An Avirulent Ralstonia Solanacearum Strain Undergoes Phenotype Conversion from a Pathogenic Strain Under Natural Environment

Deju Chen*, Haifeng Zhang, Yanli Li, Yanpin Chen, Xuefang Zheng, Jieping Wang, Jiamei Che, Bo Liu*

Institute of Agricultural Bioresources, Fujian Academy of Agricultural Sciences, Fuzhou, China

Email address: *chendeju@163.com (Deju Chen), 13600828002@126.com (Haifeng Zhang), 158759666@qq.com (Yanli Li), cpy071@yahoo.com.cn (Yanpin Chen), zhengxuefang2002@yahoo.com.cn (Xuefang Zheng), wangjieping2011@163.com (Jieping Wang), chejm2002@163.com (Jiamei Che), fzliubo@163.com (Bo Liu)

*Corresponding author

To cite this article: Deju Chen, Haifeng Zhang, Yanli Li, Yanpin Chen, Xuefang Zheng, Jieping Wang, Jiamei Che, Bo Liu. An Avirulent Ralstonia Solanacearum Strain Undergoes Phenotype Conversion from a Pathogenic Strain Under Natural Environment. American Journal of Bioscience and Bioengineering. Vol. 8, No. 3, 2020, pp. 46-58. doi: 10.11648/j.bio.20200803.13

Received: August 8, 2019; Accepted: September 5, 2019; Published: July 4, 2020

Abstract: An avirulent *R. solanacearum* strain named FJAT-1458 was isolated from living tomato vessel and it showed no toxicity to tomato, pepper and eggplant. Multilocus sequence analysis (MLSA) based on eight genes (*egl*, *hrpB*, *mutS*, *pehA*, *recA*, *rpoA*, *rpoB* and *rpoC*) and whole genome average nucleotide identity (ANI) analysis suggested that strain FJAT-1458 belong to phylotype I. Genome sequence of the strain FJAT-1458 revealed a circular chromosome and a circular megaplasmid with whole genome size of 6,059,899 bp and GC content of 66.78%. Functional annotation of FJAT-1458 showed a total of 5,442 genes, with 5,166 protein-encoding genes, 202 pseudogenes and 74 noncoding RNA genes. Among which, 3,938 protein-coding genes can be assigned to 23 COG families, and 1,521 of them had KEGG orthologs. Prophage prediction using PHASTER revealed 12 prophages, including 7 intact, 1 questionable and 4 incomplete prophages. Comparative genome analyses between GMI1000 and FJAT-1458 showed that most of the virulence factors were well conserved and only small portion of them were distinct between them. Two genes, including a methyltransferase and an ISL3 family transposase genes, were identified to be inserted immediately upstream (141 bp) of *phcA* gene, which assumed to be responsible for avirulence of strain FJAT-1458. It is suggested that strain FJAT-1458 was originated from a wild-type pathogenic strain through an accident phenotype conversion, which is like those when cultured under experimental conditions. Our study provides new insight into the evolution of virulence in *R. solanacearum* strain under natural environment.

Keywords: *Ralstonia Solanacearum*, Comparative Genomic Analysis, Virulence Factors, Single Molecular Real-time Sequencing, Phenotype Conversion

1. Introduction

*Ralstonia solanacearum* is one of the most important bacterial plant pathogens, which causes lethal wilts on more than 200 plant species belonging to over 50 different botanical families over a broad geographical range [1]. The bacterium enters plant roots, invades the xylem vessels and spreads rapidly to aerial parts of the plant through the vascular system where it reproduces in large number within a few days, leading to the death of plant [2]. In addition to its lethality, *R. solanacearum* is able to survive in soils, waters and under various abiotic stresses for a long period without losing the ability to wilt host plants [3], which makes it even harder to be controlled [4]. Several strategies were employed to control of diseases caused by *R. solanacearum*, including use healthy plant seeds [5], crop rotation for 2-5 years [6], chemical control [1, 7]. An alternative strategy was to use biological control agent such as antagonistic bacteria or avirulent mutants of *R. solanacearum*. However, the promising results could only be obtained under controlled conditions while was
not confirmed in field [7].

*R. solanacearum* have evolved an elegant and effective system to invade host and expand its plant host range. There are two major pathogenicity determinants exist in *R. solanacearum*, the type III secretion system (T3SS) and extracellular polysaccharide (EPS). T3SS injects the “effector proteins” into the plant cell cytosol to favour infection [8, 9], and EPS is largely responsible for the vascular dysfunction that causes wilt symptoms in susceptible hosts and promotes rapid systemic colonization as well [10]. Besides, *R. solanacearum* produces plenty of other factors that are potentially involved in the infection, including type II secreted plant cell wall-degrading enzymes, motility or attachment appendages, aerotaxis transducers, cellulases and pectinases [11]. The pathogenesis process was controlled through a sophisticated regulatory circuit and the LysR family transcriptional regulator PhcA plays a central role which regulates directly and indirectly many of those virulence genes [12].

Interestingly, under certain growth conditions, some members of the *R. solanacearum* population spontaneously undergo phenotype conversion (PC) from a wild-type pathogenic to a nonpathogenic form when it was allowed to grow to a high concentration [13]. Several genetic studies have revealed that PC is often resulted from mutation in phcA gene [14-17]. However, the phenomenon of PC under natural environment has never been reported probably due to that only few avirulent wild-type *R. solanacearum* strains have been identified. In our previous study, a *R. solanacearum* strain named FJAT-1458 has been isolated from living tomato vessel [18].

The objective of this study was to determine if the FJAT-1458 is born with nonpathogenic or the result of PC from a wild-type pathogenic strain. The complete genome sequence of strain FJAT-1458 was isolated with single molecular real-time sequencing (SMRT) biotechnology. The genome comparison between strain FJAT-1458 and other *R. solanacearum* strains was performed. Besides, to elucidate the reason why strain FJAT-1458 is avirulent against Solanaceae plants, the virulence factors of strain FJAT-1458 were compared with strain GMI1000.

2. Materials and Methods

2.1. Bacterial Strains and Growth Conditions

*R. solanacearum* strain FJAT-1458 was isolated from living *Solanum lycopersicum* L. vessel from Fuzhou, Fujian, China. The strain was kept frozen in 20% glycerol at -80°C. A single colony was selected from plating of a culture of FJAT-1458 on tetrazolium chloride agar medium (TZC) [19] and the pure colony was then grown at 30±2°C in liquid SPA medium (sucrose, 20 g; K2HPO4, 0.5 g; MgSO4, 0.025 g; H2O, 1000 ml; pH 7.2-7.4).

2.2. Pathogenicity Analysis

The Pathogenicity of the *R. solanacearum* strain FJAT-1458 was determined with a leaf-cutting method by using four- to six-leaf stage plants of tissue culture seedling of tomato (*Solanum lycopersicum* L. var. goldstone No. 1). The second, third and fourth leaves below the terminal bud of each seedling were cut about 1 cm length of wounds by a scissor. They were soaked in a bacterial suspension containing approximately 1×10⁷ cfu/ml for 20 minutes. The treated seedlings were incubated in a greenhouse at 30°C and 80% of relative humidity for 12 hours light/dark. SPA medium was used as negative control. Each treatment was replicated 10 times and the whole experiment was repeated twice. The disease incidence (wilt symptoms) of plants were monitored daily for 6 days.

