Chromosome Sizes of Phytoplasmas Composing Major Phylogenetic Groups and Subgroups

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ABSTRACT

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Chromosome sizes of 71 phytoplasmas belonging to 12 major phylogenetic groups including several of the aster yellows subgroups were estimated from electrophoretic mobilities of full-length chromosomes in pulsed-field gels. Considerable variation in genome size, from 660 to 1,130 kilobases (kb), was observed among aster yellows phytoplasmas. Chromosome size heterogeneity was also observed in the stolbur phytoplasma group (range 860 to 1,350 kb); in this group, isolate STOLF contains the largest chromosome found in a phytoplasma to date. A wide range of chromosome sizes, from 670 to 1,075 kb, was also identified in the X-disease group. The other phytoplasmas examined, which included

members of the apple proliferation, Italian alfalfa witches' broom, faba bean phyllody, pigeon pea witches' broom, sugarcane white leaf, Bermuda grass white leaf, ash yellows, clover proliferation, and elm yellows groups, all have chromosomes smaller than 1 megabase, and the size ranges within each of these groups is narrower than in the aster yellows, stolbur, and X-disease groups. The smallest chromosome, ~530 kb, was found in two Bermuda grass white leaf phytoplasma isolates. This not only is the smallest mollicute chromosome found to date, but also is the smallest chromosome known for any cell. More than one large DNA band was observed in several phytoplasma preparations. Possible explanations for the occurrence of more than one band may be infection of the host plant by different phytoplasmas, the presence of more than one chromosome in the same organism, or the presence of large extrachromosomal DNA elements.

Phytoplasmas are wall-less, uncultivated procaryotes in the class *Mollicutes* (trivial name mycoplasmas) that are associated with diseases of several hundred plant species (26,35). Sequence analysis of 16S ribosomal DNA (rDNA) and ribosomal protein genes showed that phytoplasmas compose a discrete monophyletic clade that is more closely related to achleplasmas than to other mollicutes (13,36). The closest identified relatives of the phytoplasmas are *Achleplasma palmarum* and *A. modicum* (19) in the anaeroplasmal clade defined by Weisburg et al. (39). According to phylogenetic data based on 16S rDNA sequences, about 20 subclades (groups) can currently be distinguished within the phytoplasma clade (35). Phytoplasma subclades are considered to represent the equivalent of distinct species under the provisional taxonomic species status *candidatus* (15). Within most subclades, further differentiation is possible by restriction fragment length polymorphism (RFLP) analysis or other methods. On the basis of RFLP analysis of polymerase chain reaction (PCR)-amplified 16S rDNA and ribosomal protein genes, the aster yellows (AY) group, currently the largest phytoplasma subclade, was divided into several subgroups (19).

Another important characteristic of these uncultivated plant pathogens is their genome size. For the culturable mollicutes, a wealth of data on this property is available. The genome sizes of culturable mollicutes range from more than 2,200 to less than 600 kilobases (kb), with overlapping values between the various mollicute genera. A particularly wide range has been observed among *Mycopl*

plasma spp., whose genome sizes range from 1,380 down to 580 kb, and *Spiroplasma* spp., which have an even greater span, from 2,220 to 780 kb. In the genus *Achleplasma*, the size range is narrower, varying between 1,500 and 1,650 kb (30). Information on genome sizes of phytoplasmas is currently available for only 12 strains, mainly from the AY and X-disease groups, and these have chromosome sizes between 1,185 and 600 kb (12,29,40). The purpose of the work reported here was to expand our knowledge of the range of genome sizes within the phytoplasma clade and to ascertain whether chromosome sizes among these groups cluster or differ in range in any characteristic manner. For this purpose, we examined representative strains from a number of major taxonomic groups and subgroups as defined by Seemüller et al. (35) and Lee et al. (19).

MATERIALS AND METHODS

Sources of phytoplasmas. The phytoplasmas examined are listed in Table 1. Most isolates were previously transmitted to periwinkle (*Catharanthus roseus* (L.) G. Don) and maintained in this host by periodic grafting in an insect-proof greenhouse. In addition, the following naturally infected plants were sampled in the field in the Campania region of southern Italy: two celery plants (isolates CeY1 and 2) from the same field, two patches of Bermuda grass (BGWL1 and 2) from different locations, and two plants of *Picris echioides* L. (bristly ox-tongue; isolates PEP and PEY).

