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Simple sequence repeats and compositional bias in the bipartite *Ralstonia solanacearum* GM11000 genome

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Abstract

Background: *Ralstonia solanacearum* is an important plant pathogen. The genome of *R. solanacearum* GM11000 is organised into two replicons (a 3.7-Mb chromosome and a 2.1-Mb megaplasmid) and this bipartite genome structure is characteristic for most *R. solanacearum* strains. To determine whether the megaplasmid was acquired via recent horizontal gene transfer or is part of an ancestral single chromosome, we compared the abundance, distribution and composition of simple sequence repeats (SSRs) between both replicons and also compared the respective compositional biases.

Results: Our data show that both replicons are very similar in respect to distribution and composition of SSRs and presence of compositional biases. Minor variations in SSR and compositional biases observed may be attributable to minor differences in gene expression and regulation of gene expression or can be attributed to the small sample numbers observed.

Conclusions: The observed similarities indicate that both replicons have shared a similar evolutionary history and thus suggest that the megaplasmid was not recently acquired from other organisms by lateral gene transfer but is a part of an ancestral *R. solanacearum* chromosome.

Background

The paradigm that bacterial genomes consist of a single circular chromosome is no longer valid. Linear chromosomes have been identified in *Borrellia burgdorferi* [1], various *Streptomyces* species [2,3], *Agrobacterium tumefaciens* [4] and various other species. In addition, it is now appreciated that genomes of several bacterial taxa consist of multiple replicons. Most organisms with a multi- or bipartite genome structure belong to the α -Proteobacteria (including *Rhodobacter sphaeroides* [5,6] and various *Rhizobium* [7,8], *Agrobacterium* [4,8], *Brucella* [9,10] and *Azospirillum* [11] species) or the β -Proteobacteria. Most isolates from species belonging to the β -proteobacterial genera *Burkholderia* and *Ralstonia* harbour multiple replicons, including members of the *Burkholderia cepacia* complex

[12–16], *Burkholderia gladioli* [15], *Burkholderia pseudomallei* [17], *Burkholderia glumae* [13], *Burkholderia glathei* [13], *Burkholderia* sp. LB400 [18], *Ralstonia pickettii* [13], *Ralstonia eutropha* [13] and *Ralstonia metallidurans* [18]. Multiple replicons may have arisen from the need to achieve higher overall replication rates [19]. The origin of these multiple replicons is at present unclear but it has been suggested that they could have their origin in gene duplication followed by divergence; in this case intrachromosomal recombinational events within a duplicated region could give rise to the formation of two stable replicons [8]. In the genus *Brucella* these rearrangements have occurred in the region containing the ribosomal RNA genes [10] but in theory the rearrangements can occur at any repeated sequence [20]. An additional

explanation is that the presence of multiple replicons within an organism involved horizontal DNA transfer [19,21,22]. This hypothesis was used to explain the presence of two chromosomes in *Vibrio cholerae*: the small chromosome was suggested to be derived from a megaplasmid captured by an ancestral *Vibrio* [23,24]. This megaplasmid probably acquired genes from diverse bacterial species before its capture by the ancestral *Vibrio*; subsequent relocation of essential genes from the chromosome to the megaplasmid completed its stable structure.

Ralstonia solanacearum is a soil-borne phytopathogen with an unusually broad host-range, causing bacterial wilt on a wide range of crops, including economically important crops like potato, tomato, ginger and banana [25]. Recently the genome sequence of *R. solanacearum* strain GMI1000 was determined [26]. It was shown that the 5.8-Mb genome is organised into two replicons, a 3.7-Mb chromosome and a 2.1-Mb megaplasmid. This bipartite genome structure is characteristic for most *R. solanacearum* strains [27] and derivatives of strain GMI1000 without the megaplasmid have not been obtained [26]. The larger replicon contains all the basic genes required for survival of the bacterium; the smaller replicon carries several metabolically essential genes also present on the chromosome (including a rDNA locus, a gene coding for the α -subunit of DNA polymerase III and the gene for protein elongation factor G) but also contains several genes coding for enzymes involved in primary metabolism (including amino acid and cofactor biosynthesis) not present on the chromosome. The smaller replicon also contains all the *hrp* genes (required to cause disease in plants) and it has been suggested that it has a significant function in overall fitness and adaptation of the organism to various environmental conditions [26]. The origin of the bipartite genome structure of *R. solanacearum* is not clear. To determine whether the megaplasmid was formed through intrachromosomal recombinational events within a duplicated region or was recently acquired from other organisms we compared the abundance, distribution and composition of simple sequence repeats between the chromosome and the megaplasmid of *R. solanacearum* GMI1000. We also compared the compositional bias of di- and tetranucleotides between both replicons.