2.3. Genomic DNA Preparation, Library Construction, Sequencing and Assembly

Bacterial cells were grown at 30°C overnight in SPA liquid medium. Genomic DNA was isolated and purified according to the manufacture’s instruction (Pacific Biosciences, Menlo Park, CA, USA). A 20-kb single-molecule real-time (SMRT) bell library was prepared with the SMRTbell template prep kit version 1.0 reagents (Pacific Biosciences, Menlo Park, CA, USA). The library was sequenced on a PacBio RS II sequencing platform using the C4 sequencing chemistry and P6 polymerase with 1 SMRT cells. The raw reads were filtered and assembled de novo following the Hierarchical Genome Assembly Process (HGAP) version 3.0 [20]. The polished assemblies were examined for circularity based on the presence of overlapping sequences at both ends of the contigs. Location of the overlapping sequence were determined using MUMmer version 3.0 [21].

2.4. Genome Annotation

The genome sequences were annotated using the NCBI prokaryotic annotation pipeline (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html). The circular map of the chromosome and plasmid were drawn by CGVIEW [22]. Prophage sequences were identified using PHASTER (http://phaster.ca) [23] and Insertion sequences were analyzed with ISEscan [24]. Functional annotation was based on BLAST searches against the NCBI RefSeq database, the Cluster of Orthologous Groups [25] and the Kyoto Encyclopedia of Genes and Genomes [26]. Protein domains were annotated using InterPro [27]. The classical secretory proteins were identified by SignalP (version 4.1, http://www.cbs.dtu.dk/services/SignalP/), and transmembrane helices were predicted by TMHMM (version 2.0, http://www.cbs.dtu.dk/services/TMHMM/).

2.5. Genomic Comparison

The complete genome sequences of FJAT-91, FQY 4, CQPS-1, CMR15, EP1, GMI1000, IBSBF1503, KACC10709, KACC10722, OE1-1, Po82, PS107, RS489, RS488, SEPPX05, UW163, UY031, YC40-M and YC45 were downloaded from the NCBI databases. The protein sequences of egl, hrbB, mutS, pehA, recA, rpoA, rpoB and rpoC genes were extracted and aligned using MUSCLE with default parameters [28]. The individual alignments were concatenated
successively to build the single super alignment. The best model for the alignment was estimated by MEGA software version 7.0 [29]. The phylogenetic tree was then constructed using Maximum Likelihood (ML) method in MEGA software version 7.0 [29]. The Jones-Taylor-Thornton (JTT) model assuming a discrete Gamma distribution (+G) with five rate categories was used for construction of a ML tree and the tree topology was evaluated by bootstrap analysis (1,000 replicates). Pairwise average nucleotide identity among these 19 strains were calculated with ANIm module of standalone version of JSpecies v1.2.1 with default parameters [30].

2.6. Nucleotide Sequence Deposition

The whole genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number CP016554 and CP016555 (BioProject ID PRJNA329182, and BioSample ID SAMN05392572).

3. Results and Discussions

3.1. Pathogenicity Test of Strain FJAT-1458

The pathogenicity of \textit{R. solanacearum} strain FJAT-1458 was determined with a leaf-cutting method by using tomato seedling with 5-6 leaves. Our results showed that strain FJAT-1458 is not able to cause wilt symptoms. However, the tomato seedlings began wilting at 4 days and reached to 58.33% incidence rate at 6 days after inoculated with \textit{R. solanacearum} strain GMI1000 (Figure 1). The pathogenicity of \textit{R. solanacearum} strain FJAT-1458 against pepper and eggplant were also tested, and it is not able to cause wilt symptoms in either of these two plants as well. Our results suggested that strain FJAT-1458 is probably avirulent.

![Figure 1](image1.png)

\textbf{Figure 1.} Pathogenicity of \textit{Ralstonia solanacearum} strains FJAT-1458 strains determined with a leaf-cutting method by using tomato seedling with 5-6 leaves after 6 days. (A) GMI1000; (B) FJAT-1458; (C) control.

3.2. General Genomic Features and Genome Annotation of Strain FJAT-1458

Whole genome sequencing was carried out with single molecular real-time sequencing (SMRT) biotechnology on the PacBio RS II platform. The completed genome of \textit{R. solanacearum} strain FJAT-1458 was 6.06 Mb with a GC content of 66.78%. It contained one circular chromosome (3.98 Mb with a GC content of 66.71%, Figure 2a) and one circular megaplasmid (2.08 Mb with a GC content of 66.92%, Figure 2b). A total of 5,166 protein-coding genes were predicted (chromosome and megaplasmid encoded 3,633 and 1,533 genes, respectively). The FJAT-1458 genome contained 12 tRNA, 58 rRNA and 4 other non-coding RNAs. Of the 5,166 CDSs, 3,938 protein-coding genes can be assigned to 23 COG families. In addition, a total of 1,521 protein-coding genes had KEGG orthologs. 548 (10.61%) of protein-coding genes were predicted as classical secretory proteins with SignalP and 1,165 (22.55%) protein-coding genes have more than one transmembrane helix (Table 1).

![Figure 2](image2.png)

\textbf{Figure 2.} Circular genome map of \textit{R. solanacearum} strain FJAT-1458. (A) chromosome of FJAT-1458; (B) megaplasmid of FJAT-1458. The distribution of the circle from inner to outer indicates, (1) circle 1: scale in kb; (2) circle 2: GC-skew (G-C/G+C ratio) using a 1 kb window with 100 bp step; (3) circle 3: GC-content using a 3 kb window with 100 bp step; (4) circle 4: COG assignments for predicted CDSs on the forward strand; (5) circle 5: COG assignments for predicted CDSs on the reverse strand.
### General genome features of the Ralstonia solanacearum strain FJAT-1458.

|                      | Chromosome | Plasmid | Total     |
|----------------------|------------|---------|-----------|
| Genome Size (bp)     | 3,984,240  | 2,075,659 | 6,059,899 |
| G+C content          | 66.71      | 66.92   | 66.78     |
| Total genes          | 3,801      | 1,641   | 5,442     |
| Protein coding genes | 3,633      | 1,533   | 5,166     |
| tRNA genes           | 9          | 3       | 12        |
| tRNA genes           | 54         | 4       | 58        |
| Other non-coding RNA genes | 4       | 0       | 4         |
| Pseudo genes         | 101        | 101     | 202       |
| Hypothetical protein genes | 898      | 473     | 1,371     |
| Genes assigned to COGs| 2,810      | 1,128   | 3,938     |
| Genes assigned to GO function | 2,027  | 790     | 2,817     |
| Genes assigned to KEGG ontology | 1,129 | 392     | 1,521     |
| Genes with PFAM domains | 2,996      | 1,206   | 4,202     |
| Genes with assigned function | 2,735  | 1,060   | 3,795     |
| Genes with signal peptides | 362        | 186     | 548       |
| Genes with transmembrane helices | 802       | 363     | 1,165     |

### 3.3. Prophages

A total of 7 intact, 1 questionable and 4 incomplete prophages were identified in the genome of *R. solanacearum* strain FJAT-1458 by PHASTER [23]. These prophages were designated as Prophage 1-12 (Supplementary Table A1). Among which, Prophage 1-10 were located on the chromosome and Prophage 11-12 were located on the mega-plasmid. Prophage size ranged between 5.17 kb and 58.9 kb in length and GC content ranged between 57.85% and 66.54%. The GC content of Prophage 1 (66.54%), Prophage 2 (66.19%), Prophage 5 (66.31%) and Prophage 6 (66.26%) were very close to the average GC content of the whole genome (66.78%), indicating that they might have been integrated into *R. solanacearum* genome long time ago.

