	NZ_CP022780	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum SL3882	NZ_CP022778	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum T101	NZ_CP022757	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum T11	NZ_CP022776	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum T110	CP023012	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum T117	NZ_CP022755	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum T12	NZ_CP022774	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum T25	CP023014	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt

Table A1. *Cont.*

Strains	GenBank	Bioproject	Origin	Host	Disease
R. solanacearum T42	NZ_CP022772	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum T51	NZ_CP022770	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum T60	NZ_CP022768	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum T78	NZ_CP022765	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum T82	NZ_CP022763	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum T95	NZ_CP022761	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum T98	NZ_CP022759	PRJNA396777	South Korea (JE)	Potato	Bacterial wilt
R. solanacearum UW163	NZ_CP012939	PRJNA297400	Peru (NA)	Plaintain	Moko disease
R. solanacearum UW386	NZ_CP039339	PRJNA531204	Nigeria	Soil	-
R. solanacearum UW576	NZ_CP051175	PRJNA591018	Senegal	Tomato	Bacterial wilt
R. solanacearum UW763	NZ_CP051173	PRJNA591018	Senegal	Tomato	Bacterial wilt
R. solanacearum UW773	NZ_CP051171	PRJNA591018	Senegal	Tomato	Bacterial wilt
R. solanacearum UW774	NZ_CP051169	PRJNA591018	Senegal	Tomato	Bacterial wilt
R. solanacearum UY031	NZ_CP012687	PRJNA278086	Uruguay	Wild potato	Brown rot
R. solanacearum YC40M	NZ_CP015850	PRJNA314427	China (GD)	Galangal	Bacterial wilt
R. solanacearum YC45	CP011997	PRJNA286156	China (GD)	Ginger	Bacterial wilt
R. solanacearum YQ	NZ_CP059489	PRJNA648113	China (ZJ)	Casuarina pine	Bacterial wilt

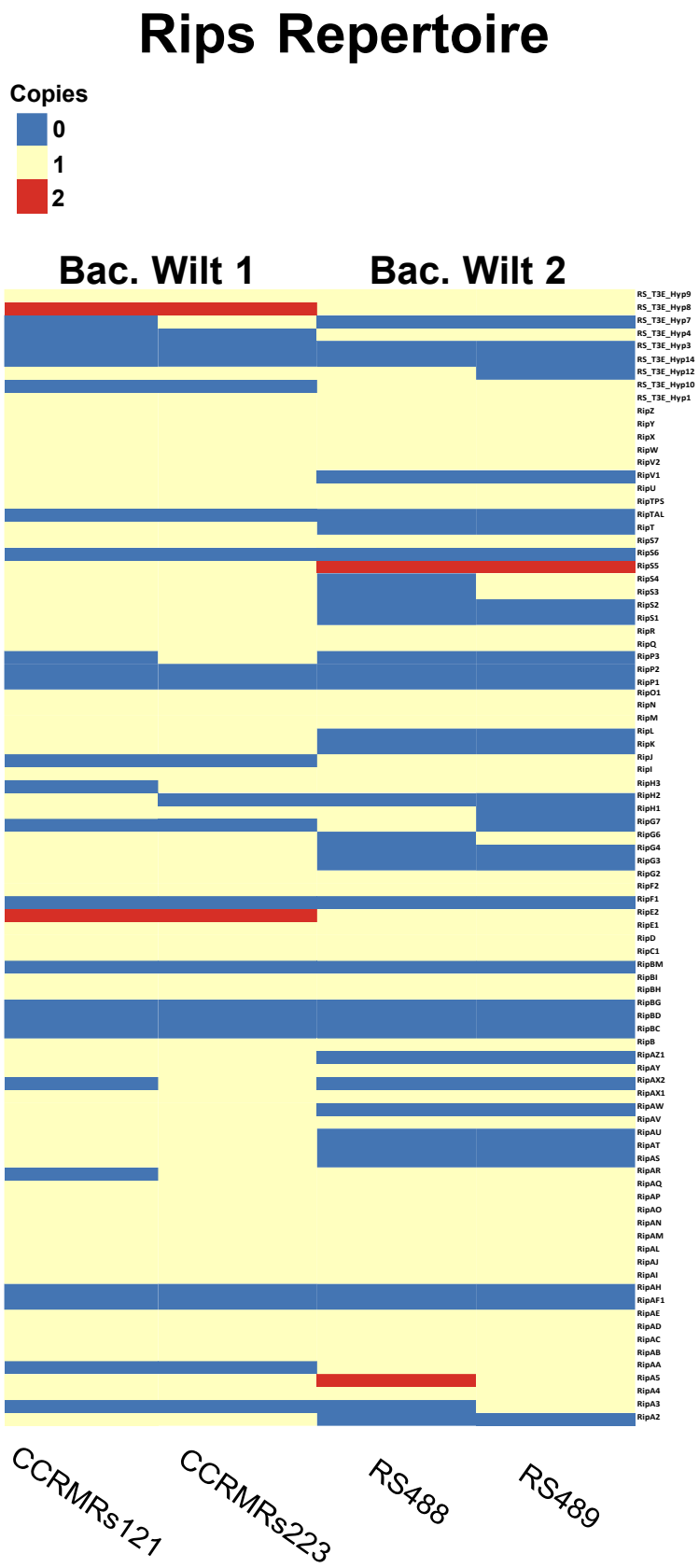

Figure A1. Heatmap of Rips repertoire of newly sequenced (Bac. Wilt 1) and publicly available (Bac. Wilt 2) genomes from isolates causing bacterial wilt in Brazil. The genomes on the right have notably fewer Rips than the newly sequenced ones, despite all causing diseases in tomato.