Repeated DNA consists of homopolymeric tracts of a single nucleotide or of small or large numbers of multimeric classes of repeats. These multimeric repeats can be homogenous (i.e. built from identical units), heterogeneous (i.e. built from mixed units) or are built from degenerate repeat sequence motifs [28]. A special category of repeats are tandem repeats which are made up of periodically repeated monomeric sequences of varying length, arranged in a 'head-to-tail' configuration [29]. Several mechanisms have been proposed for the creation of tandem repeats, in-

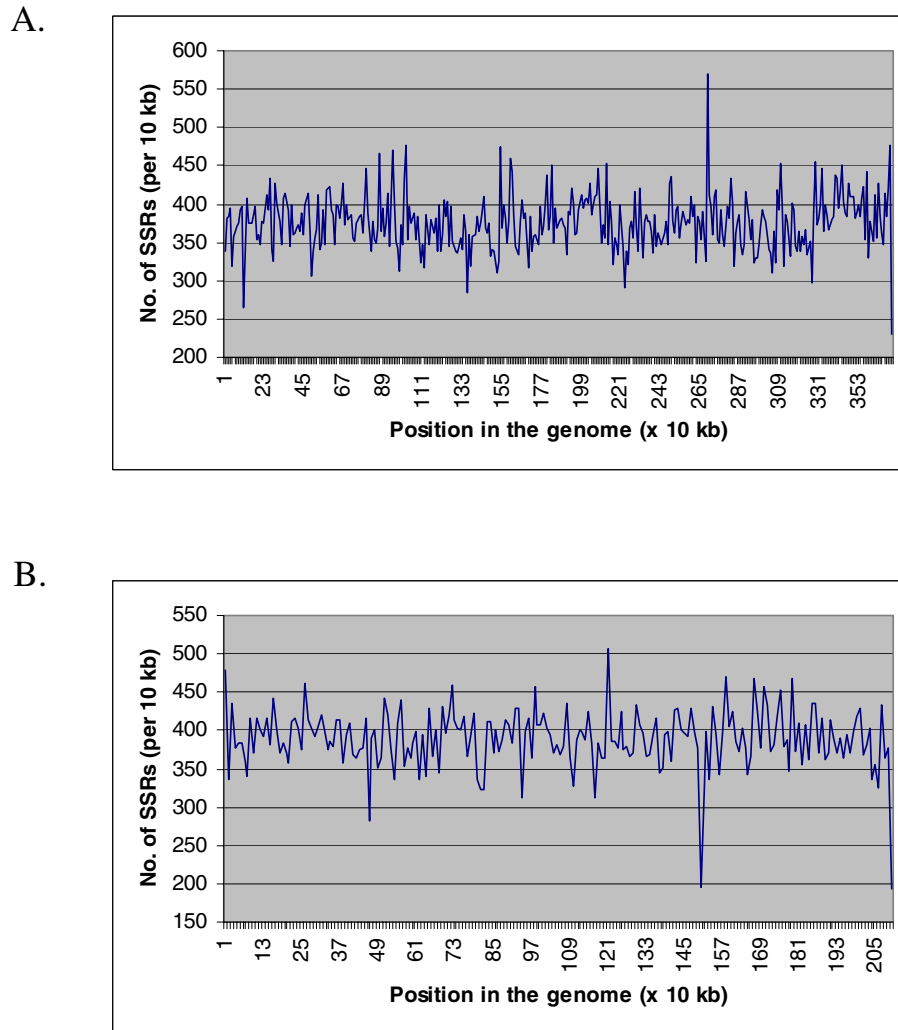
cluding 'slipped strand mispairing' in which illegitimate base-pairing during replication gives rise to addition of repeat units [30,31]. There is growing evidence that small tandem repeats (also called simple sequence repeats or SSRs) affect gene expression. A first effect of SSRs is the mediation of phase variation through the loss or gain of one or more repeats [29]. Phase variation is the process by which many bacterial species undergo reversible phenotypic changes resulting from genetic alterations in certain loci [32,33]. SSRs can also be involved in gene regulation by affecting spacing between flanking regions [34] or spacing between the -35 and -10 promoter regions [35]. Variation in abundance, distribution and composition of SSRs has been described [28] and it has been proposed that variation in SSR results in variation in gene expression and key phenotypes and hence provides an important target for natural selection and evolution [28,36].

