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Genome Announcement

Genome sequencing of four strains of Phylotype I, II and IV of *Ralstonia solanacearum* that cause potato bacterial wilt in India

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ABSTRACT

Ralstonia solanacearum is a heterogeneous species complex causing bacterial wilts in more than 450 plant species distributed in 54 families. The complexity of the genome and the wide diversity existing within the species has led to the concept of *R. solanacearum* species complex (RsSC). Here we report the genome sequence of the four strains (RS2, RS25, RS48 and RS75) belonging to three of the four phylotypes of *R. solanacearum* that cause potato bacterial wilt in India. The genome sequence data would be a valuable resource for the evolutionary, epidemiological studies and quarantine of this phytopathogen.

Genome announcement

Ralstonia solanacearum[1,2] formally known as *Pseudomonas solanacearum* and *Burkholderia solanacearum* is a gram-negative, chemo-organotroph phytopathogenic β-proteobacterium with an unusual broad host range.[3] The pathogen not only affects solanaceous but many plants of other dicot and monocot families. The extensive genetic diversity of strains responsible for various wilt diseases has in recent years led to the concept of an *R. solanacearum* "species complex" (RsSC).[4] As *R. solanacearum* strains have been isolated from virgin forest-soils of all five continents, the origin of the species complex is believed to predate the geographical separation of continents.[5] The pathogen is hierarchically classified into four phylotypes according to newly proposed phylotype sub-classification system based on 16S-23S ITS region, *egl* and *hrpB* genes and on comparative genomic hybridization (CGH) which reflect their origin as Asia (Phylotype I), America (II), Africa (III) or Indonesia (IV).[6] These phylotypes are

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Table 1 – General features and distribution of CDS, tRNA, rRNA, regulatory genes and pathogenic genes between Chromosome and Megaplsmid of *R. solanacearum* strains including Rs2, Rs25, Rs48 and Rs75.

Strain	Origin	Isolated from	Phylotype[a]	Sequence status	Genome size (Kb)[b]			GC%[c]	CDS[d]	rRNA[e]	tRNA[f]	T3E[g]	Reference
					CHR	MPL	Total						
Grenada9-1	Grenada	Banana	IIA(6)	Draft	NA	NA	5479	66.60	5365	3[h]	56[h]	NA	9
IBSBF1503	Peru	Cucumber	II(4)NPB	Draft	NA	NA	5514	66.70	5452	3[h]	54[h]	NA	
CBF1416	Costa Rica	Plantain	IIB(3)	Draft	NA	NA	5744	66.60	5722	3[h]	59[h]	NA	
Rs-09-161	India	Eggplant	I/R1b3	Complete	3741	1985	5726	66.82	5213	3[h]	66[h]	71	10
Rs-10-244	India	Chilli	I/R1b3	Complete	3716	2025	5741	66.98	5202	3[h]	63[h]	76	
RS-2[i]	**Indore (MP), India**	**Potato**	**IIB(1)**	**Draft**	**3481**	**1608**	**4768**	**57.36**	**4590**	**2**	**53**	**63**	**This study**
RS-25[j]	**Shimla (HP), India**	**Potato**	**I(45)**	**Draft**	**3065**	**1950**	**5232**	**60.11**	**4732**	**3**	**57**	**91**	
RS-48[j]	**Shimla (HP), India**	**Potato**	**I(30)**	**Draft**	**3065**	**1727**	**5300**	**60.10**	**4817**	**3**	**57**	**89**	
RS-75[i]	**Shillong (Meghalaya), India**	**Potato**	**IV(8)**	**Draft**	**2903**	**1720**	**5045**	**60.06**	**4867**	**2**	**54**	**73**	
GMI1000	Fr. Guyana	Tomato	I (18)	Complete	3716	2094	5811	67.00	5120	4	57	74	11
Y45	China	Tomato	IB	Draft	3726	1986	5712	NA	5496	5	53	ND	12
FYQ_4	China	Tomato	I	Complete	3715	2089	5805	66.82	5153	9	62	ND	13
K60	USA	Tomato	IIA(7)	Draft	3717	1773	5490	66.70	5213	3[h]	51[h]	ND	14
CFBP2957	Fr. West Indies	Tomato	IIA(36)	Complete	3539	2144	5683	69.90	5310	1	56	72	15
Molk2	Phillippines	Banana	IIB(3)	Draft	NA	NA	5961	66.70	5061	1	34	75	
CMR15	Cameroon	Tomato	III(29)	Complete	3594	1963	5593	69.90	5149	3	59	67	
Psi07	Indonesia	Tomato	IV(10)	Complete	3508	2085	5606	66.30	5247	1	49	74	
Po82	Mexico	Potato	IIB(4)	Complete	3481	1949	5430	66.65	5019	3	54	75	16
BDB R229	Indonesia	Banana	IV	Draft	3574	1585	5159	66.50	4629	1	45	57	17
R. syzygii R24	Indonesia	Clove	IV	Draft	3681	1743	5424	65.90	4867	2	50	48	