Besides phage structural genes and IS elements, prophage carries large number of genes which provide new functions to the host (Supplementary Table A2). For example, we identified five DNA methyltransferase, three methylase, three endonuclease and one exonuclease genes from these prophages, suggesting their role in DNA restriction and modification. We also identified two type III effector proteins and one type VI secretion system tip protein VgrG, which might contribute to virulence. Besides, we discovered a HicBA toxin-antitoxin II system in prophage 5, which encode a stable HicA toxin and a labile HicB antitoxin. TA systems are reported to be strongly correlated with physiological processes such as gene regulation, growth arrest, survival and apoptosis [31-34].

### 3.4. Comparative Genome Analysis

Phylogenetic relationships of *R. solanacearum* strain FJAT-1458 with other strains of *R. solanacearum* were assessed by performing Multilocus Sequence Analysis (MLSA) using eight genes (*egl*, *hrpB*, *mutS*, *pehA*, *recA*, *rpoA*, *rpoB* and *rpoC*). The result showed that strain FJAT-1458 belonged to phytype I and was closest to strain YC-45 and SEPPX05 (Figure 3).

**Figure 3.** Phylogenetic reconstruction of *R. solanacearum* strain FJAT-1458 with other 19 of *R. solanacearum* strain. The phylogenetic tree was constructed using concatenated alignments of the marker genes *egl*, *hrpB*, *mutS*, *pehA*, *recA*, *rpoA*, *rpoB* and *rpoC*. The evolutionary history was inferred by using the Maximum Likelihood method base on the JTT model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1000)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 47.47% sites). Evolutionary analyses were conducted in MEGA 7.0 (Kumar S et al., 2016). The scale bar represents branch length measured in the number of substitutions per sites. Numbers at branch-points are percentages of 1000 bootstrap re-samplings that support the tree topology.
Based on average nucleotide identity (ANI) values, strains of *R. solanacearum* were grouped into three different genomespecies (Supplementary Table A3). The first group consists of strains from phylotype I and III. The second group includes phylotype II strains and the last group comprises strains from phylotype IV. ANI value is considered as one of the most robust measurements of genomic relatedness between strains and an ANI thresholds range (95–96%) correspond to ≥ 70% DDH standard for species definition [35]. These ANI results indicate that these three genomespecies groups could be considered as separate species. Our results are consistent with previous reports [36, 37].

### 3.5. Genes Involved in Virulence

In this study, we showed that strain FJAT-1458 is avirulent to tomato, pepper and eggplant. To explore if avirulence of FJAT-1458 is due to the absence of key virulence factors, we created an inventory of 35 genes involved in virulence from GMI1000 [38], including exopolysaccharide (EPS) biosynthetic genes, cell wall degrading enzyme (CWDE) genes, response genes to the host defenses, key virulence regulator genes, chemotaxis genes, and genes involved in motility. Protein sequences predicted from the genome of strain FJAT-1458 were then searched against the database built from these virulence factors. Our results showed that these genes were well conserved between strain FJAT-1458 and GMI1000 (Supplementary Table A4), and the amino acid sequence identities were more than 99%, except *phcB* and twitching motility gene *pilA*, whose identities were 86.02% and 93.49%, respectively.

### 3.6. T2SS

Type II Secretion System (T2SS) is one means by which Gram-negative pathogens secrete proteins into the extracellular milieu and/or host organisms [39]. In *R. solanacearum* strains, lots of proteins secreted in a Type-II-dependent manner which contribute to its virulence, and *R. solanacearum* strain with a defective type II secretion system (T2SSs) is weakly virulent [40]. Similar to strain GMI1000 [41], FJAT-1458 harbors three type II secretion systems (T2SS) (Figure 4). The first one is the orthodox system which contains 12 genes in the chromosome (from 355,322 to 367,621). This gene cluster is well conserved and shares a high sequence identity with strain GMI1000 (average amino acid sequence identity of 99.32%). The other two T2SSs are unorthodox systems. One includes seven core genes in the chromosome (ranging from 1,233,265 to 1,243,218) with four hypothetical genes inserted between *gspE* and *gspD* (Figure 4b). The other possesses six core genes located in the mega-plasmid (from 1,835,267 to 1,844,143), with four hypothetical genes inserted between *gspD* and *gspE* (Figure 4c). These two gene clusters are also conserved between strain GMI1000 and strain FJAT-1458 (average amino acid sequence identity of 98.68%).

![Figure 4](image_url)

**Figure 4.** Genetic organization of T2SS gene clusters in strain FJAT-1458 and GMI1000. (A) The orthodox system of T2SS in the chromosome (from 355,322 to 367,621 bp); (B) one unorthodox system of T2SS in the chromosome (from 1,233,265 to 1,243,218 bp); (C) The other unorthodox system of T2SS in the mega-plasmid (from 1,835,267 to 1,844,143 bp).

### 3.7. T3SS

The type III secretion system (T3SS) is widely spread in gram-negative bacteria, and is responsible for delivering bacterial proteins, termed effectors, from the bacterial cytosol directly into the interior of host cells [42]. These translocated proteins facilitate bacterial pathogenesis by specifically interfering with host cell signal transduction and other cellular processes [43]. The *hrp* gene cluster of T3SS is the key virulence determinant in *R. solanacearum*. In strain FJAT-1458, it is located on the mega-plasmid and spans 29.655 kb (from 785,966 to 815,620), composed of 30 genes. Most of genes were well conserved between strain FJAT-1458 and GMI1000 (Figure 5), sharing amino acid sequence identity from 94.19% to 100.00%. One gene named *popC* was presumed to be pseudo due to internal stop codon.

A total of 68 T3es were identified in FJAT-1458 genome (Supplementary Table A5). Among which, 62 (91.18%) of them were also present in the strain GMI1000. Six T3es were present in strain FJAT-1458 but absent in strain GMI1000, while 12 T3es were absent in strain FJAT1458 but were present in strain GMI1000.
3.8. T4SS

The Type IV Secretion System (T4SS) plays diverse important roles in virulence and adaptation. In strain GMI1000, the T4SS gene cluster is comprised of 17 genes (\textit{RSc2574-RSc2588, RSp0179}, and \textit{RSp1521}) [41]. No experimental evidence is yet available in support of a role for these genes as a “fitness island” or an “ecological island” [44]. These 17 T4SS genes were searched against the genome of strain FJAT-1458, and none of them were identified in this genome.