References

1. Manda, R.R.; Addanki, V.A.; Srivastava, S. Bacterial wilt of solanaceous crops. *Int. J. Chem. Stud.* **2020**, *8*, 1048–1057. [CrossRef]
2. Yuliar, N.; Nion, Y.A.; Toyota, K. Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. *Microbes Environ.* **2015**, *30*, 1–11. [CrossRef] [PubMed]
3. Guji, M.J.; Yetayew, H.T.; Kidanu, E.D. Yield loss of ginger (*Zingiber officinale*) due to bacterial wilt (*Ralstonia solanacearum* in different wilt management systems in Ethiopia. *Agric. Food Secur.* **2019**, *8*, 5. [CrossRef]
4. Lopes, C.A.; Rossato, M. History and Status of Selected Hosts of the *Ralstonia solanacearum* Species Complex Causing Bacterial Wilt in Brazil. *Front. Microbiol.* **2018**, *9*, 1228. [CrossRef]
5. Genin, S.; Boucher, C. Lessons learned from the genome analysis of *ralstonia solanacearum*. *Annu. Rev. Phytopathol.* **2004**, *42*, 107–134. [CrossRef]
6. Costa, K.D.D.S.; dos Santos, P.R.; dos Santos, A.M.M.; Silva, A.M.F.; Chagas, J.T.B.; de Carvalho Filho, J.L.S.; Pereira, J.W.d.L.; Silva, M.D.O.; da Silva, J.R.; et al. Genetic control of tomato resistance to *Ralstonia solanacearum*. *Euphytica* **2019**, *215*, 136. [CrossRef]
7. Santiago, T.R.; Lopes, C.A.; Caetano-Anollés, G.; Mizubuti, E.S.G. Phylotype and sequevar variability of *Ralstonia solanacearum* in Brazil, an ancient centre of diversity of the pathogen. *Plant Pathol.* **2017**, *66*, 383–392. [CrossRef]
8. Genin, S.; Denny, T.P. Pathogenomics of the *Ralstonia solanacearum* Species Complex. *Annu. Rev. Phytopathol.* **2012**, *50*, 67–89. [CrossRef]
9. Safni, I.; Cleenwerck, I.; De Vos, P.; Fegan, M.; Sly, L.; Kappler, U. 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. solanacearum* phylotype IV strains as *Ralstonia syzygii* subsp. *indonesiensis* subsp. nov., banana blood disease bacterium strains as *Ralstonia syzygii* subsp. *celebesensis* subsp. nov. and *R. solanacearum* phylotype I and III strains as *Ralstonia pseudosolanacearum* sp. nov. *Int. J. Syst. Evol. Microbiol.* **2014**, *64*, 3087–3103. [CrossRef]
10. Zhang, Y.; Qiu, S. Phylogenomic analysis of the genus *Ralstonia* based on 686 single-copy genes. *Antonie Van Leeuwenhoek* **2016**, *109*, 71–82. [CrossRef]
11. Santiago, T.R.; Lopes, C.A.; Mizubuti, E.S. Diversidade e Variabilidade de *Ralstonia* spp. In *Estado da Arte em Fitobacterioses Tropicais*; EDFRPE-Editora Universitária da UFRPE: Recife, Pernambuco, Brasil, 2016; pp. 243–256.
12. Albuquerque, G.M.R.; Santos, L.A.; Felix, K.C.S.; Rollemberg, C.L.; Silva, A.M.F.; Souza, E.B.; Cellier, G.; Prior, P.; Mariano, R.L.R. Moko Disease-Causing Strains of *Ralstonia solanacearum* from Brazil Extend Known Diversity in Paraphyletic Phylotype II. *Phytopathology®* **2014**, *104*, 1175–1182. [CrossRef] [PubMed]
13. Sabbagh, C.R.R.; Carrere, S.; Lonjon, F.; Vailleau, F.; Macho, A.P.; Genin, S.; Peeters, N. Pangenomic type III effector database of the plant pathogenic *Ralstonia* spp. *PeerJ* **2019**, *7*, e7346. [CrossRef] [PubMed]
14. Chen, L.; Dahal, A.; Zhang, Y.; Rokunuzzaman, M.; Kiba, A.; Hikichi, Y.; Ohnishi, K. Involvement of avirulence genes avrA and popP1 of Japanese *Ralstonia solanacearum* strains in the pathogenicity to tobacco. *Physiol. Mol. Plant Pathol.* **2018**, *102*, 154–162. [CrossRef]
15. Bocsanczy, A.M.; Bonants, P.; van der Wolf, J.; Bergsma-Vlami, M.; Norman, D.J. Identification of candidate type 3 effectors that determine host specificity associated with emerging *Ralstonia pseudosolanacearum* strains. *Eur. J. Plant Pathol.* **2022**, *163*, 35–50. [CrossRef]
16. Chen, L.; Lei, N.; Kiba, A.; Hikichi, Y.; Ohnishi, K. Contribution of RipS type III effector family of *Ralstonia solanacearum* Japanese strain OE1-1 to disease development in eggplant. *J. Gen. Plant Pathol.* **2021**, *87*, 77–82. [CrossRef]
17. Moon, H.; Pandey, A.; Yoon, H.; Choi, S.; Jeon, H.; Prokchorchik, M.; Jung, G.; Witek, K.; Valls, M.; McCann, H.C.; et al. Identification of RipAZ1 as an avirulence determinant of *Ralstonia solanacearum* in *Solanum americanum* . *Mol. Plant Pathol.* **2021**, *22*, 317–333. [CrossRef]
18. Landry, D.; González-Fuente, M.; Deslandes, L.; Peeters, N. The large, diverse, and robust arsenal of *Ralstonia solanacearum* type III effectors and their in planta functions. *Mol. Plant Pathol.* **2020**, *21*, 1377–1388. [CrossRef]
19. Bioinformatics, B. *FastQC: A Quality Control Tool for High Throughput Sequence Data*; Babraham Institute: Cambridge, UK, 2011.
20. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.M.; Nikolenko, S.I.; Pham, S.; Prjibelski, A.D.; et al. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *J. Comput. Biol.* **2012**, *19*, 455–477. [CrossRef]
21. Simão, F.A.; Waterhouse, R.M.; Ioannidis, P.; Kriventseva, E.V.; Zdobnov, E.M. BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **2015**, *31*, 3210–3212. [CrossRef]
22. Gurevich, A.; Saveliev, V.; Vyahhi, N.; Tesler, G. QUAST: Quality assessment tool for genome assemblies. *Bioinformatics* **2013**, *29*, 1072–1075. [CrossRef]
23. Seemann, T. Prokka: Rapid prokaryotic genome annotation. *Bioinformatics* **2014**, *30*, 2068–2069. [CrossRef] [PubMed]
24. Rodrigues, D.L.; Ariute, J.C; Rodrigues, F.; Benko-Iseppon, A.; Barh, D.; Azevedo, V.; Aburjaile F. PanViTa-Pan Virulence and resisTance Analysis. *Front. Bioinform.* **2023**, *3*, 1–4. Available online: https://github.com/dlnrodrigues/panvita (accessed on 26 July 2020). [CrossRef] [PubMed]
25. Liu, B.; Zheng, D.; Zhou, S.; Chen, L.; Yang, J. VFDB 2022: A general classification scheme for bacterial virulence factors. *Nucleic Acids Res.* **2022**, *50*, D912–D917. [CrossRef] [PubMed]