[a] Sequevar numbers are in parenthesis.
[b] CHR – chromosome, MLP – Megaplsmid, ND – not available.
[c] G + C content in percent.
[d] CDS – number of coding sequences.
[e] Number of genes coding for ribosomal RNAs.
[f] Number of genes coding for transfer RNAs.
[g] Number of predicted type III effectors.
[h] ARAGON tRNA detection.
[i] Isolated from the infected stem.
[j] Isolated from infected tubers.

further classified into sequevars, containing isolates with similar virulence patterns or common geographic origin.[3] Despite their considerable diversity, *R. solanacearum* strains are unified by their common etiology resulting in disease.[7] Three of the four phylotypes of *R. solanacearum* are known to cause bacterial wilt of potato in India.[8] In the present study four strains, RS2 (Phylotype II), RS25 and RS48 (Phylotype I) and RS75 (Phylotype IV) isolated from brown-rot infected potato tubers obtained from different parts of the country were taken for complete genome sequencing and to analyze their relationship complexity.

We sequenced the genomes of all four strains using shotgun approach and Roche-454, GSflx-Titanium platform yielding appx. 2.88 million reads (>500 bp) of which nearly 99.5% reads were of high quality. The genome coverage ranged from 18X (RS48) to 76X (RS75). The high quality reads were aligned using GS *De Novo* Assembler (version 2.5.3) and gene prediction using the prokaryotic GeneMark.hmm (Version 2.2a) and AUGUSTUS (http://bioinf.uni-greifswald.de/augustus/submission/) revealed a total of 4590, 4732, 4817 and 4867 protein coding regions (CDSs) respectively for RS2, RS25, RS48 and RS75 spread over megaplasmid as well as chromosomal genomes. High quality reads were mapped on to publically available reference genomes, GMI1000 (RS25 & RS48), Po82 (RS2) and PSI07 (RS75) (www.ncbi.nlm.nih.gov/genome/) using gsMapper with optimized mapping parameters and obtained total genome coverage and per cent GC content for all four strains. The total protein coding regions, rRNA and tRNA coding, regulatory and pathogenicity genes including the Type III secretary genes were obtained from the consensus using the .gff (from public database) file with the help of in-house perl scripts (Table 1). The presence of repetitive elements was analyzed using MISA (http://pgrc.ipk-gatersleben.de/misa/) and was observed that nearly 90% of the elements were of di or tri and 7.3% hexa mer repeats. Chromosomes carried higher portion (60–70%) of the repeat elements than megaplsmids in all the four strains. The availability of the reference genomes of more and more strains of *RsSC* would greatly aid in epidemiological/quarantine studies and in gaining understanding on their origin, evolution, intra and inter-relationship within the complex and their interactions with plants.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at NCBI/GenBank under Bio-Project PRJNA221562 with Accession Nos. SRX360515, SRX365373, SRX365374 and SRX365375.

Conflicts of interest

The authors declare no conflicts of interest.

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