Heterogeneity of Genome Sizes within the Genus *Spiroplasma*

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Organisms belonging to the genus *Spiroplasma* are currently classified into 23 groups, 17 of which have been assigned species epithets. We determined the genome sizes of representatives of 20 groups by using pulsed-field gel electrophoresis. Each genome size was deduced from the mobility of linear nonrestricted DNA, as well as from the sum of the sizes of restriction fragments obtained after digestion with *NotI*, a restriction endonuclease with a limited number of restriction sites in *spiroplasma* DNA. The values which we obtained indicated that the genome sizes of members of the genus *Spiroplasma* range from 940 to 2,220 kbp.

Organisms belonging to the class *Mollicutes* are among the simplest self-replicating organisms known. These wall-less eubacteria are characterized by small genomes that have low DNA guanine-plus-cytosine (G+C) contents. The class *Mollicutes* is currently divided into eight genera: *Asteroleplasma*, *Anaeroplasm*, *Acholeplasma*, *Entomoplasm*, *Mesoplasm*, *Spiroplasma*, *Mycoplasma*, and *Ureaplasma* (36).

Measurement of the genome sizes of bacterial species has been greatly facilitated by the development of pulsed-field gel electrophoresis (PFGE) (32). This technique, associated with restriction enzyme digestion and Southern blot hybridization, has made it possible to obtain physical and genomic maps of numerous bacterial species (33, 34). PFGE has been used to determine the genome sizes of several mollicute species (9, 13, 14, 19, 21, 24, 26, 28, 29, 35, 39, 41, 49, 52, 53). A continuum of sizes has been found, ranging from 580 kbp for the smallest genome, the *Mycoplasma genitalium* genome (9, 35), to 1,820 kbp for the largest genome, the *Spiroplasma citri* genome (53).

Spiroplasma citri, the causative agent of citrus stubborn disease, was the first *Spiroplasma* species to be described (30). Today, numerous *spiroplasmas* have been isolated from plants and arthropods, mainly insects (50). An approach to *spiroplasma* classification based on serologic relatedness data, electrophoretic patterns of proteins, G+C DNA contents, and levels of DNA-DNA homology was first proposed in 1980 (18). Up to 1993, 24 groups were recognized (3, 15, 37) but recently, the group XVII *spiroplasmas* have been shown to be members of group VIII (12); thus, group XVII contains no organisms at the present time. Groups may be divided into subgroups on the basis of DNA-DNA homology, serological relatedness, and host range data (44).

Previously, the genome sizes of the following *Spiroplasma* species have been determined: *Spiroplasma citri* (1,820 kbp), *Spiroplasma apis* (1,350 kbp), *Spiroplasma melliferum* (1,460 kbp), *Spiroplasma insolitum* (1,850 kbp), *Spiroplasma clarkii* (1,770 kbp), and *Spiroplasma monobiae* (940 kbp) (13, 16, 47, 48, 52, 53). In this study we determined, by using PFGE, the genome sizes of representatives of all *spiroplasma* groups except groups II, XIV, and XXIII. We also determined the genome sizes of representatives of all eight subgroups of group I.

***Spiroplasma* strains and cultivation procedures.** All of the

spiroplasma strains which we studied (Table 1) were grown in 100 ml of SP4 or MIA medium (43).

Genome size determination. Cultures at the late log phase of growth were incubated with 100 µg of chloramphenicol per ml for 90 min at 37°C in order to complete ongoing rounds of replication and to prevent the initiation of new rounds. The cells were then collected by centrifugation for 30 min at 20,000 × g.

The pelleted cells were embedded in LMP agarose (Geneline; Beckman, Palo Alto, Calif.) and were treated as described previously (27), except that the cellular pellet was resuspended in STE (100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) instead of phosphate-buffered saline (53). Genome sizes were determined by using full-length (nonrestricted) chromosomes that were linearized by random breakage during the extraction procedure (14). In addition, restriction enzyme *NotI* was used as recommended by the manufacturer (New England Biolabs, Beverly, Mass.). Electrophoresis was performed with a Geneline II apparatus (Beckman) by using a transverse alternating field electrophoresis system and Tris-borate-EDTA as the running buffer. With the exception of nonrestricted genomic DNA from *Spiroplasma ixodetis* Y32^T (T = type strain), the genomic linear molecules and *NotI* restriction fragments were separated on a 1% LE agarose gel (Beckman) under the following electrophoresis conditions: 370 mA; three steps of 12 h each, with pulse times of 1, 2, and 3 min; 8°C. The sizes of the resulting fragments were evaluated by comparing them with *Saccharomyces cerevisiae* chromosomal DNA fragments ranging in size from 215 to 2,200 kbp (Bio-Rad, Hercules, Calif.) and lambda phage DNA concatemers (New England Biolabs). For *Spiroplasma ixodetis* Y32^T, nonrestricted genomic DNA was subjected to PFGE on a 0.6% LE agarose gel by using the conditions described above, except that there was an additional step of 9 h with a pulse time of 4 min. In this case, the size of the genomic DNA was determined by comparing its mobility with the mobilities of chromosomes obtained from *Candida albicans* (1,010 to 3,000 kbp) and *Saccharomyces cerevisiae* (215 to 2,200 kbp).