26. Pal, C.; Bengtsson-Palme, J.; Rensing, C.; Kristiansson, E.; Larsson, D.J. BacMet: Antibacterial biocide and metal resistance genes database. *Nucleic Acids Res.* **2014**, *42*, D737–D743. [CrossRef]
27. Peeters, N.; Carrère, S.; Anisimova, M.; Plener, L.; Cazalé, A.C.; Genin, S. Repertoire, unified nomenclature and evolution of the Type III effector gene set in the *Ralstonia solanacearum* species complex. *BMC Genom.* **2013**, *14*, 859. [CrossRef]
28. Ailloud, F.; Lowe, T.; Cellier, G.; Roche, D.; Allen, C.; Prior, P. Comparative genomic analysis of *Ralstonia solanacearum* reveals candidate genes for host specificity. *BMC Genom.* **2015**, *16*, 270. [CrossRef]
29. Page, A.J.; Cummins, C.A.; Hunt, M.; Wong, V.K.; Reuter, S.; Holden, M.T.; Fookes, M.; Falush, D.; Keane, J.A.; Parkhill, J. Roary: Rapid large-scale prokaryote pan genome analysis. *Bioinformatics* **2015**, *31*, 3691–3693. [CrossRef]
30. Hadfield, J.; Croucher, N.J.; Goater, R.J.; Abudahab, K.; Aanensen, D.M.; Harris, S.R. Phandango: An interactive viewer for bacterial population genomics. *Bioinformatics* **2018**, *34*, 292–293. [CrossRef]
31. Emms, D.M.; Kelly, S. OrthoFinder: Phylogenetic orthology inference for comparative genomics. *Genome Biol.* **2019**, *20*, 238. [CrossRef]
32. Letunic, I.; Bork, P. Interactive Tree Of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* **2021**, *49*, W293–W296. [CrossRef]
33. Costa, S.S.; Guimarães, L.C.; Silva, A.; Soares, S.C.; Baraúna, R.A. First Steps in the Analysis of Prokaryotic Pan-Genomes. *Bioinform. Biol. Insights* **2020**, *14*, 1177932220938064. [CrossRef] [PubMed]
34. Tettelin, H.; Riley, D.; Cattuto, C.; Medini, D. Comparative genomics: The bacterial pan-genome. *Curr. Opin. Microbiol.* **2008**, *11*, 472–477. [CrossRef] [PubMed]
35. Pritchard, L.; Glover, R.H.; Humphris, S.; Elphinstone, J.G.; Toth, I.K. Genomics and taxonomy in diagnostics for food security: Soft-rotting enterobacterial plant pathogens. *Anal. Methods* **2016**, *8*, 12–24. [CrossRef]
36. Meier-Kolthoff, J.P.; Carbasse, J.S.; Peinado-Olarte, R.L.; Göker, M. TYGS and LPSN: A database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Res.* **2022**, *50*, D801–D807. [CrossRef]
37. Meier-Kolthoff, J.P.; Auch, A.F.; Klenk, H.P.; Göker, M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinform.* **2013**, *14*, 60. [CrossRef]
38. Meier-Kolthoff, J.P.; Hahnke, R.L.; Petersen, J.; Scheuner, C.; Michael, V.; Fiebig, A.; Rohde, C.; Rohde, M.; Fartmann, B.; Goodwin, L.A.; et al. Complete genome sequence of DSM 30083T, the type strain (U5/41T) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. *Stand. Genom. Sci.* **2014**, *9*, 2. [CrossRef]
39. Cellier, G.; Remenant, B.; Chiroleu, F.; Lefeuvre, P.; Prior, P. Phylogeny and Population Structure of Brown Rot- and Moko Disease-Causing Strains of Ralstonia solanacearum Phylotype II. *Appl. Environ. Microbiol.* **2012**, *78*, 2367–2375. [CrossRef]
40. Paudel, S.; Dobhal, S.; Alvarez, A.M.; Arif, M. Taxonomy and Phylogenetic Research on *Ralstonia solanacearum* Species Complex: A Complex Pathogen with Extraordinary Economic Consequences. *Pathogens* **2020**, *9*, 886. [CrossRef]
41. Sharma, P.; Johnson, M.A.; Mazloom, R.; Allen, C.; Heath, L.S.; Lowe-Power, T.M.; Vinatzer, B.A. Meta-analysis of the *Ralstonia solanacearum* species complex (RSSC) based on comparative evolutionary genomics and reverse ecology. *Microb. Genom.* **2022**, *8*, 000791. [CrossRef]
42. Geng, R.; Cheng, L.; Cao, C.; Liu, Z.; Liu, D.; Xiao, Z.; Wu, X.; Huang, Z.; Feng, Q.; Luo, C.; et al. Comprehensive Analysis Reveals the Genetic and Pathogenic Diversity of *Ralstonia solanacearum* Species Complex and Benefits Its Taxonomic Classification. *Front. Microbiol.* **2022**, *13*. [CrossRef]
43. Mergeay, M.; Monchy, S.; Vallaeys, T.; Auquier, V.; Benotmane, A.; Bertin, P.; Taghavi, S.; Dunn, J.; van der Lelie, D.; Wattiez, R. *Ralstonia metallidurans* , a bacterium specifically adapted to toxic metals: Towards a catalogue of metal-responsive genes. *FEMS Microbiol. Rev.* **2003**, *27*, 385–410. [CrossRef] [PubMed]
44. Pugazhendhi, A.; Boovaragamoorthy, G.M.; Ranganathan, K.; Naushad, M.; Kaliannan, T. New insight into effective biosorption of lead from aqueous solution using *Ralstonia solanacearum*: Characterization and mechanism studies. *J. Clean. Prod.* **2018**, *174*, 1234–1239. [CrossRef]
45. Dang, F.; Lin, J.; Chen, Y.; Li, G.X.; Guan, D.; Zheng, S.J.; He, S. A feedback loop between *CaWRKY41* and H_2O_2 coordinates the response to *Ralstonia solanacearum* and excess cadmium in pepper. *J. Exp. Bot.* **2019**, *70*, 1581–1595. [CrossRef] [PubMed]
46. Sarowar, S.; Kim, Y.J.; Kim, E.N.; Kim, K.D.; Hwang, B.K.; Islam, R.; Shin, J.S. Overexpression of a pepper basic pathogenesis-related protein 1 gene in tobacco plants enhances resistance to heavy metal and pathogen stresses. *Plant Cell Rep.* **2005**, *24*, 216–224. [CrossRef] [PubMed]
47. Wicker, E.; Lefeuvre, P.; de Cambiaire, J.C.; Lemaire, C.; Poussier, S.; Prior, P. Contrasting recombination patterns and demographic histories of the plant pathogen *Ralstonia solanacearum* inferred from MLSA. *ISME J.* **2012**, *6*, 961–974. [CrossRef] [PubMed]
48. Santiago, T.R.; Lopes, C.A.; Caetano-Anollés, G.; Mizubuti, E.S.G. Genetic Structure of *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum* in Brazil. *Plant Dis.* **2020**, *104*, 1019–1025. [CrossRef]
49. de Pedro-Jové, R.; Puigvert, M.; Sebastià, P.; Macho, A.P.; Monteiro, J.S.; Coll, N.S.; Setúbal, J.C.; Valls, M. Dynamic expression of *Ralstonia solanacearum* virulence factors and metabolism-controlling genes during plant infection. *BMC Genom.* **2021**, *22*, 170. [CrossRef]
50. Ghosh, S.; O'Connor, T.J. Beyond Paralogs: The Multiple Layers of Redundancy in Bacterial Pathogenesis. *Front. Cell. Infect. Microbiol.* **2017**, *7*, 467. [CrossRef]
51. Nakano, M.; Mukaihara, T. Comprehensive Identification of PTI Suppressors in Type III Effector Repertoire Reveals that *Ralstonia solanacearum* Activates Jasmonate Signaling at Two Different Steps. *Int. J. Mol. Sci.* **2019**, *20*, 5992. [CrossRef]

52. Solé, M.; Popa, C.; Mith, O.; Sohn, K.H.; Jones, J.D.G.; Deslandes, L.; Valls, M. The awr Gene Family Encodes a Novel Class of *Ralstonia solanacearum* Type III Effectors Displaying Virulence and Avirulence Activities. *Mol.-Plant-Microbe Interact.* **2012**, *25*, 941–953. [CrossRef]
53. Cho, H.; Song, E.S.; Heu, S.; Baek, J.; Lee, Y.K.; Lee, S.; Lee, S.W.; Park, D.S.; Lee, T.H.; Kim, J.G.; et al. Prediction of Host-Specific Genes by Pan-Genome Analyses of the Korean *Ralstonia solanacearum* Species Complex. *Front. Microbiol.* **2019**, *10*, 506. [CrossRef] [PubMed]
54. Ailloud, F.; Lowe, T.M.; Robène, I.; Cruveiller, S.; Allen, C.; Prior, P. *In planta* comparative transcriptomics of host-adapted strains of *Ralstonia solanacearum*. *PeerJ* **2016**, *4*, e1549. [CrossRef] [PubMed]
55. Cellier, G.; Prior, P. Deciphering phenotypic diversity of *Ralstonia solanacearum* strains pathogenic to potato. *Phytopathology* **2010**, *100*, 1250–1261. [CrossRef] [PubMed]
56. Ramírez, M.; Moncada, R.N.; Villegas-Escobar, V.; Jackson, R.W.; Ramírez, C.A. Phylogenetic and pathogenic variability of strains of *Ralstonia solanacearum* causing moko disease in Colombia. *Plant Pathol.* **2020**, *69*, 360–369. [CrossRef]

Capítulo II – Enriquecimento Funcional e Redes de Interação entre T3SS e Rips de *R. solanacearum* causadoras de Moko-da-bananeira e Murcha bacteriana em tomateiro.