The comparison of genome-wide compositional biases as a tool to study bacterial evolution has been introduced by Karlin and co-workers [37–39]. It is thought that dinucleotide relative abundance values are constant within a genome because the factors that work on them are constant throughout the genome; and it has been postulated that the set of dinucleotide relative abundance values constitute a genomic signature that reflects the pressures of these factors [38]. Differences in genome signature between different organisms can be attributed to differences in context-dependent mutation rates generated by the replication-repair system and differences in efficiency of the replication machinery on different sequences. In addition, many DNA structural properties (including curvature, flexibility and helix stability), which may play an important role in biological processes like replication, are determined by dinucleotide arrangements [38,40]. Tetranucleotide relative abundances are also characteristic for a given genome [39]. It has been postulated that frequent tetranucleotides may include parts of repetitive structural, regulatory and transposable elements, while low values for some palindromic tetranucleotides have been attributed to restriction avoidance [39].

Results

Distribution and composition of SSRs in the *R. solanacearum* genome

A total of 221729 SSRs with a motif length between 1 and 10 bp and minimum three repeats were found in the entire *R. solanacearum* genome. Of those, 139993 (63.14%) were located on the chromosome (Table 1) and 81736 (36.86%) were located on the megaplasmid (Table 2). This corresponds well with the size distribution between both replicons (63.96% of all bases are in the chromosome, 36.04% are in the megaplasmid). The SSRs were evenly distributed both over the chromosome as over the megaplasmid (Fig. 1). The total number of repeats is

**Figure 1**

Distribution of simple sequence repeats in the *R. solanacearum* chromosome (panel A) and megaplasmid (panel B).

lower than expected by chance; especially the number of mononucleotide repeats is significantly lower than expected (Tables 1 and 2). Trinucleotide repeats occur more than expected by chance alone, both in the chromosome and the megaplasmid (Tables 1 and 2). Mononucleotide repeats of length = 3 bp and dinucleotide repeats are dis-

tributed over coding and non-coding regions as expected, both in the chromosome and the megaplasmid. As mononucleotide repeats become larger, there is more and more deviation from the expected distribution; these larger mononucleotide repeats are almost exclusively located in non-coding regions. Our data also show that trinucle-

Table 1: Number of simple sequence repeats of given structure in the chromosome of *R. solanacearum* GM11000

No. of repeats	Motif length										Total
	1	2	3	4	5	6	7	8	9	10	
3	98068 ⁻	12307 ⁺	3712 ⁺	90	21	11	1	-	3	1	114214 ⁻
4	17943 ⁻	2122 ⁺	213 ⁺	-	-	-	-	-	-	-	20278 ⁻
5	4053 ⁻	277	17	-	-	-	-	-	2	-	4349 ⁻
6	859 ⁻	18	-	-	1	-	-	1	1	-	880 ⁻
7	199 ⁻	3	-	-	1	1	-	-	1	-	205 ⁻
8	45 ⁻	-	-	-	-	-	-	-	-	-	45 ⁻
9	14 ⁻	-	-	-	-	-	-	-	-	-	14 ⁻
10	2	-	-	-	-	-	-	-	-	-	2
11	4	-	-	-	-	-	-	-	-	-	4
12	-	-	-	-	-	-	-	-	-	-	-
13	2	-	-	-	-	-	-	-	-	-	2
Total	121189 ⁻	14727 ⁺	3942 ⁺	90	23	12	1	1	7	1	139993 ⁺

⁺ significantly overrepresented compared to mean frequencies in computer-generated randomised genomes ($P < 0.001$) ⁻ significantly underrepresented compared to mean frequencies in computer-generated randomised genomes ($P < 0.001$)

Table 2: Number of simple sequence repeats of given structure in the megaplasmid of *R. solanacearum* GM11000