3.9. T6SS

The type VI secretion system (T6SS) is a complex and widespread gram-negative bacterial export pathway with the capacity to translocate protein effectors into a diversity of target cell types [45]. Previous study has showed that T6SS could contribute to pathogenicity, swimming motility and mediate biofilm formation [46]. In strain GMI1000, the T6SS locus spans an approximate 45.2-kb region in the mega-plasmid, comprised of 15 core genes and 16 additional genes. In strain FJAT-1458, the T6SS locus located in a 46 kb region in the mega-plasmid, containing 15 core genes and 19 additional genes. The core T6SS genes between these two strains were conserved, with average amino acid identity up to 99.11% (Figure 6). The inserted ORFs between \textit{vgrGA2} and \textit{impA} varied significantly (8 additional ORFs in strain GMI1000, while 11 additional ORFs in strain FJAT-1458). Among these 11 ORFs, four of them were identified as transposon, suggesting this region to be a hotspot for insertion.

3.10. A Fragment was Inserted Immediately Upstream of Phca Gene

Comparison of virulence factors between strain GMI1000 and strain FJAT-1458 revealed that most of them are well conserved between them, and only small portion of virulence factors are distinct. These distinct virulence factors might lead to weakened virulence instead of loss of virulence. Previous study revealed that the LysR family transcriptional regulator PhcA plays a central role which regulates directly and indirectly many of those virulence genes [12] and inactivation of \textit{phcA} gene resulted in loss of virulence [14-16]. A previous genetic study carried out with strain AW1 showed that spontaneous PC can be attributed to insertions with \textit{phcA} gene (2-bp, 200-bp or 1-kb insertions) [14]. Another independent research with three spontaneous PC mutation strains of ACH0158 also showed that inactivation of \textit{phcA} gene (an insertion of \textit{ISRso4}, a 132bp-deletion and a 2-bp insertion) results in loss of virulence [16]. Thus, we carefully examined the \textit{phcA} gene and its flanking sequence. We didn’t find any insertion sequence in \textit{phcA} gene and it is well conserved between strain GMI1000 and strain FJAT-1458 (coverage 100.00%, identity 99.71%). However, we did find two genes, including a methyltransferase and an ISL3 family transposase genes, inserted immediately upstream (141 bp) of \textit{phcA} gene (Figure 7). This insertion sequence might suppress the expression of \textit{phcA} gene as it is just located in the promoter region of \textit{phcA} gene. The low levels of functional PhcA could not be able to activate the transcription of its downstream genes associated with the virulence of the pathogen [47], which finally resulted in avirulence of FJAT-1458. Further experiments (such as transcriptome analysis and genetic compensation experiment) are still required to confirm.
4. Conclusions

An avirulent *R. solanacearum* strain named FJAT-1458 was isolated from living tomato vessel and it showed no toxicity to tomato, pepper and eggplant. Comparative genome analyses between GMI1000 and FJAT-1458 revealed that a fragment, containing a methyltransferase and an ISL3 family transposase genes, was inserted immediately upstream (141 bp) of *phcA* gene, which assumed to be responsible for avirulence of FJAT-1458. It is suggested that strain FJAT-1458 was originated from a wild-type pathogenic strain through an accident phenotype conversion, which is like those when cultured under experimental conditions. Our study provides new insight into the evolution of virulence in *R. solanacearum*

Appendix

| Strains       | ANI (%) |
|---------------|---------|
| FJAT-1458     | 98.56   |
| KACC10709     | 98.56   |
| KACC10722     | 98.55   |
| PS107         | 98.51   |
| UY031         | 98.49   |
| RS488         | 98.49   |
| OEI-1         | 98.48   |
| EP1           | 98.47   |
| YC45          | 98.46   |
| GM1000        | 98.45   |
| SEPPX05       | 98.44   |

Author Contributions

CD, LB and ZH conceived and designed the experiments. CD, LY, CY, CJ performed the experiments. CD, CJ and WJ generated and analyzed the data. CD wrote the paper.

Acknowledgements

This work was funded by the Fujian Natural Science Foundation (2017J01049).

Table A1. Whole genome average nucleotide identity (ANI) analysis of 20 strains from *R. solanacearum* species.

| Strains       | ANI (%) |
|---------------|---------|
| FJAT-1458     | 98.50   |
| KACC10709     | 98.76   |
| FJAT-91       | 98.52   |
| FYQ_4         | 98.65   |
| YC40-M        | 98.57   |
| OEI-1         | 98.98   |
| EP1           | 98.58   |
| YC45          | 98.57   |
| GM1000        | 98.57   |
| SEPPX05       | 98.57   |

Table A1. Continued.

| Strains       | ANI (%) |
|---------------|---------|
| FJAT-1458     | 98.50   |
| KACC10709     | 98.76   |
| FJAT-91       | 98.52   |
| FYQ_4         | 98.65   |
| YC40-M        | 98.57   |
| OEI-1         | 98.98   |
| EP1           | 98.58   |
| YC45          | 98.57   |
| GM1000        | 98.57   |
| SEPPX05       | 98.57   |

Table A1. Whole genome average nucleotide identity (ANI) analysis of 20 strains from *R. solanacearum* species.
Table A2. Summary of prophages predicted in FJAT-1458.

| ID     | Location       | Completeness | Start            | End              | Length  | Total Protein numbers | GC Content | ATT Sites |
|--------|----------------|--------------|------------------|------------------|---------|------------------------|------------|-----------|
| Prophage 1 | Chromosome     | questionable(90) | 220332          | 236365          | 16034   | 21                     | 66.54%     | No        |
| Prophage 2 | Chromosome     | intact(150)   | 426995          | 463041          | 36047   | 49                     | 66.19%     | Yes       |
| Prophage 3 | Chromosome     | intact(122)   | 1622955         | 1666443         | 43489   | 48                     | 64.40%     | No        |
| Prophage 4 | Chromosome     | intact(150)   | 1875020         | 1933963         | 58944   | 53                     | 64.61%     | Yes       |
| Prophage 5 | Chromosome     | intact(150)   | 1938002         | 1958812         | 20811   | 26                     | 66.31%     | No        |
| Prophage 6 | Chromosome     | intact(133)   | 2168497         | 2190188         | 21692   | 17                     | 64.90%     | Yes       |
| Prophage 7 | Chromosome     | intact(97)    | 2596449         | 2641654         | 45206   | 41                     | 63.96%     | Yes       |
| Prophage 8 | Chromosome     | intact(150)   | 2726121         | 2771275         | 45155   | 45                     | 59.20%     | No        |
| Prophage 9 | Chromosome     | incomplete(40) | 2957099        | 2976375         | 19277   | 18                     | 66.26%     | No        |
| Prophage 10 | Chromosome    | incomplete(40) | 3037730        | 3052587         | 14585   | 10                    | 64.67%     | No        |
| Prophage 11 | Megaplasmid   | incomplete(50) | 337007         | 345420          | 8414    | 11                    | 60.78%     | No        |
| Prophage 12 | Megaplasmid   | incomplete(60) | 1661567        | 1666733         | 5167    | 8                     | 57.85%     | No        |
| Total   |                |              |                  |                  |         |                        |            |           |

321513 | 347

Table A3. Functional annotation of genes predicted in prophages of FJAT-1458.