1. Introdução

O aparato do sistema de secreção bacteriano do tipo III, codificado majoritariamente por proteínas dos clusters *hrp* e *hrc*, exige uma complexa organização espaço-temporal por ser imprescindível no processo de patogenicidade no hospedeiro vegetal. Tal regulação depende tanto de estímulos do microambiente vegetal quanto de proteínas regulatórias. Fitopatógenos do gênero *Ralstonia* têm sua regulação gênica pautada na proteína HrpB, um regulador transcricional do tipo AraC que induz não apenas a expressão gênica do T3SS e seus efetores, mas também de pelo menos outros três operons gênicos relacionados à virulência da bactéria (CUNNAC; BOUCHER; GENIN, 2004; GENIN et al., 1992).

As interações proteína-proteína constituem uma peça-chave ao se tentar compreender o contexto em que determinado organismo está inserido, sobretudo os de patogenicidade. Por meio desse tipo de análise, é possível predizer qual a função de uma determinada proteína ainda não conhecida e também prever seu nível de participação com o enriquecimento de dados de transcriptômica, por exemplo (RAO et al., 2014).

Diante do exposto, os objetivos deste capítulo foram (i) enriquecer a anotação dos novos genomas sequenciados de *R. solanacearum*; (ii) predizer as interações entre proteínas de grande relevância para a patogenicidade de *R. solanacearum*, a fim de minerar efetores de virulência que atuam em conjunto num contexto de infecção.

2. Metodologia

Foram utilizados 120 genomas, sendo 2 de *R. pseudosolanacearum*, e 118 de *R. solanacearum*, dos quais 14 são novos genomas montados, para enriquecimento funcional utilizando o eggNOG-MAPPER (CANTALAPIEDRA et al., 2021), bem como anotação com base no banco de dados do KEGG (Kyoto Encyclopedia of Genes and Genomes) (KANEHISA, 2004). Para a visualização da via de síntese do sistema de secreção tipo III, foi utilizado o KEGG Mapper Color (KANEHISA; SATO; KAWASHIMA, 2022). Para a reconstrução da interação entre efetores do tipo III, reguladores e o T3SS, foram feitas redes proteína-proteína de interações não-direcionadas no STRING (SZKLARCZYK et al., 2021) utilizando as sequências de aminoácidos de Rips preditas previamente, as sequências de reguladores AraC e

proteínas Hrc/Hrp preditas pelo eggNOG-MAPPER, considerando apenas o respectivo *match* de maior identidade e *bitscore*.

3. Resultados

Após enriquecer as anotações com os bancos de dados do KEGG, foi possível verificar que todos os novos isolados sequenciados apresentavam os genes presentes na via de referência de *R. solanacearum* associados ao T3SS (rso03070). Contudo, o gene *flr*A – que compõe o flagelo da espécie atrelado ao T3SS (rso02040) – não estava presente em nenhuma das amostras. Também não foram encontrados genes referentes à agulha do T3SS (K03221, K04056, K04057 e K04059). No que diz respeito às interações registradas entre as Rips, proteínas estruturais e reguladores transcricionais do T3SS o acesso *R. solanacearum* G1000 apresentou maior similaridade (*matches*) considerando-se a base de dados do STRING, seguido por *R. solanacearum* PSI07. A diferença entre sequências de *input* e *matches* encontrados para cada interação corresponde a sequências de Rips, especialmente T3Es hipotéticos. O isolado CCRMRs91 foi que teve o maior grau de interação média por nó (8,3), enquanto o B4 foi o que possui o menor grau (6,04). O isolado B106 foi o que apresentou o maior número nós (87) e interações (336) presentes na via. Os valores de sequências utilizadas como *input*, número de matches no organismo modelo, nós e interações para cada isolado pode ser encontrado na Tabela 1. De maneira geral, a maioria das interações foi apoiada por mineração de texto, co-ocorrência e proximidade gênica. O regulador *hrp*B possuía interações com todos os genes dos grupos *hrc/hrp*, com exceção do regulador *hrc*A. Entretanto, não eram constatadas interações entre muitas das Rips, mesmo para aquelas que já possuíam estrutura predita no banco de dados, como a RipTAL do isolado B106 (RSp0304). Os reguladores com domínios AraC, incluindo o gene *ada*, possuíam apenas interações entre si.

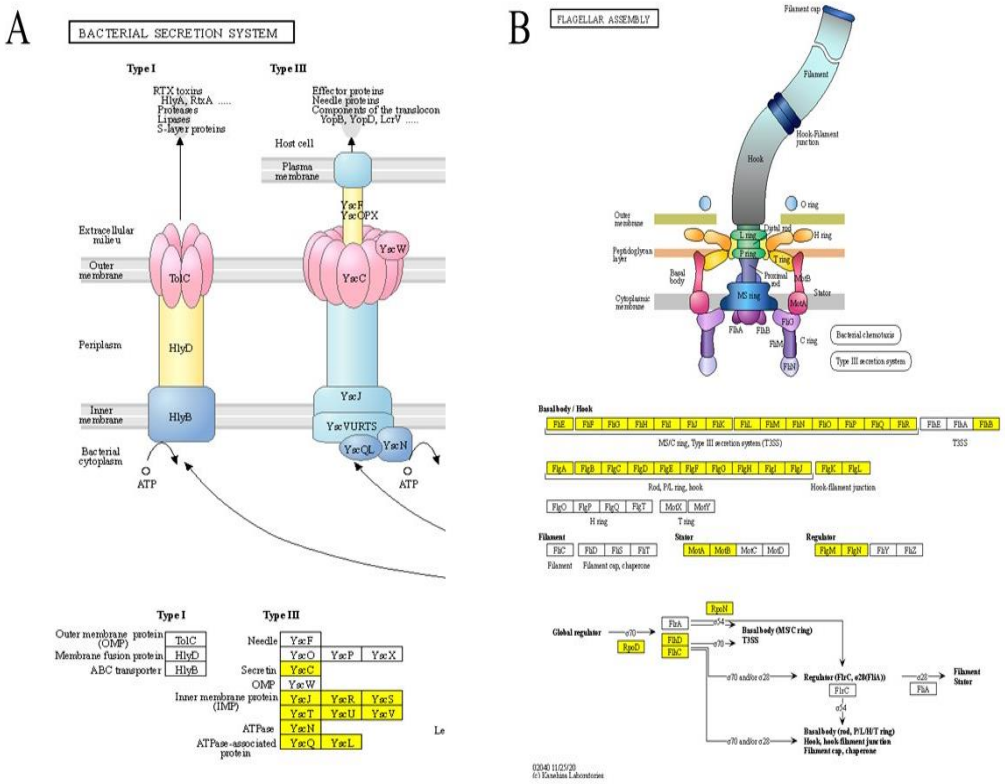

Figura 2. Genes estruturais que compõem o sistema de secreção tipo III nos novos isolados sequenciados.

Tabela 1. Número de nós e interações obtidos para os respectivos isolados com base no organismo modelo determinado.