Pulsed-field gel electrophoresis (PFGE) analysis. Leaf mid-ribs were taken for phytoplasma extraction. Samples were prepared and PFGE was carried out as described by Neimark and Kirkpatrick (29), except that the lysis solution was changed several times, SeaPlaque agarose (FMC BioProducts, Rockland, ME) was used, and the blocks were not gamma-irradiated. The PFGE was carried out in a CHEF DRIII apparatus (Bio-Rad Laboratories, Rich-

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TABLE 1. Genome size of phytoplasmas of various taxonomic groups and subgroups

Phytoplasma (geographic origin)	Strain/isolate	Taxonomic group/subgroup ^a	Reference/further information	Genome size (kb)
Chrysanthemum yellows (Germany)	CHRYM	Aster yellows/A	Obtained from R. Marwitz	780
Cuscuta odorata latent (Germany)	COL	Aster yellows/A	31	780
Plantago virescence (Germany)	PVM	Aster yellows/A	31	810
American aster yellows (United States)	AAY	Aster yellows/B	35	915 ^b
Cactus virescence (probably United States)	CC	Aster yellows/B	35	935 ^b
Catharanthus virescence (Peru)	CVL	Aster yellows/B	31	680
Delphinium virescence (Germany)	DEV	Aster yellows/B	31	800
Diploaxis virescence (Spain)	DIV	Aster yellows/B	31	1,000
Eastern American aster yellows (United States)	AYW	Aster yellows/B	31	1,040
French Aster yellows (France)	AYM1	Aster yellows/B	Obtained from G. Morvan	1,120 ^b
Gladiolus witches' broom (France)	GLAWC	Aster yellows/B	1	1,080, 790 ^b
Hydrangea phyllody (Belgium)	HYDB	Aster yellows/B	31	660
Hydrangea phyllody (France)	HYDF	Aster yellows/B	7	800
Plantago virescence (Germany)	PVW	Aster yellows/B	31	910
Rape virescence	RV	Aster yellows/B	31	1,130
Safflower phyllody (Israel)	SAFP	Aster yellows/B	31	740
Sandal spike (India)	SAS	Aster yellows/B	31	690
Clover phyllody (Germany)	KVG	Aster yellows/C	34	830
Clover phyllody (France)	KVF	Aster yellows/C	8	750
From grapevine (Germany)	GY	Aster yellows/C	21	830
Leafhopper-borne (Germany)	CVA	Aster yellows/C	31	880
Potato purple top (France)	PPT	Aster yellows/C	5	758
From apricot (Spain)	AYA	Aster yellows/F	35	740 ^c
Leafhopper-borne (Germany)	CVB	Aster yellows/F	31	910, 680
Aster yellows (Germany)	AV976	Aster yellows/L	Obtained from R. Marwitz	1,095, 790
Aster yellows (Germany)	AV2192	Aster yellows/L	31	1,125
From Bermuda grass (Thailand)	AYBG	Aster yellows/L	31	780
Primula yellows (Germany)	PRIVA	Aster yellows/L	31	830 ^b
Aster yellows (Germany)	AVUT	Aster yellows/M	Obtained from R. Marwitz	910 ^b
Celery yellows (Italy)	CeY1	Stolbur	C. Marcone, <i>unpublished data</i>	980
Celery yellows (Italy)	CeY2	Stolbur	C. Marcone, <i>unpublished data</i>	980
Lavender decline (France)	DEPL	Stolbur	6	990
Grapevine yellows (Italy)	CA-1	Stolbur	24	1,070
Grapevine yellows (Italy)	CH-1	Stolbur	24	945
Grapevine yellows (Italy)	SA-1	Stolbur	24	1,020
Grapevine yellows (Italy)	SA-2	Stolbur	24	1,080
Grapevine yellows (Germany)	GGY1	Stolbur	24	860
From sour cherry (France)	MOL	Stolbur	31	1,220
Stolbur of pepper (Serbia)	STOL	Stolbur	35	900
Tomato stolbur (France)	STOLF	Stolbur	31	1,350
Apple proliferation (Germany)	AT	Apple proliferation	31	645
Apple proliferation (Italy)	AP15	Apple proliferation	31	690
European stone fruit yellows (Germany)	GSFY1	Apple proliferation	23	630
European stone fruit yellows (Germany)	GSFY2	Apple proliferation	23	630
European stone fruit yellows (Italy)	ESFY1	Apple proliferation	20	630
Pear decline (Germany)	PD1	Apple proliferation	23	660
From grapevine (Italy)	FDI	X-disease	31	705
Vaccinium witches' broom (Germany)	VAC	X-disease	31	1,075
Western X-disease (United States)	GVX	X-disease	31	670
Western X-disease (United States)	PYLR	X-disease	31	670
Picris echioides phyllody (Italy)	PEP	Italian alfalfa witches' broom	35	605
Cleome phyllody (Thailand)	CLP	Faba bean phyllody	35	720
Cotton phyllody (Burkina Faso)	CoP	Faba bean phyllody	35	790
Crotalaria saltiana phyllody (Sudan)	CSP	Faba bean phyllody	35	660
Faba bean phyllody (Sudan)	FBP	Faba bean phyllody	32	660
Sesame phyllody (Thailand)	SEPN	Faba bean phyllody	32	660 ^b
Sesame phyllody (Thailand)	SEPT	Faba bean phyllody	32	700
Soybean phyllody (Thailand)	SoyP	Faba bean phyllody	32	735
Sunn hemp witches' broom (Thailand)	SUNHP	Faba bean phyllody	32	680
Sunn hemp phyllody (Thailand)	CROP	Faba bean phyllody	35	670
Tomato big bud (Australia)	TBB	Faba bean phyllody	35	690
Picris echioides yellows (Italy)	PEY	Pigeon pea witches' broom	35	740 ^b
Leafhopper-borne (Germany)	BVK	Sugarcane white leaf	35	700
Bermuda grass white leaf (Italy)	BGWL1	Bermuda grass white leaf	25	530
Bermuda grass white leaf (Italy)	BGWL2	Bermuda grass white leaf	25	530
Ash yellows (United States)	ASHY1	Ash yellows	35	620
Beet leafhopper-transmitted virescence agent (United States)	BLTVA	Clover proliferation	35	675
Alder yellows (Italy)	ALY	Elm yellows	35	750
Elm yellows (United States)	EY	Elm yellows	31	730
Elm yellows (France)	ULW	Elm yellows	35	680
Rubus stunt (Italy)	RuS	Elm yellows	35	820