The genome sizes of the *spiroplasmas* which we examined are shown in Table 2. For most of the *spiroplasma* genomes, a single band was obtained after digestion with *NotI*, indicating either that there were no sites for this enzyme or that a single site was present. For two *spiroplasmas*, *Spiroplasma* sp. strain EA1 and *Spiroplasma sabaudiense* AR-1343^T, three bands were obtained after digestion with *NotI*, indicating that there were three restriction sites for *NotI*.

In every case, the genome size estimated from *NotI* digestion

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TABLE 1. Spiroplasmas whose genome sizes have been determined

Group or subgroup	Species or trivial name	Strain(s)	Principal host(s)	Reference
I-1	<i>Spiroplasma citri</i>	R8A2 ^T = (ATCC 27556 ^T)	Citrus leafhoppers	30
I-2	<i>Spiroplasma melliferum</i>	BC-3 ^T = ATCC 33219 ^T)	<i>Apis mellifera</i>	8
I-3	<i>Spiroplasma kunkelii</i>	E-275 ^T = ATCC 29320 ^T)	<i>Zea mays</i> leafhoppers	46
I-4	Rabbit tick spiroplasma	277F = ATCC 29761)	<i>Haemaphysalis leporispalustris</i>	25
I-5	Green leaf bug spiroplasma	LB-12 = ATCC 33649)	<i>Trigonotylus ruficornis</i>	22
I-6	<i>Spiroplasma insolitum</i>	M-55 ^T = ATCC 33502 ^T)	<i>Bidens</i> sp.	16
I-7	Cocos spiroplasma	N-525 = ATCC 33287)	<i>Cocos nucifera</i>	11
I-8	<i>Spiroplasma phoeniceum</i>	P-40 ^T = ATCC 43115 ^T)	<i>Catharanthus roseus</i>	31
III	<i>Spiroplasma floricola</i>	OBMG = ATCC 33221)	Insects, flowers	10
IV	<i>Spiroplasma apis</i>	B31 ^T = ATCC 33834 ^T)	<i>Apis mellifera</i>	23
V	<i>Spiroplasma mirum</i>	SMCA ^T = ATCC 29335 ^T)	<i>Haemaphysalis leporispalustris</i>	40
VI	<i>Spiroplasma ixodetis</i>	Y32 ^T = ATCC 33835 ^T)	<i>Ixodes pacificus</i>	38
VII	<i>Spiroplasma monobiae</i>	MQ1 ^T = ATCC 33825 ^T), MMG, CUAS-1	<i>Monobia quadridens</i>	47
VIII-1	Syrphid spiroplasma	EA-1 = ATCC 33826)	<i>Eristalis arbustorum</i>	4
VIII-2	Deerfly spiroplasma	DF-1 = ATCC 43209)	<i>Chrysops</i> sp.	7
IX	<i>Spiroplasma clarkii</i>	CN-5 ^T = ATCC 33827 ^T)	<i>Cotinus nitida</i>	48
X	<i>Spiroplasma culicicola</i>	AES-1 ^T = ATCC 35112 ^T)	<i>Aedes sollicitans</i>	17
XI	<i>Spiroplasma velocicrescens</i>	MQ4 ^T = ATCC 35262 ^T)	<i>Monobia quadridens</i>	20
XII	Cucumber beetle spiroplasma	DU-1 = ATCC 43210)	<i>Diabrotica undecimpunctata</i>	5
XIII	<i>Spiroplasma sabaudiense</i>	Ar-1343 ^T = ATCC 43303 ^T)	<i>Aedes</i> sp.	1
XV	Leafhopper spiroplasma	I-25 = ATCC 43262)	<i>Cicadulina bipunctella</i>	37
XVI-2	<i>Spiroplasma cantharicola</i>	CB-1 ^T = ATCC 43207 ^T)	<i>Cantharis bilineatus</i>	45
XVIII	Tabanid spiroplasma	TN-1 = ATCC 43211)	<i>Tabanus nigrovittatus</i>	7
XIX	Firefly spiroplasma	PUP-1 = ATCC 43206)	<i>Photuris pennsylvanicus</i>	5
XX	Colorado potato beetle spiroplasma	LD-1 = ATCC 43213)	<i>Leptinotarsa decemlineata</i>	4
XXI	Flower spiroplasma	W115 = ATCC 43260)	<i>Prunus</i> sp.	6
XXII	<i>Spiroplasma taiwanense</i>	CT-1 ^T = ATCC 43302 ^T)	<i>Culex tritaeniorhynchus</i>	2
XXIV	<i>Spiroplasma chinense</i>	CCH ^T = ATCC 43960 ^T)	<i>Calystegia hederacea</i>	15

products was the same as the size determined with nondigested linear molecules.