ISOLATE	INPUT	MODEL	MATCHES	NODES	EDGES	AVERAGE NODE DEGREE
CCRMRs91	98	*R. solanacearum* GM1000	83	80	332	8.3
CCRMRs314	93	-	82	78	310	7.95
CCRMRs339	100	-	86	84	330	7.86
CCRMRs302	91	-	81	76	295	7.76
B106	105	-	90	87	336	7.72
B75	101	-	87	82	313	7.63
CCRMRs317	101	-	85	82	313	7.63
CCRMRs294	102	-	86	83	314	7.57
CCRMRs279	92	-	81	77	286	7.46
CCRMRs223	97	-	82	78	285	7.31
CCRMRs286	101	-	85	82	297	7.24
CCRMRs283	100	-	84	81	289	7.14
CCRMRs121	92	-	78	76	234	6.16
B4	89	-	73	70	211	6.03

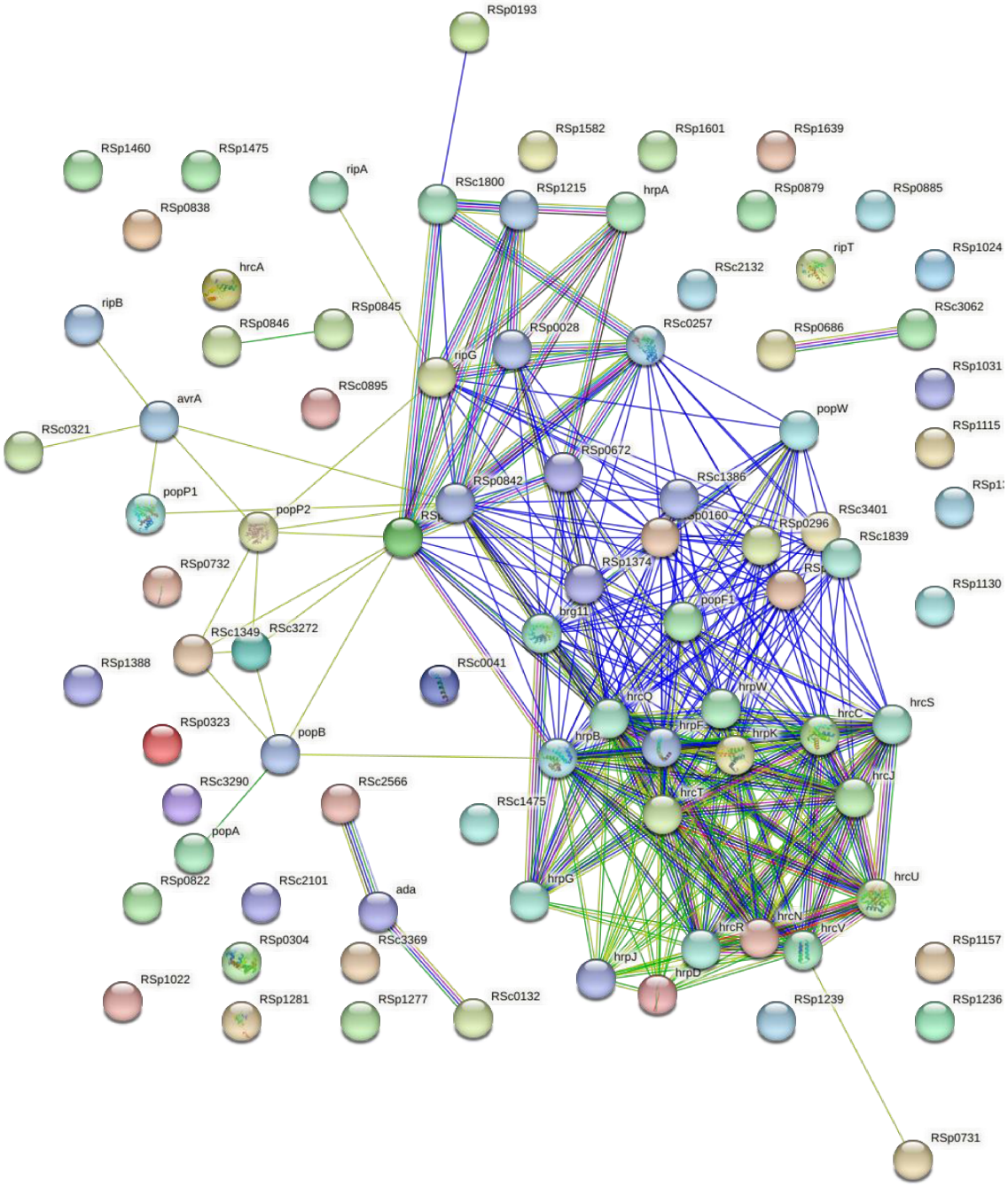

Figura 3. Rede de interação entre proteínas Hrp, Hrc e Rips do isolado B106. As interações em verde representam genes vizinhos, em azul co-ocorrência gênica, em amarelo evidência por mineração de texto, e em roxa evidência experimental.

6. DISCUSSÃO

A taxogenômica vem sendo adotada como solução para espécies bacterianas que possuem classificação taxonômica controversa (ARAHAL, 2014; CIUFO et al., 2018). Em especial, a identidade média de nucleotídeos (ANI) vem sido utilizada para elucidar melhor as relações taxonômicas existentes em outros fitopatógenos bacterianos além do RSSC, como *Erwinia*, *Dickeya*, *Pectobacterium*, *Xanthomonas*, *Xyllela* e *Pseudomonas* (CONSTANTIN et al., 2016; LU et al., 2022; MARCELLETTI; SCORTICHINI, 2016; PRITCHARD et al., 2016). Considerando os dados apresentados no Capítulo I com informações congruentes em relação à classificação taxonômica dos novos isolados sequenciados e dos genomas públicos disponíveis no NCBI, a técnica de ANI se revela como tão fidedigna quanto a PCR-Multiplex utilizada em estudos anteriores para classificação de RSSC (PRIOR; FEGAN, 2005; SANTIAGO et al., 2017).

No entanto, a classificação taxonômica por hibridização DNA-DNA *in silico* não se mostrou como uma opção suficientemente sensível para separação de isolados do filotipo IIA e IIB em espécies distintas, como sugerido anteriormente por Zhang e Qiu (2016). A pangenômica também vem auxiliado no desenvolvimento de kits diagnósticos para doenças causadas por fitopatógenos (POWNEY et al., 2011). Assim, visto que foi possível separar os cinco grupos existentes no conjunto de dados deste trabalho, a pangenômica pode auxiliar na mineração de alvos para o desenvolvimento de kits diagnósticos específicos para cada um dos filotipos. Isso é extremamente importante, por exemplo, para isolados pertencentes ao filotipo II, visto que sua diversidade de ecótipos é maior e, portanto, podem afetar mais culturas agrícolas.

O modelo escolhido como melhor opção para a geração de redes de interação foi *R. solanacearum* GM1000, a qual pertence ao filotipo I, diferente de *R. solanacearum* PSI07 que está posicionado no filotipo IV. Nenhum dos dois modelos podem ser considerados da mesma espécie taxonômica considerando-se os novos isolados sequenciados, conforme análises feitas ao longo do Capítulo I. O isolado GM1000 foi considerado, por muito tempo, a “linhagem tipo” de *R. solanacearum* em ensaios de patogenicidade (GONZÁLEZ et al., 2007; VALLS; GENIN; BOUCHER, 2006; VAN GIJSEGEM et al., 1998). Tendo em vista a ampla diversidade de Rips existentes dentro de RSSC e a própria diversidade do complexo, demonstrada por Landry (2020), Sabbagh (2019) e colaboradores, bem como por Safni (2014) e colaboradores, é imprescindível que sejam escolhidos modelos adequeados para estudos futuros com as espécies desse grupo.

A regulação da expressão gênica do T3SS e seus efetores mediante genes *hrp* é bem estudada para fitopatógenos, em especial *Xanthomonas* e *Ralstonia* (CUNNAC; BOUCHER; GENIN, 2004; GÜRLEBECK; THIEME; BONAS, 2006). A ausência de interações na rede entre os efetores preditos e o regulador *hrp*B são indicativos de dados ausentes que consideram novas Rips descritas, seja considerando-se a co-ocorrência em isolados estudados ou ainda o desenho experimental de experimentos visando testar a expressão dos efetores pós silenciamento do *hrp*B. É válido ressaltar que a incapacidade da plataforma em reconhecer alguns efetores também reflete a ausência de sequências depositadas, principalmente para RipG, RipH, RipA e RipS, que possuem numerosas subfamílias, denotando a importância de pesquisas adicionais nesse sentido.