No. of repeats	Motif length										Total
	1	2	3	4	5	6	7	8	9	10	
3	57345 ⁻	6440	1986 ⁺	49	10	8	-	3	-	-	65841 ⁻
4	11216 ⁻	1096	108 ⁺	2	1	4	2	2	2	-	12433 ⁻
5	2560 ⁻	146	10	-	-	2	-	3	-	-	2721 ⁻
6	529 ⁻	9	1	-	-	2	-	1	-	-	542 ⁻
7	141 ⁻	1	-	-	-	-	-	1	-	-	143
8	41 ⁻	1	-	-	-	-	-	-	-	-	42 ⁻
9	11	-	-	-	-	-	-	-	-	-	11
10	2	-	-	-	-	-	-	-	-	-	2
11	1	-	-	-	-	-	-	-	-	-	1
Total	71846 ⁻	7693	2105 ⁺	51	11	16	2	4	8	0	81736

⁺ significantly overrepresented compared to mean frequencies in computer-generated randomised genomes ($P < 0.001$) ⁻ significantly underrepresented compared to mean frequencies in computer-generated randomised genomes ($P < 0.001$)

otides are overrepresented in protein-coding regions of both replicons (Table 3). The nucleotide composition of the SSR tracts in the *R. solanacearum* chromosome and megaplasmid are shown in Tables 4 and 5, respectively. Our data show that (i) the G+C composition of mononucleotide repeats in both replicons is significantly lower than the overall composition, but this difference can exclusively be attributed to non-coding regions; (ii) G and C mononucleotide repeats are underrepresented in coding and non-coding regions of both replicons and (iii) CG and GC dinucleotide repeats are vastly overrepresented both in coding and non-coding regions of both replicons, while other dinucleotide repeats are underrepresented.

Compositional biases in the *R. solanacearum* genome

Dinucleotide relative abundances are shown in Table 6. The dinucleotides TA and AT are strongly underrepresented in both replicons while GC is moderately overrepresented in both replicons. CC and GG are moderately underrepresented in the chromosome. The average absolute dinucleotide relative abundance difference (δ^*) between both replicons is 9.78. To assess the variability of dinucleotide relative abundances within a replicon, both replicons were divided into 12 and 7 (for the chromosome and the megaplasmid, respectively) equally-sized, nonoverlapping fragments and ρ^*_{XY} values were calculated for each fragment. $\delta^*(f,g)$ values within repli-

Table 3: Distribution of simple sequence repeats among protein coding and non-coding regions of the *R. solanacearum* genome

	Total no.	Chromosome				Megaplasmid				
		Coding regions		Non-coding regions		Total no.	Coding regions		Non-coding regions	
		No.	%	No.	%		No.	%	No.	%
Mononucleotides										
3 bp	98068	81805	83.4	16263	16.6	57345	47087	82.1	10258	17.9
4 bp	17943	13192	73.5	4751	26.5	11216	8509	75.9	2707	24.1
5 bp	4053	2555	63.0	1498	37.0	2560	1752	68.4	808	31.6
6 bp	859	373	43.4	486	56.6	529	282	53.3	247	46.7
7 bp	199	62	31.2	137	68.8	141	49	34.8	92	65.2
8 bp	45	7	15.6	38	84.4	41	16	39.0	25	61.0
9 bp	14	1	7.1	13	92.9	11	3	27.3	8	72.7
10 bp	2	1	50.0	1	50.0	2	0	0	2	100
11 bp	4	-	0	4	100	1	0	0	2	100
13 bp	2	-	0	4	100	-	-	-	-	-
Dinucleotides ≥ 6 bp	14727	13090	88.9	1637	11.1	7693	6699	87.1	994	12.9
Trinucleotides ≥ 9 bp	3942	3672	93.2	270	6.8	2105	1933	91.8	172	8.2
Tetranucleotides	90	77	85.6	13	14.4	51	37	72.5	14	27.5
Genome partition			87.8		12.2			86.5		13.5

Table 4: Nucleotide composition of simple sequence repeats in the *R. solanacearum* chromosome