The sizes of spiroplasma chromosomes range from 940 kbp for the smallest chromosome, the *Spiroplasma monobiae* MQ-1^T (group VII) chromosome, to 2,220 kbp for the largest chromosome, the *Spiroplasma ixodetis* Y32^T (group VI) chromosome. Thus, the genome of *Spiroplasma ixodetis* Y32^T appears to be more than twice as large as the genome of the spiroplasma with the smallest genome and is the largest genome described so far for a mollicute species. The genome of this organism is four times larger than the genome of the mollicute with the smallest genome, *Mycoplasma genitalium* (9, 35).

Figure 1 shows the distribution of genome sizes in different genera of the class *Mollicutes*. Genome sizes determined by PFGE are not available for members of the genera *Asterole-*

plasma and *Anaeroplasm*. Many genome sizes fall in the range from 800 to 1,300 kbp. Only four *Mycoplasma* strains and five *Ureaplasma* strains have genomes that are smaller than 800 kbp (9, 19, 24, 29, 35). More than one-half of the *Spiroplasma* species, including all of the group I species, and most of the *Acholeplasma* species examined have genomes that are larger than 1,300 kbp (24, 29, 41). In general, *Spiroplasma* and *Acholeplasma* species, which are considered phylogenetically "early" mollicutes, have larger genomes than *Mycoplasma* and *Ureaplasma* species, which are considered more "recent" mollicutes (42). This is consistent with the idea that *Mollicutes* evolved by regressive evolution (i.e., by genome losses).

In view of the results presented above and elsewhere, it is clear that in the class *Mollicutes*, genome size heterogeneity occurs at every level of classification. This is particularly true within genera; in one genus certain species may have genomes

FIG. 1. Distribution of genome sizes within the class *Mollicutes*. Each diamond represents a mollicute species. Data are from this study and references 9, 13, 14, 19, 21, 26, 28, 29, 35, 39, 41, 49, 52, and 53.

TABLE 2. Genome sizes of *Spiroplasma* spp.

Group or subgroup	Strain	Genome size (kbp)	
		Undigested linear molecule	Sum of <i>NorI</i> restriction fragment sizes
I-1	<i>Spiroplasma citri</i> R8A2 ^T	1,820 ^a	1,820 ^a
I-2	<i>Spiroplasma melliferum</i> BC-3 ^T	1,460 ^a	1,460 ^a
I-3	<i>Spiroplasma kunkelii</i> E-275 ^T	1,610	1,580
I-4	<i>Spiroplasma</i> sp. strain 277F	1,620	1,580
I-5	<i>Spiroplasma</i> sp. strain LB-12	2,020	ND ^b
I-6	<i>Spiroplasma insolitum</i> M-55 ^T	1,810	1,760
I-7	<i>Spiroplasma</i> sp. strain N-525	1,780	ND
I-8	<i>Spiroplasma phoeniceum</i> P-40 ^T	1,860	1,840
III	<i>Spiroplasma floricola</i> OBMG	1,270	1,325
IV	<i>Spiroplasma apis</i> B31 ^T	1,300 ^c	ND
V	<i>Spiroplasma mirum</i> SMCA ^T	1,300	1,270
VI	<i>Spiroplasma ixodetis</i> Y32 ^T	2,220	2,220
VII	<i>Spiroplasma monobiae</i> MQ1 ^T	940	980
	<i>Spiroplasma monobiae</i> MMG	995	ND
	<i>Spiroplasma monobiae</i> CUAS-1	1,080	ND
VIII-1	<i>Spiroplasma</i> sp. strain EA-1	1,230	1,150
VIII-2	<i>Spiroplasma</i> sp. strain DF-1	1,270	1,250
IX	<i>Spiroplasma clarkii</i> CN-5 ^T	1,720	1,660
X	<i>Spiroplasma culicicola</i> AES-1 ^T	1,350	1,320
XI	<i>Spiroplasma</i> sp. strain MQ4	1,480	ND
XII	<i>Spiroplasma</i> sp. strain DU-1	1,350	1,280
XIII	<i>Spiroplasma sabaudiense</i> Ar-1343 ^T	1,175	1,095
XV	<i>Spiroplasma</i> sp. strain I-25	1,380	1,340
XVI-2	<i>Spiroplasma cantharicola</i> CB-1 ^T	1,320	1,320
XVIII	<i>Spiroplasma</i> sp. strain TN-1	1,370	1,390
XIX	<i>Spiroplasma</i> sp. strain PUP-1	1,375	1,350
XX	<i>Spiroplasma</i> sp. strain LD-1	1,085	ND
XXI	<i>Spiroplasma</i> sp. strain W115	980	1,000
XXII	<i>Spiroplasma taiwanense</i> CT-1 ^T	1,195	1,220
XXIV	<i>Spiroplasma chinense</i> CCH ^T	1,530	1,580

^a Data from reference 52.^b ND, not determined.^c Data from reference 13.

that are twice as large as the genomes of other species. Genome size heterogeneity also occurs at the species level; in *Ureaplasma urealyticum*, *Mycoplasma mycoides*, *Mycoplasma hominis*, and *Spiroplasma citri*, variations of up to 30% in genome size have been observed for different strains of the same species (21, 29, 49, 51).

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