No que tange à predição de efetores tipo III, os efetores hipotéticos correspondem a homólogos de efetores presentes em *Xanthomonas* e *Acidovorax* (PEETERS et al., 2013d). Sabendo que os efetores tipo III regulados por *hrp* necessitam de um motivo de interação específico flanqueado por sequências TTCG (LIPSCOMB; SCHELL, 2011), essa seria uma das maneiras de testar a interação entre os efetores com o *hrp*B. Contudo, *R. solanacearum* ainda é a espécie mais bem estudada de fitopatógeno com efetores do tipo III.

Além da especificidade de hospedeiro e da robustez genética que um amplo repertório de Rips pode conferir aos isolados do RSSC, também é discutido como alguns desses efetores são essenciais ao escape de imunidade em hospedeiros que se tornaram resistentes com interações ao longo de diversas gerações. Por exemplo, foi demonstrado que RipAY e RipAC possuem papel na inibição de reações de hipersensibilidade causadas por RipE1 em *N. benthamiana* e *A. thaliana* por interferirem na via de degradação de glutationa e supressão da imunidade vegetal (SANG et al., 2016, 2020; YU et al., 2020). Contudo, mesmo com esse feito já sendo registrado em artigos científicos publicados, a RipAY do isolado B106 (Rsp1022) aparece sem nenhuma interação na rede mostrada na Figura 3 do Capítulo II. A peculiaridade dos efetores de agirem em conjunto para exercer a patogênese no hospedeiro corrobora com a hipótese apresentada no Capítulo I de que um repertório de Rips contribui mais para a especificidade de hospedeiros do que a presença ou ausência de um determinado efetor no isolado. Considerando-se também a dificuldade de estabelecer variáveis melhoradas geneticamente no Brasil para resistência à murcha bacteriana (LOPES; BOITEUX, 2016), explorar o papel supressor e inibitório dos efetores se revela elucidador tanto para aqueles que são ativadores quanto para desencadeadores de sinais moleculares.

7. CONCLUSÕES GERAIS

O presente estudo mostrou o quão enriquecedora a abordagem bioinformática e a aplicação de métodos *in silico* podem ser, quando aplicadas a dados genômicos, revelando candidatos essenciais para futuras inovações tecnológicas e aplicações agronômicas, com ênfase para a defesa vegetal. As análises por taxogenômica não apenas confirmaram que os novos isolados sequenciados pertencem ao filotipo II do complexo específico (RSSC) de *R. solanacearum*, como também mostraram a diversidade de isolados existentes para o ecótipo Moko das regiões Norte e Nordeste do Brasil. Ademais, o presente estudo trouxe indícios de que atribuir um determinado conjunto de Rips a um ecótipo específico pode ser infundado quando são feitas análises comparativas em larga escala. Em contrapartida, as análises de pangenômica mostraram que, pelo perfil de um pangenoma próximo a se fechar, a descoberta de novas sequências gênicas, incluindo novos efetores do tipo III, tem baixa probabilidade. A análise do secretoma dos isolados utilizados neste trabalho é capaz de prover novas interações de co-ocorrência para o banco de dados via mineração de texto, ajudando a enriquecer informações específicas para *R. solanacearum* em meta-análises no âmbito de outros estudos. Compreender como o secretoma participa em diferentes contextos de infecção deve determinar não apenas quais efetores são responsáveis pela especificidade de hospedeiros, mas também qual deles contribui mais para o processo infeccioso naquele hospedeiro. Por fim, o presente trabalho enriquece o conhecimento a nível molecular dos isolados brasileiros de *R. solanacearum*.

8. PERSPECTIVAS

- Avaliar a contribuição de eventos de transferência horizontal para a dispersão dos efetores do tipo III nas populações de *R. solanacearum* que compartilham ou não o mesmo ecótipo;
- Minerar alvos para criação de kits diagnósticos rápidos para serem utilizados no campo, visando à classificação taxonômica adequada de isolados que acometem as lavouras;
- Realizar ensaios em diferentes hospedeiros para confirmar que a predição do repertório de Rips ajuda a determinar o espectro de hospedeiros de isolados de *R. solanacearum*;
- Desenhar de *primers* para testar via PCR (Reação em Cadeia da Polimerase) a presença de efetores hipotéticos nos isolados de *R. solanacearum*.

REFERÊNCIAS

ALBUQUERQUE, G. M. R. et al. Sequevar distribution of *Ralstonia* spp. in Solanaceae in the semiarid climate of the Pernambuco state, Brazil. **European Journal of Plant Pathology**, v. 159, n. 1, p. 13–25, jan. 2021.

ARAHAL, D. R. Chapter 6 - Whole-Genome Analyses: Average Nucleotide Identity. Em: GOODFELLOW, M.; SUTCLIFFE, I.; CHUN, J. (Eds.). **Methods in Microbiology**. New Approaches to Prokaryotic Systematics. [s.l.] Academic Press, 2014. v. 41p. 103–122.

CANTALAPIEDRA, C. P. et al. eggNOG-mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale. **Molecular Biology and Evolution**, v. 38, n. 12, p. 5825–5829, 9 dez. 2021.

CASTILLO, J. A.; GREENBERG, J. T. Evolutionary Dynamics of *Ralstonia solanacearum*. **Applied and Environmental Microbiology**, v. 73, n. 4, p. 1225–1238, 15 fev. 2007.

CIANCIOTTO, N. P. Type II secretion: a protein secretion system for all seasons. **Trends in Microbiology**, v. 13, n. 12, p. 581–588, dez. 2005.

CIUFO, S. et al. Using average nucleotide identity to improve taxonomic assignments in prokaryotic genomes at the NCBI. **International Journal of Systematic and Evolutionary Microbiology**, v. 68, n. 7, p. 2386–2392, jul. 2018.

COLLMER, A.; BAUER, D. W. *Erwinia chrysanthemi* and *Pseudomonas syringae* : Plant Pathogens Trafficking in Extracellular Virulence Proteins. Em: **Bacterial Pathogenesis of Plants and Animals**. Current Topics in Microbiology and Immunology. Berlin, Heidelberg: Springer Berlin Heidelberg, 1994. v. 192p. 43–79.

CONSTANTIN, E. C. et al. Genetic characterization of strains named as *Xanthomonas axonopodis* pv. *dieffenbachiae* leads to a taxonomic revision of the *X. axonopodis* species complex. **Plant Pathology**, v. 65, n. 5, p. 792–806, jun. 2016.

COSTA, K. D. DA S. et al. Genetic control of tomato resistance to *Ralstonia solanacearum* . **Euphytica**, v. 215, n. 7, p. 136, jul. 2019.

CUNNAC, S.; BOUCHER, C.; GENIN, S. Characterization of the cis-Acting Regulatory Element Controlling HrpB-Mediated Activation of the Type III Secretion System and Effector Genes in *Ralstonia solanacearum* . **Journal of Bacteriology**, v. 186, n. 8, p. 2309–2318, 15 abr. 2004.

DANGL, J. L. The Enigmatic Avirulence Genes of Phytopathogenic Bacteria. Em: DANGL, J. L. (Ed.). **Bacterial Pathogenesis of Plants and Animals**. Current Topics in Microbiology and Immunology. Berlin, Heidelberg: Springer Berlin Heidelberg, 1994. v. 192p. 99–118.

GAMA, M. A. S. et al. Genética da interação bactéria-planta. Em: GUIMARÃES, L. M. P. et al. (Eds.). **Estado da Arte em Fitobacterioses Tropicais**. Recife, Pernambuco, Brasil: EDFRPE - Editora Universitária da UFRPE, 2016. p. 43–64.

GENIN, S. et al. Evidence that the hrpB gene encodes a positive regulator of pathogenicity genes from Pseudomonas solanacearum. **Molecular Microbiology**, v. 6, n. 20, p. 3065–3076, 1992.