	Total		Coding		Non-coding	
	No.	%	No.	%	No.	%
Genome composition						
A	608615	16.37	543922	16.62	64693	14.53
C	1238438	33.32	1112352	34.00	126086	28.31
G	1252933	33.71	1099390	33.60	153543	34.47
T	616427	16.58	515362	15.75	101065	22.70
Mononucleotide SSRs ≥ 6 bp						
Total	1125		444		681	
A	299	26.58	109	24.55	190	27.90
C	220	19.56	106	23.87	114	16.74
G	261	23.20	153	34.46	108	15.86
T	345	30.67	76	17.12	269	39.50
G+C	481	42.76	259	58.33	222	32.60
A+T	644	57.24	185	41.67	459	67.40
Dinucleotide SSRs ≥ 6 bp						
Total	14764		13121		1643	
AC/CA	278	1.88	205	0.15	73	4.44
AG/GA	205	1.39	80	0.61	125	7.61
AT/TA	47	0.32	11	0.08	36	2.19
CG/GC	13710	92.86	12521	95.43	1189	72.37
CT/TC	210	1.42	130	0.99	80	4.87
GT/TG	323	2.19	174	1.33	149	9.07

cons ranged from 6.63 to 31.77 (mean \pm standard deviation: 14.49 ± 5.35) (for the chromosome) and from 4.83 to 20.63 (13.11 ± 4.55) (for the megaplasmid). These differences are not significantly smaller than the between-replicon differences (data not shown). Significantly over- or underrepresented tetranucleotides are shown in Table 7. CTAG, AATT, CATG, GATA and TATA are underrepresented in both replicons. GTAG and TTAA are overrepresented in both replicons.

Discussion

To study the origin of the bipartite genome structure of *R. solanacearum* GMI1000 we compared the abundance, distribution and composition of simple sequence repeats and differences in compositional biases between the chromosome and the megaplasmid of *R. solanacearum* GMI1000.

Occurrence of simple sequence repeats

Our data clearly show that the *R. solanacearum* genome contains numerous SSRs with a motif length between 1

and 10 bp, although not as many as expected by chance alone. Mutations in SSRs are thought to be the result of slipped strand mispairing during DNA replication; slipped strand mispairing can occur because the tertiary structure of SSRs allows mismatching and repeats can be inserted or excised during DNA duplication [41–43]. The observation of upper limits for SSR length in *Escherichia coli* suggested that the tendency for repeat length to arise via mutation is counteracted by selection [36]. We observed similar upper limits: the upper limit for total length of mononucleotide SSRs is 13 bp and 11 bp for the chromosome and megaplasmid, respectively and, in addition, very few other SSRs with a total length >15 bp (for the chromosome) or >18 bp (for the megaplasmid) are observed. Both strand separation and slippage are more likely for mononucleotide SSRs, explaining why mononucleotide SSRs are more likely to undergo slipped strand mispairing; longer SSRs with a lower repeat number have less opportunity to undergo slipped strand mispairing and there will be less mutability in their repeat number [36]. This may explain why larger mononucleotide SSRs are overrepresented in non-coding regions of the *R. solanacearum* genome as selection has ample opportunity to operate against these larger repeats that cause frameshift and nonsense mutations in coding regions. This hypothesis is supported by the fact that poly(A) and poly(T) SSRs are overrepresented, especially in the non-coding regions, in both replicons (Tables 4 and 5): strand separation for these poly(A) and poly(T) tracts is considerably easier than for poly(G) or poly(C) tracts, increasing the possibility of slipped strand mispairing.

Compositional biases

The dinucleotide TA is underrepresented in both replicons. TA is underrepresented in almost all prokaryotic genomes; this could be due to the fact that (i) TA forms the thermodynamically least stable DNA (allowing unwinding of the helix), (ii) RNases preferentially degrade UA dinucleotides in mRNA, and/or (iii) TA is part of many regulatory sequences [38]. AT is significantly underrepresented in the *R. solanacearum* genome but is overrepresented in the genome of most α -Proteobacteria and in the genomes of the β -proteobacterial species *R. eutropha* and *Bordetella pertussis* [39]. CC and GG are slightly underrepresented in the chromosome but not in the megaplasmid, although the differences in relative abundances are small (Table 6). The dinucleotide GC is overrepresented in both replicons; this is also the case in most other β -Proteobacteria and γ -Proteobacteria [39]. In general, within species $\delta^*(f,g)$ -differences among nonoverlapping 50 kb contigs of bacteria are in the range 18–43 [39] and genome signatures of chromosomes and plasmids from the same host are at least weakly similar to each other [$\delta^*(f,g) < 115$] [44,45]. $\delta^*(f,g)$ values reported for the multiple chromosomes of *A. tumefaciens*, *Deinococcus radiodurans*,