^a Group designation proposed by Seemüller et al. (35); AY subgroup designation according to Lee et al. (19) and C. Marcone (*unpublished data*).

^b Preparation showed one or more additional bands with sizes of less than 400 kb that did not hybridize or hybridized only weakly with the ribosomal DNA probe.

^c Two additional bands of approximately 490 and 300 kb hybridized with a ribosomal DNA probe.

mond, CA) employing the following protocol: temperature, 14°C; voltage, 6 V/cm; pulse parameters, 20 to 120 s; running time, 21 h; angle, 120°; agarose, 1%; and buffer, 0.5× Tris-borate-EDTA. Size markers were *Hansenula wingei* chromosomes (Bio-Rad Laboratories), *Saccharomyces cerevisiae* chromosomes (Bio-Rad Laboratories and Pharmacia Biotechnology, Inc., Uppsala, Sweden), and concatameric lambda DNA ladder (Bio-Rad Laboratories). All preparations were examined by PFGE at least twice. Chromosomal DNA of each phytoplasma was blotted onto nylon membrane as described by Neimark and Kirkpatrick (29) and hybridized as described by Hoheisel et al. (14) with a rDNA probe prepared from phytoplasma strain AAY by PCR amplification using primer pair P1/P7 (37). This probe encompasses the entire 16S rRNA gene, the 16S/23S rDNA spacer region, and the 5' region of the 23S rRNA gene. The probe was purified by extracting the PCR-amplified DNA band from agarose using the JETsorb gel extraction kit (GenoMed, Bad Oynhausen, Germany) and labeling with ³²P-dATP using the Prime-It II kit (Stratagene Inc., La Jolla, CA).

RESULTS AND DISCUSSION

Effect of strains and plant sources on chromosome isolation.

A total of about 100 phytoplasma strains obtained mainly from the

collection of the Dossenheim Institute was examined. In preparations from 71 phytoplasmas belonging to 12 of the 20 taxonomic groups proposed by Seemüller et al. (35), at least one discrete full-length phytoplasma chromosome band was obtained (Table 1). These full-length chromosome bands were obtained without utilizing gamma irradiation, presumably by using physical shearing, endogenous nuclease activity, or both during preparation to obtain linearized chromosomes. Previously, where ample amounts of material were available, we used shearing and endogenous nuclease activity instead of gamma irradiation to introduce single double-strand breaks into chromosomes, but we were cautious in recommending this procedure for precious or irreplaceable samples (4). The large amounts of fragmented DNA visible in the lower portion of the lanes is a reflection of this procedure (Figs. 1 and 2). The best results usually were obtained when plants were fully symptomatic but did not yet show signs of decline. Preparations from ≈30 isolates did not yield any bands or the bands observed were not reproducible, in spite of the fact that samples were taken at different stages of disease development. No relationship was observed between isolates that failed to produce bands and their group affiliation. The failure to demonstrate chromosome bands in these phytoplasma preparations may have been because these isolates did not reach sufficiently high titers in periwinkle. Firrao et al.