GENIN, S.; BOUCHER, C. LESSONS LEARNED FROM THE GENOME ANALYSIS OF *RALSTONIA SOLANACEARUM*. **Annual Review of Phytopathology**, v. 42, n. 1, p. 107–134, set. 2004.

GENIN, S.; DENNY, T. P. Pathogenomics of the *Ralstonia solanacearum* Species Complex. **Annual Review of Phytopathology**, v. 50, n. 1, p. 67–89, 8 set. 2012.

GONZÁLEZ, E. T. et al. Using the *Ralstonia solanacearum* Tat Secretome To Identify Bacterial Wilt Virulence Factors. **Applied and Environmental Microbiology**, v. 73, n. 12, p. 3779–3786, 15 jun. 2007.

GÜRLEBECK, D.; THIEME, F.; BONAS, U. Type III effector proteins from the plant pathogen *Xanthomonas* and their role in the interaction with the host plant. **Journal of Plant Physiology**, v. 163, n. 3, p. 233–255, 10 fev. 2006.

KANEHISA, M. The KEGG resource for deciphering the genome. **Nucleic Acids Research**, v. 32, n. 90001, p. 277D – 280, jan. 2004.

KANEHISA, M.; SATO, Y.; KAWASHIMA, M. KEGG mapping tools for uncovering hidden features in biological data. **Protein Science**, v. 31, n. 1, p. 47–53, 2022.

LINDGREN, P. B. THE ROLE OF *hrp* GENES DURING PLANT-BACTERIAL INTERACTIONS. **Annual Review of Phytopathology**, v. 35, n. 1, p. 129–152, set. 1997.

LIPSCOMB, L.; SCHELL, M. A. Elucidation of the Regulon and cis-Acting Regulatory Element of HrpB, the AraC-Type Regulator of a Plant Pathogen-Like Type III Secretion System in Burkholderia pseudomallei. **Journal of Bacteriology**, v. 193, n. 8, p. 1991–2001, 15 abr. 2011.

LOPES, C. A.; BOITEUX, L. S. Melhoramento Genético Visando Resistência à Murcha Bacteriana. Em: GAMA, M. A. S. et al. (Eds.). **Estado da Arte em Fitobacterioses Tropicais**. Recife, Pernambuco, Brasil: EDFRPE - Editora Universitária da UFRPE, 2016. p. 257–274.

LOPES, C. A.; ROSSATO, M. History and Status of Selected Hosts of the *Ralstonia solanacearum* Species Complex Causing Bacterial Wilt in Brazil. **Frontiers in Microbiology**, v. 9, p. 1228, 13 jun. 2018.

LU, C.-H. et al. *Pseudomonas lijiangensis* sp. nov., a novel phytopathogenic bacterium isolated from black spots of tobacco. **International Journal of Systematic and Evolutionary Microbiology**, v. 72, n. 10, 25 out. 2022.

MAGALHÃES, O. *Phytomonas solanaceara* (Erw. Smith 1896) Bergey et al. 1930. **Rev. Med.**, v. 40, p. 223–226, 1932.

MARCELLETTI, S.; SCORTICHINI, M. Genome-wide comparison and taxonomic relatedness of multiple *Xylella fastidiosa* strains reveal the occurrence of three subspecies and a new *Xylella* species. **Archives of Microbiology**, v. 198, n. 8, p. 803–812, 1 out. 2016.

MORRIS, C. E.; MONIER, J.-M. THE ECOLOGICAL SIGNIFICANCE OF BIOFILM FORMATION BY PLANT-ASSOCIATED BACTERIA. **Annual Review of Phytopathology**, v. 41, n. 1, p. 429–453, set. 2003.

PEETERS, N. et al. Repertoire, unified nomenclature and evolution of the Type III effector gene set in the *Ralstonia solanacearum* species complex. **BMC Genomics**, v. 14, n. 1, p. 859, 6 dez. 2013.

POWNEY, R. et al. An analysis of the *Erwinia amylovora* pan-genome identifies novel chromosomal targets for molecular diagnostics. **Acta Horticulturae**, n. 896, p. 45–50, maio 2011.

PRASANNATH, K. Pathogenicity and Virulence Factors of Phytobacteria. **Scholars Academic Journal of Biosciences (SAJB)**, v. 1, n. 1, p. 24–33, 2013.

PRIOR, P.; FEGAN, M. Recent developments in the phylogeny and classification of *Ralstonia solanacearum* . **Acta Horticulturae**, n. 695, p. 127–136, nov. 2005.

PRITCHARD, L. et al. Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. **Analytical Methods**, v. 8, n. 1, p. 12–24, 2016.

RAO, V. S. et al. Protein-Protein Interaction Detection: Methods and Analysis. **International Journal of Proteomics**, v. 2014, p. e147648, 17 fev. 2014.

ROUX, Brice et al. Genomics and transcriptomics of *Xanthomonas campestris* species challenge the concept of core type III effectome. **BMC genomics**, v. 16, n. 1, p. 1-19, 2015.

SAFNI, I. et al. 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. solanacearum* phylotype IV strains as *Ralstonia syzygii* subsp. *indonesiensis* subsp. nov., banana blood disease bacterium strains as *Ralstonia syzygii* subsp. *celebesensis* subsp. nov. and *R. solanacearum* phylotype I and III strains as *Ralstonia pseudosolanacearum* sp. nov. **International Journal of Systematic and Evolutionary Microbiology**, v. 64, n. Pt_9, p. 3087–3103, 1 set. 2014.

SANG, Y. et al. The *Ralstonia solanacearum* type III effector RipAY targets plant redox regulators to suppress immune responses. **Molecular Plant Pathology**, v. 19, n. 1, p. 129–142, 27 dez. 2016.

SANG, Y. et al. Intra-strain Elicitation and Suppression of Plant Immunity by *Ralstonia solanacearum* Type-III Effectors in *Nicotiana benthamiana* . **Plant Communications**, Special Issue on Plant-Pathogen Interactions (Organizing Editors: Paul Birch, Savithramma Dinesh-Kumar, Hui-Shan Guo, Ping He, Xin Li, Frank Takken, Yuanchao Wang). v. 1, n. 4, p. 100025, 13 jul. 2020.

SANTIAGO, T. R. et al. Phylotype and sequevar variability of *Ralstonia solanacearum* in Brazil, an ancient centre of diversity of the pathogen. **Plant Pathology**, v. 66, n. 3, p. 383–392, abr. 2017.

SANTIAGO, T. R.; LOPES, C. A.; MIZUBUTI, E. S. Diversidade e Variabilidade de *Ralstonia* spp. Em: GAMA, M. A. S. et al. (Eds.). **Estado da Arte em Fitobacterioses Tropicais**. Recife, Pernambuco, Brasil: EDFRPE - Editora Universitária da UFRPE, 2016. p. 243–256.

SZKLARCZYK, D. et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. **Nucleic Acids Research**, v. 49, n. D1, p. D605–D612, 8 jan. 2021.

TANG, X.; XIAO, Y.; ZHOU, J.-M. Regulation of the Type III Secretion System in Phytopathogenic Bacteria. **Molecular Plant-Microbe Interactions®**, v. 19, n. 11, p. 1159–1166, nov. 2006.

VALLS, M.; GENIN, S.; BOUCHER, C. Integrated Regulation of the Type III Secretion System and Other Virulence Determinants in *Ralstonia solanacearum*. **PLoS Pathogens**, v. 2, n. 8, p. e82, 25 ago. 2006.

VAN GIJSEGEM, F. et al. The *Ralstonia solanacearum* hrp Gene Region: Role of the Encoded Proteins in Interactions with Plants and Regulation of Gene Expression. Em: PRIOR, P.; ALLEN, C.; ELPHINSTONE, J. (Eds.). **Bacterial Wilt Disease: Molecular and Ecological Aspects**. Berlin, Heidelberg: Springer, 1998. p. 178–183.