| ID | Location | Start | End | Strand | Annotation                   |
|----|----------|-------|-----|--------|------------------------------|
| 1  | Chromosome | 220332 | 221732 | +     | DNA modification methylase   |
| 2  | Chromosome | 221729 | 222949 | +     | DNA methylase N-4            |
| 3  | Chromosome | 223129 | 224094 | -     | ISS-like element IS1405 family transposase |
| 4  | Chromosome | 224170 | 224535 | -     | hypothetical protein         |
| 5  | Chromosome | 224703 | 224897 | +     | hypothetical protein         |
| 6  | Chromosome | 225059 | 225427 | +     | hypothetical protein         |
| 7  | Chromosome | 225582 | 225770 | -     | hypothetical protein         |
| 8  | Chromosome | 225892 | 226386 | -     | DUF3489 domain-containing protein |
| 9  | Chromosome | 226476 | 226745 | +     | hypothetical protein         |
| 10 | Chromosome | 226842 | 227378 | +     | elements of external origin  |
| 11 | Chromosome | 227378 | 229345 | +     | phage terminase large subunit family protein |
| 12 | Chromosome | 229389 | 229898 | +     | capsid-related protein       |
| 13 | Chromosome | 229909 | 230301 | +     | tail fiber                   |
| 14 | Chromosome | 230302 | 230523 | +     | head-tail connector          |
| 15 | Chromosome | 230523 | 232049 | +     | lambda-like phage portal protein |
| 16 | Chromosome | 232059 | 233309 | +     | putative head maturation protease |
| 17 | Chromosome | 233319 | 233696 | +     | head decoration protein D     |
| 18 | Chromosome | 233705 | 234709 | +     | minor capsid protein E       |
| 19 | Chromosome | 234712 | 235014 | +     | hypothetical protein         |
| 20 | Chromosome | 235209 | 235466 | +     | hypothetical protein         |
| 21 | Chromosome | 235592 | 236365 | +     | hypothetical protein         |

Table A4. Comparative analysis of known and candidate virulence factors (except for Type III effectors) between strain FJAT-1458 and GMI1000.

| Virulence_Category | GMI1000 | Length | Start | End |
|-------------------|---------|--------|-------|-----|
| EPS biosynthetic genes |          | 418    | 1     | 418 |
| Rsp1014 | epsF  | EPS I polysaccharide export inner membrane protein | 418 | 1 | 418 |
| Rsp1015 | epsE  | EPS I polysaccharide export inner membrane protein | 436 | 1 | 436 |
| Rsp1016 | epsD  | NDP-N-acetyl-D-galactosaminuronic acid dehydrogenase | 423 | 1 | 423 |
| Rsp1017 | epsC  | UDP-N-acetylgalactosamine 2-epimerase | 375 | 1 | 375 |
| Rsp1018 | epsB  | Tyrosine-protein kinase epsB (EPS I polysaccharide export protein epsB) | 751 | 1 | 751 |
| Rsp1019 | epsP  | Low molecular weight protein-tyrosine-phosphatase epsP | 145 | 1 | 145 |
| Rsp1020 | epsA  | EPS I polysaccharide export outer membrane protein | 381 | 1 | 381 |
| Rsp0338 | epsR  | Negative regulator of exopolysaccharide production | 222 | 1 | 222 |
| Rsc1756 | pehB  | Exo-poly-galacturonidase | 702 | 1 | 702 |
| Cell Wall Degradation |          | 680    | 1     | 680 |
| RSp0833 | pehC  | Polygalacturonase | 680 | 1 | 680 |
| enzymes (CWDE) |          | 436    | 1     | 436 |
| Rsp0612 | egl   | endoglucanase | 436 | 1 | 436 |
| Rsc0319 | plcN  | non hemolytic phospholipase C | 700 | 1 | 700 |
| Response to host defenses |          | 509    | 1     | 509 |
| RSp1581 | katE  | catalase hydroperoxidase Hpl oxidoreductase | 509 | 1 | 509 |
| Rsc2690 | oxyR  | Hydrogen peroxide-inducible gene activator | 314 | 1 | 314 |
| Rsp0283 | dinF  | DNA-damage-inducible SOS response protein | 457 | 1 | 457 |
### Table A4. Continued.

| Virulence_Category       | GM11000 | FJAT-1458 | Alignment_Length | Identity |
|--------------------------|---------|-----------|-----------------|----------|
| **Locus_Tag**            |         |           |                 |          |
| **GM11000**              |         |           |                 |          |
| **Description**          |         |           |                 |          |
| **Length**               |         |           |                 |          |
| **Start**                |         |           |                 |          |
| **End**                  |         |           |                 |          |
| **Type III effectors & putative effectors** | RSp0914 | GALA1 | LRR F-box protein, Interaction with SKP1-like proteins | 661 | 1 | 661 |
|                          | RSp0672 | GALA2 | LRR F-box protein | 1035 | 55 | 1035 |
|                          | RSp0028 | GALA3 | LRR F-box protein, Interaction with SKP1-like proteins | 518 | 1 | 518 |

### Table A5. Comparative analysis of Type III effectors between strain FJAT-1458 and GM11000.

| Virulence_Category       | GM11000 | FJAT-1458 | Alignment_Length | Identity |
|--------------------------|---------|-----------|-----------------|----------|
| **Locus_Tag**            |         |           |                 |          |
| **GM11000**              |         |           |                 |          |
| **Description**          |         |           |                 |          |
| **Length**               |         |           |                 |          |
| **Start**                |         |           |                 |          |
| **End**                  |         |           |                 |          |
| **Type III effectors & putative effectors** | RSp0914 | GALA1 | LRR F-box protein, Interaction with SKP1-like proteins | 661 | 1 | 661 |
|                          | RSp0672 | GALA2 | LRR F-box protein | 1035 | 55 | 1035 |
|                          | RSp0028 | GALA3 | LRR F-box protein, Interaction with SKP1-like proteins | 518 | 1 | 518 |

### Table 1. Key virulence regulators.

- **Putative effectors**
  - Type III effectors & putative effectors
- **Virulence Category**
  - Twitching motility
  - Swimming motility
  - Chemotaxis

**Response to host defenses**

- **Enzymes (CWDE)**
- **Cell Wall Degradation**
- **EPS biosynthetic genes**
- **Chemotaxis**
- **Swimming motility**
- **Twitching motility**

**Response to host defenses**

- **Key virulence regulators**

**GM11000**

| Locus Tag | Symbol | Description | Length | Start | End |
|-----------|--------|-------------|--------|-------|-----|
| RSp0103   | xpsR   |            | 306    | 1     | 306 |
| RSp2808   | pehS   | sensor histidine kinase transcription regulator | 682 | 1 | 682 |
| RSp2807   | pehR   | response regulator, FIS type, sigma 54 interacting region | 560 | 1 | 560 |
| RSp3286   | solI   |            | 204    | 1     | 204 |
| RSp3287   | solR   | Transcriptional activator | 236 | 1 | 236 |
| RSp1408   | cheA   |            | 726    | 1     | 726 |
| RSp1407   | cheW   | chemotaxis protein, regulation | 163 | 1 | 163 |
| RSp0382   | flIC   |            | 273    | 1     | 273 |
| RSp0340   | flagM  |            | 106    | 1     | 106 |
| RSp0558   | pilA   |            | 168    | 1     | 168 |
| RSp2972   | pilP   |            | 181    | 1     | 181 |