Fig. 1. Full-length phytoplasma chromosomes isolated by pulsed-field gel electrophoresis. The size markers were M, concatameric lambda DNA ladder; M1, *Saccharomyces cerevisiae* chromosomes; M2, *Hansenula wingei* chromosomes; and M3, *S. cerevisiae* chromosomes. **A**, COL through CVB, aster yellows strains. **B**, STOL through CeY2, stolbur strains. **C**, BGWL1 and 2, Bermuda grass white leaf; PEP, *Picris echioides* phyllody; ASHY1, ash yellows; ALY, alder yellows; ULW and EY1, elm yellows; RuS, rubus stunt; GSFY1, European stone fruit yellows; PD1, pear decline; and AT and AP15, apple proliferation. **D**, BVK, leafhopper-borne; TBB, tomato big bud; FBP, faba bean phyllody; SUNHP, sunn hemp witches' broom; SoyP, soybean phyllody; SEPT and SEPN, sesame phyllody; CoP, cotton phyllody; CROP, sunn hemp phyllody; PEY, *Picris echioides* yellows; PYLR and GVX, western X-disease; and FDI, from grapevine.

(10) obtained much stronger chromosomal DNA bands of the western X-disease phytoplasma following extraction from diseased celery than from diseased periwinkle. In our work, celery also proved to be an excellent phytoplasma host.

AY group. Of the isolates examined, 28 were AY group members belonging to subgroups A, B, C, and F, as proposed by Lee et al. (19) or to subgroups L and M, recently identified on the basis of RFLP analysis of PCR-amplified rRNA and *tuf* gene DNA (C. Marcone, unpublished data) (Table 1; Figs. 1A and 2A). Within subgroup B, the largest subdivision of the AY group (35), genome sizes are quite heterogeneous and range from 1,130 kb (isolate RV) down to 660 kb (isolate HYDB). Subgroup L, although it currently contains only four isolates, was heterogeneous in chromosome size (range from 1,095 to 780 kb). Subgroups C may be composed of isolates with a relatively narrow size range (880 to 750 kb), but, in contrast to chromosome size heterogeneity, a narrow size range cannot be given much weight as a property of a subgroup until several subgroup members have been examined. Obviously, too few members of subgroups A, F, and M have been examined to estimate their subgroup properties. The AY phytoplasmas examined differ greatly in virulence for periwinkle and cause a wide variety of symptoms in this host including virescence, phyllody, small and faintly colored flowers, flower mal-

formations, shortening of internodes, elongation and etiolation of internodes, small and deformed leaves, yellowing, and decline. However, we did not observe any relation between symptoms or virulence and genome size among AY isolates.

Previously, Neimark and Kirkpatrick (29) examined 11 phytoplasmas including eight virescence agents (seven AY strains with chromosome sizes between 1,185 and 850 kb and strain BLTVA with a size of 675 kb). These authors found polymorphism in genome sizes among these "virescence phytoplasmas" and noted a difference in size between them and the small genomes of three X-disease phytoplasmas associated with decline diseases of woody plants ("decline phytoplasmas"). Our results show that chromosome sizes below 850 kb are common among AY phytoplasmas (Table 1), and these genome sizes overlap those of the so-called decline phytoplasmas. Even AY isolates with chromosome sizes smaller than 700 kb including CVL, HYDB, and SAS cause virescence and phyllody in periwinkle.

Stolbur group. The stolbur group is composed of isolates with chromosomes mainly at the upper end of the size range and, up to now, no isolate has a chromosome smaller than 860 kb; thus, stolbur isolate chromosomes, on average, are somewhat larger than those of AY isolates and definitely larger than those of any other currently constituted group (Table 1; Figs. 1B and 2B). The group

Fig. 2. Southern blot hybridization of the pulsed-field gel electrophoresis gels. The size markers were M, concatameric lambda DNA ladder; M1, *Saccharomyces cerevisiae* chromosomes; M2, *Hansenula wingei* chromosomes; and M3, *S. cerevisiae* chromosomes. **A**, COL through CVB, aster yellows strains. **B**, STOL through CeY2, stolbur strains. **C**, BGWL1 and 2, Bermuda grass white leaf; PEP, Picris echioides phyllody; ASHY1, ash yellows; ALY, alder yellows; ULW and EY1, elm yellows; RuS, rubus stunt; GSFY1, European stone fruit yellows; PD1, pear decline; and AT and AP15, apple proliferation. **D**, BVK, leafhopper-borne; TBB, tomato big bud; FBP, faba bean phyllody; SUNHP, sunn hemp witches' broom; SoyP, soybean phyllody; SEPT and SEPN, sesame phyllody; CoP, cotton phyllody; CROP, sunn hemp phyllody; PEY, Picris echioides yellows; PYLR and GVX, western X-disease; and FDI, from grapevine.