VON PARSEVAL, M. **Uma doenca do fumo e da batata ingleza no municipio de Santa Cruz neste Estado causado pelo *Bacterium solanacearum* .** Porto Alegre: Instituto Borges de Medeiros, 1922.

WICKER, E. et al. Contrasting recombination patterns and demographic histories of the plant pathogen *Ralstonia solanacearum* inferred from MLSA. **The ISME Journal**, v. 6, n. 5, p. 961–974, maio 2012.

YU, G. et al. A bacterial effector protein prevents MAPK-mediated phosphorylation of SGT1 to suppress plant immunity. **PLOS Pathogens**, v. 16, n. 9, p. e1008933, 25 set. 2020.

ZHANG, Y.; QIU, S. Phylogenomic analysis of the genus *Ralstonia* based on 686 single-copy genes. **Antonie van Leeuwenhoek**, v. 109, n. 1, p. 71–82, jan. 2016.

ANEXOS

Ao decorrer do mestrado, foram desenvolvidas atividades acadêmicas diversas, as quais estão citadas no texto a seguir referente ao currículo *lattes*.

- Um (1) artigo publicado como primeiro autor:

- **Ariute, J. C**., Rodrigues, D. L. N., Soares, S. D. C., Azevedo, V., Benko-Iseppon, A. M., & Aburjaile, F. F. (2022). Comparative Genomic Analysis of Phytopathogenic *Xanthomonas* Species Suggests High Level of Genome Plasticity Related to Virulence and Host Adaptation. *Bacteria*, *1*(4), 218-241. Disponível em: https://doi.org/10.3390/bacteria1040017

- Dois (2) artigos submetidos, sendo um (1) como primeiro autor:

- **ARIUTE, J.** C.; FELICE, A. G. ; SOARES, S.; DA GAMA, M. A. S.; SOUZA, E. B.; AZEVEDO, V.; BRENIG, B.; ABURJAILE, F.; BENKO-ISEPPON, A. M. Characterization and association of Rips repertoire to host range of novel *Ralstonia solanacearum* strains by in silico approaches. *Microorganisms* 2023. Disponível em: https://doi.org/10.3390/microorganisms11040954

- Diego Lucas Neres Rodrigues, **Juan Carlos Ariute**, Francielly Morais Rodrigues, Ana Maria Benko-Iseppon, Debmalya Barh, Vasco Ariston De Carvalho Azevedo and Flavia Figueira Aburjaile*. PanViTa: Pan Virulence and resisTance Analysis. Front. Bioinform. - *Integrative Bioinformatics* 2023. Disponível em: https://doi.org/10.3389/fbinf.2023.1070406

- Um (1) capítulo de livro publicado como co-autor:

- ABURJAILE, F. F. ; FERREIRA NETO, J. R. ; AZEVEDO, T. ; **ARIUTE, J. C.** ; BARBOZA, J. ; LANE, R. ; PANDOLFI, V. ; BENKO-ISEPPON, A. M. . Impact of endosymbionts on antimicrobial properties of medicinal plants: a review. In: Mahendra Rai; Christiane Mendes Feitosa. (Org.). Eco-Friendly Biobased Products Used in Microbial Diseases. 1ed.Boca Raton, FL, 33487-2742: CRC Press, Taylor & Francis Group, 2022, p. 296-320.

- Um (1) capítulo de livro submetido como primeiro autor:

- Juan Carlos Ariute Oliveira, Lucas Gabriel Rodrigues Gomes, Arun Kumar Jaiswal, Sandeep Tiwari, Vasco Azevedo, Ana Maria Benko Iseppon, and Flávia Figueira Aburjaile. The Secretome Landscape of *Ralstonia.* Submetido em 2022 a edição especial Microbial Genetics (CRC Press – Taylor & Francis Group).

- Quatro (4) trabalhos apresentados em eventos científicos:

- **OLIVEIRA, J. C. A.**; ABURJAILE, F. F. ; SOUZA, E. B. ; AZEVEDO, V. ; GAMA, M. A. S. ; BENKO-ISEPPON, A. M. . COMPARATIVE GENOMICS OF RALSTONIA SOLANACEARUM ISOLATES FROM PERNAMBUCO, BRAZIL. In: 66th Brazilian Congress of Genetics - Genética 2021, 2021. GENÉTICA 2021 - 66 Brazilian Congress of Genetics, 2021. p. 1-828.
- **OLIVEIRA, J. C. A.**; GAMA, M. A. S. ; SOUZA, E. B. ; AZEVEDO, V. ; BRENIG, B. ; ABURJAILE, F. ; BENKO-ISEPPON, A. M. . PANGENOME APPROACHES REVEAL NEW INSIGHTS OF THE BRAZILIAN ISOLATES FROM RALSTONIA SOLANACEARUM SPECIES COMPLEX (RSSC). In: 67th Brazilian Congress of

Genetics - Genética 2022, 2022, Natal. GENÉTICA 2022 - 67th Brazilian Congress of Genetics, 2022.

- **OLIVEIRA, J. C. A.**; GAMA, M. A. S. ; SOUZA, E. B. ; AZEVEDO, V. ; BRENIG, B. ; ABURJAILE, F. ; BENKO-ISEPPON, A. M.. MÉTODOS *IN SILICO* PARA CLASSIFICAÇÃO ACURADA DE ISOLADOS DO COMPLEXO DE ESPÉCIES *R. SOLANACEARUM* (RSCC). IX Simpósio de Microbiologia da UFMG, 2022.
- DINIZ, C. A. A. ; **ARIUTE, J. C.** ; CARNEIRO, D. V. D. ; GAMA, M. ; SOUZA, E. ; BRENIG, B. ; AZEVEDO, V. ; BENKO-ISEPPON, A. M. ; ABURJAILE, F. . GENOMIC CHARACTERIZATION OF CITROBACTER FREUNDII ISOLATED FROM LEGUMINOUS BACTERIAL CANKER IN BRAZIL. In: 67th Brazilian Congress of Genetics - Genética 2022, 2022, Natal. GENÉTICA 2022 - 67th Brazilian Congress of Genetics, 2022.

➢ Participação como ouvinte em seis (6) eventos científicos:

- 3rd International Associated Laboratoires Meeting - LIA 2022 BACT-INFLAM: CLOSING SEMINARS. 2022. (Outra).
- 67th Brazilian Congress of Genetics 2022. (Congresso).
- IX Simpósio de Microbiologia da UFMG - A Microbiologia no Brasil: A ciência de hoje para soluções dos problemas do amanhã. 2022. (Simpósio).
- 32ª Reunião de Genética de Microrganismos (REGEM-32) / GENÉTICA 2021 - Brazilian Congress of Genetics. 2021. (Congresso).
- 66th Brazilian Congress of Genetics - Genética 2021. (Congresso).
- International Society for Computational Biology (ISCB) Student Coundcil Symposiun 2021021. 2021. (Simpósio).

➢ Participação na comissão organizadora de dois (2) eventos científicos:

- AZEVEDO, V. A. C. ; ABURJAILE, F. F. ; **OLIVEIRA, J. C. A.** . 3rd International Associated Laboratoires Meeting - LIA 2022 BACT-INFLAM: CLOSING SEMINARS. 2022. (Outro).
- FRANCO, G. R. ; MINARDI, R. ; NETO, A. G. ; **OLIVEIRA, J. C. A.** . VI Curso de Verão em Bioinformática da UFMG. 2022. (Outro).

➢ Conclusão de um (1) Curso de extensão universitária com 54 horas (aproveitado para obtenção de créditos):

From Gene to Trait (2021), oferecido pelo GGRCG (Genomics for Cliamte Change Research Center), associado a EMBRAPA, UNICAMP e FAPESP.