**GM11000**

| Locus Tag | Symbol | Description | Length | Start | End |
|-----------|--------|-------------|--------|-------|-----|
| BCR16_RS23200 | 418 | component of acridine efflux pump, multidrug efflux system | 398 | 1 | 398 |
| BCR16_RS23205 | 436 | component of acridine efflux pump, multidrug efflux system | 1049 | 1 | 1049 |
| BCR16_RS23210 | 423 |            | 347 | 1 | 347 |
| BCR16_RS23215 | 375 |            | 467 | 1 | 467 |
| BCR16_RS23220 | 351 | regulatory protein, SAM-dependent methyltransferase domain | 474 | 1 | 474 |
| BCR16_RS23225 | 381 | Transmembrane sensor kinase | 474 | 1 | 474 |
| BCR16_RS23230 | 222 | response regulator, LuxR family | 210 | 1 | 210 |
| BCR16_RS23235 | 702 |            | 611 | 1 | 611 |
| BCR16_RS23240 | 509 |            | 161 | 1 | 161 |
| BCR16_RS23245 | 314 |            | 181 | 1 | 181 |

**Key virulence regulators**

- **Twitching motility**
- **Swimming motility**
- **Chemotaxis**

**GM11000**

| Locus Tag | Symbol | Description | Length | Start | End |
|-----------|--------|-------------|--------|-------|-----|
| RSp0914   | GALA1  | LRR F-box protein, Interaction with SKP1-like proteins | 661 | 1 | 661 |
| RSp0672   | GALA2  | LRR F-box protein | 1035 | 55 | 1035 |
| RSp0028   | GALA3  | LRR F-box protein, Interaction with SKP1-like proteins | 518 | 1 | 518 |
### Virulence Category

| **Locus tag** | **Symbol** | **Description** | **Length** | **Start** | **End** |
|---------------|------------|-----------------|------------|-----------|--------|
| Rsc1800       | GALA4      | LRR F-box protein | 401        | 1         | 401    |
| Rsc1801       | GALA5      | LRR F-box protein, Interaction with SKP1-like proteins | 522        | 1         | 522    |
| Rsc1356       | GALA6      | LRR F-box protein, Interaction with SKP1-like proteins | 620        | 1         | 620    |
| Rsc1357       | GALA7      | LRR F-box protein, Host specificity factor on medicago truncatula | 647        | 1         | 647    |
| Rsc3401       | SKWP1      | Heat/armadillo-related repeats | 2353       | 1         | 2353   |
| Rsp1374       | SKWP2      | Heat/armadillo-related repeats | 2483       | 1         | 2487   |
| Rsp0930       | SKWP3      | Heat/armadillo-related repeats | 2208       | 1         | 2208   |
| Rsc1839       | SKWP4      | Heat/armadillo-related repeats | 2497       | 1         | 2497   |
| Rsp0296       | SKWP5      | Heat/armadillo-related repeats | 2338       | 1         | 2338   |
| Rsc2130       | SKWP6      | Heat/armadillo-related repeats | 721        | 1         | 721    |
| Rsc1386       | HLK1       |                | 765        | 1         | 765    |
| Rsp0215       | HLK2       |                | 754        | 1         | 754    |
| Rsp0160       | HLK3       |                | 719        | 1         | 719    |
| Rsc1349       | SspH1 family |                | 685        | 1         | 685    |
| Rsc0245       | ripB       | Nucleoside hydrolase domain | 492        | 1         | 492    |
| Rsc2775       | popW       | Harpin with pectate lyase domain | 380        | 1         | 380    |
| Rsp0304       | AvrPphD family |                | 643        | 1         | 643    |
| Rsp0323       | HopG1 family |                | 451        | 1         | 451    |
| Rsp0732       | HopAV1 family |                | 830        | 1         | 830    |
| Rsp0875       | popC       | Leucine Rich repeats | 1742       | 1         | 1742   |
| Rsp1281       | HopR1 family |                | 368        | 1         | 368    |
| Rsc0826       | PopP1 (YopJ family) | S/T acetyltransferase domain, avirulence factor on some petunia lines | 356        | 1         | 356    |
| Rsc1815       | AvrBs3 family | 35AA repeats units | 321        | 1         | 321    |
| Rsc3212       | RipT (YopT family) | Cysteine protease domain | 1742       | 1         | 1742   |
| Rsc3290       | HopH1 family |                | 584        | 1         | 584    |
| Rsc3369       | AvrPphE family |                | 154        | 1         | 154    |
| Rsp0572       | HopH1 family |                | 557        | 1         | 557    |
| Rsp0822       | AvrPphF family |                | 577        | 1         | 577    |
| Rsp1239       | -          |                | 557        | 1         | 557    |
| Rsp1277       | HopAA1 family |                | 361        | 1         | 361    |
| Rsc0041       | -          |                | 430        | 1         | 430    |
| Rsc0608       | AvrA       | Avirulence factor on Nicotiana species | 584        | 1         | 584    |
| Rsc1475       | -          |                | 154        | 1         | 154    |
| Rsp0731       | -          |                | 557        | 1         | 557    |
| Rsp0845       | -          |                | 154        | 1         | 154    |
| Rsp0876       | popB       |                | 174        | 1         | 174    |
| Rsp0877       | popA       | Harpin, Formation of ion-conducting pores | 344        | 1         | 344    |
| Rsp0882       | -          |                | 299        | 1         | 299    |
| Rsp1022       | -          |                | 346        | 1         | 346    |
| Rsp1236       | -          |                | 540        | 1         | 540    |
| Rsp0099       | AWR2, RipA | Required for full virulence on tomato | 1127       | 1         | 1127   |
| Rsp0846       | AWR3       |                | 1193       | 1         | 1193   |
| Rsp0847       | AWR4       |                | 1330       | 1         | 1330   |
| Rsp1024       | AWR5       |                | 1146       | 1         | 1146   |
| Rsc2139       | AWR1       |                | 1063       | 1         | 1063   |
| Rsp0257       | -          | Ankyrin repeats | 277        | 1         | 277    |
| Rsc0321       | -          |                | 98         | 1         | 98     |
| Rsc0824       | -          |                | 488        | 1         | 488    |
| Rsc0868       | PopP2      | Functionnal NLS, S/T acetyltransferase domain, avirulence factor on Arabidopsis | 96         | 1         | 96     |
| Rsc1475       | -          |                | 306        | 1         | 306    |
| Rsc2101       | -          |                | 739        | 1         | 739    |
| Rsc2359       | -          |                | 739        | 1         | 739    |
| Rsc3174       | -          |                | 1359       | 1         | 1359   |
| Rsp0193       | -          | Pentatricopeptide repeat domain | 311        | 1         | 311    |
| Rsp0218       | -          | S/T kinase domain | 703        | 1         | 703    |
| Rsp0885       | -          | oxydoreductase domain | 1310       | 1         | 1310   |
| Rsp1031       | -          | Coiled-coil domain | 878        | 1         | 878    |
| Virulence_Category | GM11000 |
|-------------------|---------|
| Locus_tag | Symbol | Description | Length | Start | End |
| RSp1388 | - | | 461 | 83 | 461 |
| RSp1460 | - | | 274 | 1 | 274 |
| RSp1475 | - | | 369 | 1 | 369 |
| RSp1582 | - | | | | |
| RSp1601 | - | | 248 | 1 | 248 |
| RSc2132 | - | | 487 | 1 | 487 |
| RSp0879 | - | | | | |
| RSp1130 | - | Nudix hydrolase domain | 474 | 1 | 474 |