V. cholerae and *B. melitensis* were between 27.0 and 30.8 [45]. A comparison of both *R. solanacearum* replicons based on dinucleotide relative abundances indicates that they are very similar with $\delta^*(f,g) = 9.78$. A comparison of $\delta^*(f,g)$ values within and between replicons revealed that the variability in $\delta^*(f,g)$ within a replicon is not significantly smaller than the difference in $\delta^*(f,g)$ between both replicons. CTAG is significantly underrepresented in the *R. solanacearum* genome as it is in most proteobacterial organisms. Possible reasons for the underrepresentation of this tetranucleotide include structural defects or special functional roles associated with CTAG [38]. AATT, CATG, GATA and TATA are underrepresented in both replicons while GTAG and TTAA are overrepresented. ATTG, CATC and TTGG occur slightly less than expected in the megaplasmid but their relative abundance in the chromosome is in the normal range. The general mechanisms underlying tetranucleotide extremes are unclear but besides the above-mentioned structural defects or functional roles associated with specific tetranucleotides, it has been suggested that restriction avoidance may play an important role in the maintenance of tetranucleotide extremes [39]).

Conclusions

It can be concluded that both replicons that constitute the *R. solanacearum* genome are very similar in respect to distribution and composition of SSRs and presence of compositional biases, although minor differences between both replicons are present. The megaplasmid carries the *hrp* genes required to cause disease in plants, genes coding for constituents of the flagellum and genes involved in exopolysaccharide production; it also contains 315 genes of unknown function [26]. The minor variations in SSR and compositional biases observed between both replicons may therefore be attributable to minor differences in gene expression and regulation of gene expression between both replicons. Alternatively, it is not unlikely that some of the observed differences are the result of the small sample numbers observed (for example the minor differences in tetranucleotide SSR distribution over coding and non-coding regions in both replicons [Table 3]). At present no completely sequenced and fully annotated genomes of other β -Proteobacteria with multiple replicons are available for comparison and therefore it is difficult to place the observed differences in a broader perspective. Nevertheless, the observed similarities in SSRs and compositional biases indicate that both replicons have shared a similar evolutionary history and suggest that the megaplasmid was not recently acquired from other organisms by lateral gene transfer but is a part of an ancestral *R. solanacearum* chromosome. Alternatively, the hypothesis of an ancient acquisition by lateral gene transfer followed by a long co-evolution with the chromosome cannot be completely ruled out.

Table 5: Nucleotide composition of simple sequence repeats in the *R. solanacearum* megaplasmid

	Total		Coding		Non-coding	
	No.	%	No.	%	No.	%
Genome composition						
A	347472	16.58	301622	16.62	45850	16.34
C	699983	33.41	613428	33.81	86555	30.83
G	700536	33.44	610348	33.64	90188	32.13
T	346518	16.54	288441	15.90	58077	20.69
Mononucleotide SSRs ≥ 6 bp						
Total	722		350		372	
A	172	23.82	83	23.71	89	23.93
C	169	23.41	94	26.86	75	20.16
G	197	27.29	130	37.14	67	18.01
T	184	25.49	43	12.29	141	37.90
G+C	366	50.69	224	64.00	142	38.17
A+T	356	49.31	126	36.00	230	61.83
Dinucleotide SSRs ≥ 6 bp						
Total	7708		6727		981	
AC/CA	190	2.47	143	2.13	47	4.79
AG/GA	135	1.75	71	1.06	64	6.52
AT/TA	38	0.49	15	0.22	23	2.35
CG/GC	7127	92.46	6297	93.61	830	84.61
CT/TC	129	1.67	82	1.22	47	4.79
GT/TG	166	2.15	119	1.77	47	4.79

Table 6: Dinucleotide relative abundances in the *R. solanacearum* genome

XY	ρ* _{XY}	Chromosome		Megaplasmid	
			Over/under represented?		Over/under represented?
AA	1.0713			1.0738	
AC	0.9014			0.8928	
AG	0.8775			0.8722	
AT	0.6787		--	0.6957	--
CA	1.1702			1.1778	
CC	0.7750		-	0.7879	
CG	1.2029			1.1880	
CT	0.8775			0.8722	
GA	1.0601			1.0446	
GC	1.2454		+	1.2440	+
GG	0.7750		-	0.7879	
GT	0.9014			0.8928	
TA	0.4624		---	0.4808	---
TC	1.0601			1.0446	
TG	1.1702			1.1778	
TT	1.0713			1.0738	

Methods

DNA sequences

The sequences of the chromosome (AL646052) and the megaplasmid (AL646053) of *R. solanacearum* strain GMI1000 were downloaded from the GenBank database.