Table A5. Continued.

| Virulence_Category | FJAT-1458 |
|-------------------|-----------|
| Locus_Tag | Length | Start | End | Alignment_Length | Identity |
| BCR16_RS22160 | 589 | 1 | 589 | 661 | 88.35 |
| BCR16_RS21450 | 981 | 1 | 981 | 981 | 97.76 |
| BCR16_RS26065 | 522 | 1 | 522 | 522 | 95.98 |
| BCR16_RS10075 | 401 | 1 | 401 | 401 | 99.25 |
| BCR16_RS10080 | 522 | 1 | 522 | 522 | 98.47 |
| BCR16_RS11095 | 620 | 1 | 620 | 620 | 97.26 |
| BCR16_RS11090 | 647 | 1 | 647 | 647 | 96.91 |
| BCR16_RS00145 | 2353 | 1 | 2353 | 2353 | 99.19 |
| BCR16_RS24375 | 2512 | 1 | 2469 | 2469 | 98.95 |
| BCR16_RS22045 | 2208 | 1 | 2208 | 2208 | 98.60 |
| BCR16_RS10265 | 2495 | 1 | 2495 | 2497 | 98.00 |
| BCR16_RS27125 | 2338 | 1 | 2338 | 2338 | 99.02 |
| BCR16_RS06900 | 721 | 1 | 721 | 721 | 99.03 |
| BCR16_RS10945 | 765 | 1 | 765 | 765 | 98.69 |
| BCR16_RS26710 | 742 | 1 | 742 | 754 | 96.42 |
| BCR16_RS26430 | 719 | 1 | 719 | 719 | 98.89 |
| BCR16_RS11130 | 685 | 1 | 685 | 685 | 97.52 |
| BCR16_RS17735 | 492 | 1 | 492 | 492 | 99.39 |
| BCR16_RS03925 | 380 | 1 | 380 | 380 | 99.47 |
| BCR16_RS27145 | 641 | 1 | 641 | 643 | 96.73 |
| BCR16_RS19125 | 501 | 1 | 501 | 501 | 98.67 |
| BCR16_RS23055 | 830 | 1 | 830 | 830 | 99.52 |
| BCR16_RS20755 | 1742 | 1 | 1742 | 1742 | 99.25 |
| BCR16_RS14705 | 368 | 1 | 368 | 368 | 95.11 |
| BCR16_RS14580 | 321 | 1 | 321 | 321 | 99.69 |
| BCR16_RS00305 | 425 | 1 | 425 | 425 | 99.06 |
| BCR16_RS20560 | 183 | 1 | 183 | 183 | 79.23 |
| BCR16_RS22600 | 292 | 1 | 292 | 292 | 95.55 |
| BCR16_RS20960 | 837 | 1 | 837 | 837 | 98.69 |
| BCR16_RS20775 | 361 | 1 | 361 | 361 | 99.17 |
| BCR16_RS18800 | 430 | 1 | 430 | 430 | 99.07 |
| BCR16_RS10490 | 584 | 1 | 584 | 584 | 98.97 |
| BCR16_RS00820 | 154 | 1 | 154 | 154 | 99.35 |
| BCR16_RS23060 | 499 | 1 | 499 | 499 | 99.60 |
| BCR16_RS22480 | 1390 | 1 | 1390 | 1390 | 99.50 |
| BCR16_RS22325 | 174 | 1 | 174 | 174 | 98.85 |
| BCR16_RS22320 | 330 | 1 | 330 | 344 | 94.19 |
| BCR16_RS22295 | 299 | 1 | 299 | 299 | 100.00 |
| BCR16_RS23240 | 411 | 71 | 411 | 346 | 95.38 |
| BCR16_RS24280 | 540 | 1 | 540 | 540 | 98.33 |
| BCR16_RS26190 | 1126 | 1 | 1126 | 1127 | 99.47 |
| BCR16_RS22475 | 1189 | 1 | 1189 | 1193 | 98.41 |
| BCR16_RS22470 | 1333 | 1 | 1333 | 1334 | 98.58 |
| BCR16_RS23255 | 1146 | 1 | 1146 | 1146 | 99.21 |
| BCR16_RS06850 | 1067 | 1 | 1067 | 1067 | 98.59 |
| BCR16_RS26945 | 280 | 1 | 280 | 280 | 98.93 |
| BCR16_RS14710 | 98 | 1 | 98 | 98 | 100.00 |
| BCR16_RS01205 | 488 | 1 | 488 | 488 | 98.57 |
| BCR16_RS14380 | 96 | 1 | 96 | 96 | 100.00 |
| BCR16_RS07055 | 306 | 1 | 306 | 306 | 99.35 |
| BCR16_RS05785 | 739 | 1 | 739 | 739 | 99.73 |
| BCR16_RS26610 | 1337 | 1 | 1323 | 1360 | 94.63 |
| BCR16_RS26725 | 866 | 1 | 307 | 307 | 98.37 |

Type III effectors & putative effectors
References

[1] Denny TP: Plant pathogenic Ralstonia species. In plant-Associated Bacteria, ed. SS Gnanamanickam: Dordrecht, The Netherlands: Springer; 2006.

[2] Genin S: Molecular traits controlling host range and adaptation to plants in Ralstonia solanacearum. The New phytologist 2010, 187 (4): 920-928.

[3] van Overbeek LS, Bergervoet JH, Jacobs FH, van Elsas JD: The Low-Temperature-Induced Viable-But-Nonculturable State Affects the Virulence of Ralstonia solanacearum Biovar 2. Phytopathology 2004, 94 (5): 463-469.

[4] Hayward AC: Biology and epidemiology of bacterial wilt caused by pseudomonas solanacearum. Annual review of phytopathology 1991, 29: 65-87.

[5] Yuliar, Nion YA, Toyota K: Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. Microbes and environments / JSME 2015, 30 (1): 1-11.

[6] Lopes MM, G. BE: Potato bacterial wilt management: new prospects for an old problem, in Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex edn: Saint Paul, MN: APS Press; 2005.

[7] Saddler GS: Management of bacterial wilt disease, in Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex edn: Saint Paul, MN: APS press; 2005.

[8] Zheng XA, Li XJ, Wang BS, Cheng D, Li YP, Li WH, Huang MH, Tan XD, Zhao GZ, Song BT, Macho AP, Chen HL, Xie CH: A systematic screen of conserved Ralstonia solanacearum effectors reveals the role of RipAB, a nuclear-localized effector that suppresses immune responses in potato. Molecular Plant Pathology, 2019, 20 (4), 547-561.

[9] Tampakaki AP, Skandalis N, Gazi AD, Bastaki MN, Sarris PF, Charova SN, Kokkinidis M, Panopoulos NJ: Playing the “Harpa”: evolution of our understanding of hrp/hrc genes. Annual review of phytopathology 2010, 48: 347-370.

[10] Hayashi K, Kai K, Mori Y, Ishikawa S, Uijita Y, Ohnishi K, Kiba A, Hikichi Y: Contribution of a lectin, LecM, to the quorum sensing signalling pathway of Ralstonia solanacearum strain OE1. Molecular Plant Pathology, 2019, 20 (3), 334-345.

[11] Genin S, Denny TP: Pathogenomics of the Ralstonia solanacearum species complex. Annual review of phytopathology 2012, 50: 67-89.

[12] Peeters N, Guidot A, Vailleau F, Valls M: Ralstonia solanacearum, a widespread bacterial plant pathogen in the post-genomic era. Molecular plant pathology 2013, 14 (7): 651-662.

[13] Mori T, Fujiyoshi T, Inada T, Matsusaki H, Ogawa K, Matsuzoe N: Phenotypic Conversion of Ralstonia solanacearum in Susceptible and Resistant Solanum Plants. Environment Control in Biology 2011, 49: 165-176.

[14] Zhang Y, Li JM, Zhang WQ, Shi HL, Luo F, Hikich Y, Shi XJ, Ohnishi K: A putative LysR type transcriptional regulator Pho positively regulates the type III secretion system and contributes to the virulence of Ralstonia solanacearum. Molecular Plant Pathology, 2018, 19 (8): 1808–1819.

[15] Khokhani D, Lowe-Power TM, Tran TM, Allen C: A Single Regulator Mediates Strategic Switching between Attachment/Spread and Growth/Virulence in the Plant Pathogen Ralstonia solanacearum. mBio, 2017, 8 (5): e00895-17.

[16] Jeong EL, Timmis JN: Novel insertion sequence elements associated with genetic heterogeneity and phenotype conversion in Ralstonia solanacearum. Journal of bacteriology 2000, 182 (16): 4673-4676.

[17] Poussier S, Thoquet P, Traigael-Demery D, Barhet S, Meyer D, Arlat M, Traigael A: Host plant-dependent phenotypic reversion of Ralstonia solanacearum from non-pathogenic to pathogenic forms via alterations in the phcA gene. Molecular microbiology 2003, 49 (4): 991-1003.

[18] Zheng X, Zhu Y, Liu B, Yu Q, Lin N: Rapid differentiation of Ralstonia solanacearum by cell fractioning of an isolate using high performance liquid chromatography. Microbial pathogenesis 2016, 90: 84-92.

[19] Kelman A, Hruschka J: The role of motility and aerotaxis in the selective increase of avirulent bacteria in still broth cultures of Pseudomonas solanacearum. Journal of general microbiology 1973, 76 (1): 177-188.

[20] Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE et al: Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nature methods 2013, 10 (6): 563-569.
[21] Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL: Versatile and open software for comparing large genomes. *Genome biology* 2004, 5 (2): R12.

[22] Stothard P, Wishart DS: Circular genome visualization and exploration using CGView. *Bioinformatics* 2005, 21 (4): 537-539.

[23] Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS: PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic acids research* 2016, 44 (W1): W16-21.

[24] Xie Z, Tang H: ISEScan: automated identification of insertion sequence elements in prokaryotic genomes. *Bioinformatics* 2017, 33 (21): 3340-3347.

[25] Tatusov RL, Fedorova ND, Jackson JD, Jacobs AR, K(Json B, Koonin EV, Krylov DM, Mazumder R, Medigue C, Mekhedov SL, Nikolskaya AN et al: The COG database: an updated version includes eukaryotes. *BMC bioinformatics* 2003, 4: 41.

[26] Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M: KAAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic acids research* 2007, 35 (Web Server issue): W182-185.

[27] Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G et al: InterProScan 5: genome-scale protein function classification. *Bioinformatics* 2014, 30 (9): 1236-1240.

[28] Edgar RC: MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research* 2004, 32 (5): 1792-1797.

[29] Kumar S, Stecher G, Tamura K: MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular biology and evolution* 2016, 33 (7): 1870-1874.

[30] Richter M, Rossello-Mora R: Shifting the genomic gold standard for the prokaryotic species definition. *Proceedings of the National Academy of Sciences of the United States of America* 2009, 106 (45): 19126-19131.

[31] Gerdes K, Christensen SK, Lohner-Olesen A: Prokaryotic toxin-antitoxin stress response loci. *Nature reviews Microbiology* 2005, 3 (5): 371-382.

[32] Van Melderen L: Toxin-antitoxin systems: why so many, what for? *Current opinion in microbiology* 2010, 13 (6): 781-785.

[33] Hayes F, Van Melderen L: Toxins-antitoxins: diversity, evolution and function. *Critical reviews in biochemistry and molecular biology* 2011, 46 (5): 386-408.

[34] Buts L, Lah J, Dao-Thi MH, Wyns L, Loris R: Toxin-antitoxin modules as bacterial metabolic stress managers. *Trends in biochemical sciences* 2005, 30 (12): 672-679.

[35] Goris J, Konstandinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM: DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *International journal of systematic and evolutionary microbiology* 2007, 57 (Pt 1): 81-91.

[36] Prior P, Ailloud F, Dalsing BL, Remenat B, Sanchez B, Allen C: Genomic and proteomic evidence supporting the division of the plant pathogen Ralstonia solanacearum into three species. *BMC genomics* 2016, 17: 90.

[37] Remenat B, de Cambiaire JC, Cellier G, Jacobs JM, Mangenot S, Barbe V, Lajus A, Vallenot D, Medigue C, Fegan M et al: Ralstonia syzygii, the Blood Disease Bacterium and some Asian R. solanacearum strains form a single genomic species despite divergent lifestyles. *PloS one* 2011, 6 (9): e24356.

[38] Remenat B, Coupe-Goutaland B, Guidot A, Cellier G, Wicker E, Allen C, Fegan M, Pruvost O, Elbaz M, Calteau A et al: Genomes of three tomato pathogens within the Ralstonia solanacearum species complex reveal significant evolutionary divergence. *BMC genomics* 2010, 11: 379.

[39] Cianciotto NP, White RC: Expanding Role of Type II Secretion in Bacterial Pathogenesis and Beyond. *Infection and immunity* 2017, 85 (5).

[40] Liu H, Zhang S, Schell MA, Denny TP: 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* 2005, 18 (12): 1296-1305.

[41] Genin S, Boucher C: Lessons learned from the genome analysis of ralstonia solanacearum. *Annual review of phytopathology* 2004, 42: 107-134.

[42] Ghosh P: Process of protein transport by the type III secretion system. *Microbiology and molecular biology reviews: MMBR* 2004, 68 (4): 771-795.

[43] Hueck CJ: Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiology and molecular biology reviews: MMBR* 1998, 62 (2): 379-433.

[44] Hacker J, Carniel E: Ecological fitness, genomic islands and bacterial pathogenicity. A Darwinian view of the evolution of microbes. *EMBO reports* 2001, 2 (5): 376-381.

[45] Silverman JM, Brunet YR, Cascales E, Mougous JD: Structure of ralstonia solanacearum. *Molecular plant-microbe interactions: MPMI* 2005, 18 (12): 1296-1305.

[46] Zhang L, Xu J, Zhang H, He L, Feng J: TssB is essential for virulence and required for type VI secretion system in Ralstonia solanacearum. *Microbial pathogenesis* 2014, 74: 1-7.

[47] Hikichi Y, Mori Y, Ishikawa S, Hayashi K, Ohnishi K, Kiba A, Kai K: Regulation Involved in Colonization of Intercellular Spaces of Host Plants in Ralstonia solanacearum. *Frontiers in plant science* 2017, 8: 967.