Analysis of SSRs

We used the software developed by Gur-Arie et al. [36] to screen the entire genome of *R. solanacearum* for SSRs with a motif length between 1 and 10 bp and a minimal number of three repeats. This software can be downloaded from ftp://ftp.technion.ac.il/supported/biotech/ssr.exe and reports motif, motif length, repeat number and genomic location of all SSRs. To determine whether the ob-

Table 7: Singificantly over- or underrepresented tetranucleotides in the *R. solanacearum* genome

XYZW	Chromosome		Megaplasmid	
	τ^*_{XYZW}	Over/under represented?	τ^*_{XYZW}	Over/under represented?
AATT	0.6965	--	0.6560	--
ATTG	1.0732		0.7270	-
CATC	0.7878		0.7738	-
CATG	0.7129	-	0.7167	-
CTAG	0.2747		0.3442	
GATA	0.7165	-	0.7182	-
GTAG	1.2469	+	1.3802	++
TATA	0.7440	-	0.6723	--
TTAA	1.3314	++	1.4219	++
TTGG	0.8569		0.6476	--

served SSR frequencies of a given motif length and repeat number occurred as expected by chance, they were compared with the mean frequencies observed in three randomly shuffled genomes. Randomised sequences were generated with *shuffleseq* (part of the EMBOSS package, <http://www.hgmp.mrc.ac.uk/software/EMBOSS>). Statistical significance was tested with two-tailed *t*-tests using SPSS 11.0.1 (SPSS). To determine the distribution of SSRs between coding and non-coding regions of the genome, all coding regions were extracted from the sequence using Artemis 4.0 [46] and parsed into a new sequence file using *seqret* (EMBOSS).

Analysis of compositional bias

We determined the compositional bias in di- and tetranucleotides in the chromosome and megaplasmid of *R. solanacearum* GMI1000. Both sequences were concatenated with their inverted complementary sequence using *revseq*, *yank* and *union* (EMBOSS). Mononucleotide frequencies were calculated using Artemis 4.0 [46], di-, tri- and tetra-nucleotide frequencies were calculated using *compseq* (EMBOSS). Dinucleotide relative abundances ρ^*_{XY} were calculated using the equation $\rho^*_{XY} = f_{XY}/f_X f_Y$ where f_{XY} denotes the frequency of dinucleotide XY and f_X and f_Y denote the frequencies of X and Y, respectively [38]. Similarly, the corresponding fourth-order oligonucleotide measures (which factor out all lower-order biases) is given by $\tau^*_{XYZW} = (f^*_{XYZW} f^*_{XY} f^*_{XNZ} f^*_{XN1N2W} f^*_{YZ} f^*_{YNW} f^*_{ZW}) / (f^*_{XYZ} f^*_{XYNW} f^*_{YZW} f^*_{X} f^*_{Y} f^*_{Z} f^*_{W})$ where N is any nucleotide and X, Y, Z and W are each one of A, C, G and T [38]. Statistical theory and data from previous studies [38,39] indicate that the normal range of ρ^*_{XY} is between 0.78 and 1.23. In this study we used the refined criteria of discrimination proposed by Karlin et al. [38]. Overrepresentation is indicated by + ($1.23 = \rho^* < 1.30$), ++ ($1.30 = \rho^* < 1.50$) and +++ ($\rho^* \geq 1.50$), while underrepresentation is indicated by - ($0.70 < \rho^* = 0.78$), -- ($0.50 < \rho^* = 0.70$)

and --- ($\rho^* = 0.50$). The dissimilarities in relative abundance of dinucleotides between both sequences were calculated using the equation described by Karlin et al. [38]: $\delta^*(f,g) = 1/16 \sum |\rho^*_{XY}(f) - \rho^*_{XY}(g)|$ (multiplied by 1000 for convenience), where the sum extends over all dinucleotides. To assess the variability of dinucleotide relative abundances within a replicon, both replicons were divided into 12 and 7 (for the chromosome and the megaplasmid, respectively) non-overlapping fragments and ρ^*_{XY} values were calculated for each fragment. The average $\delta^*(f,g)$ within each replicon was also calculated.

List of Abbreviations

SSR : simple sequence repeat

Authors' Contribution

TC conceived the study and carried out the computational analyses. PV participated in experimental design. Both authors read and approved the final manuscript.

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