includes isolate STOLF, which contains the largest phytoplasma chromosome identified so far, exceeding the largest phytoplasma chromosomes found previously (29) by 155 kb. While the genome sizes of the two celery yellows isolates were identical, the sizes of the grapevine isolates, which are all from the same geographic area of Italy, were all different. It is interesting to note that the two isolates with the largest genomes, MOL and STOLF, were much less virulent for periwinkle than the other stolbur isolates.

X-disease group. In the X-disease group, the genome size of strains GVX and PYLR of the X-disease phytoplasma (Table 1) is identical to that of the western X-disease strain used for chromosome mapping by Firrao et al. (10). Similarly, genome sizes of two other X-disease group members, the phytoplasmas causing peach rosette and Italian clover phyllody, 670 and 630 kb, respectively (9), are within the same range. However, a considerably larger chromosome of approximately 1,075 kb was identified in strain VAC. This size confirms the value previously obtained by B. Schneider and E. Seemüller (*unpublished data*) but is larger than that reported by Firrao et al. (9).

Other phytoplasma groups. All the examined members of the remaining 10 phytoplasma groups have genome sizes of less than 1 megabase (Table 1; Figs. 1C and D and 2C and D). Size variation within these groups is less than within the AY, stolbur, and X-disease groups. A particularly narrow size range of from 690 to 645 kb was observed among the apple proliferation (AP) group agents; in this group, all the European stone fruit yellows isolates have the same genome size, whereas the genome sizes of the two isolates from apple that were examined are different (Fig. 1C). Genome sizes of the faba bean phyllody group isolates examined are between 790 and 660 kb (Table 1). Group member of the species *candidatus*, *Phytoplasma aurantifolia* is in the same range (40), whereas the chromosome size of the sweet potato little leaf phytoplasma was estimated to be only 600 kb (12). Until this study, the sweet potato little leaf phytoplasma was the smallest known phytoplasma genome.

Two samples from Bermuda grass infected with the Bermuda grass white leaf phytoplasma yielded a similar-sized band of approximately 530 kb (Table 1; Figs. 1C and 2C). These bands hybridized strongly with the rDNA probe, and no other bands were observed; therefore, there is no doubt that the 530-kb band represents the linearized chromosomes of these isolates. Hence, the chromosome of the Bermuda grass white leaf agent is not only the smallest phytoplasma genome but also appears to be the smallest genome known for any self-replicating organism. Previously, the 580-kb chromosome of *Mycoplasma genitalium* was considered the smallest genome (11). These values are near the calculated 562-kb upper size for a minimal self-sufficient organism (16), while theoretical estimates for a minimal gene complement are considerably lower (16,22,28).

Multiple banding patterns. In preparations from AY isolates GLAWC, CVB, and AV976, we detected two DNA bands that, from their size and their hybridization to the rDNA probe, appear to be full-length phytoplasma chromosomes (Table 1; Figs. 1A and 2A). Similar observations were made by Neimark and Kirkpatrick (29) in analyzing samples from other AY phytoplasma-infected plants. The simplest explanation for the occurrence of two bands is that the plants from which the samples were prepared were infected with two distinct phytoplasmas containing different-sized chromosomes. Apparent multiple phytoplasma infections in a single plant have also been identified by RFLP analysis of PCR-amplified ribosomal DNA by Lee et al. (17,18) and Berges et al. (3). Their effect on virulence and symptomatology is not clear.

More difficult to explain are three bands observed in strain AYA that showed, in addition to a 740-kb band presumed to be the main chromosome, two other bands with estimated sizes of 490 and 300 kb (Figs. 1A and 2A). Since both smaller bands hybridized with the rDNA probe, it is unlikely that they are plasmids because, to our knowledge, no plasmid has been described that carries a rRNA cistron. Similar-sized bands observed previously in other AY phyto-

plasma preparations did not hybridize with rRNA gene probes and were considered to be extrachromosomal replicons or "megaplasmids" (29). Possibly, either of the smaller AYA bands could represent a second smaller AYA chromosome. Two chromosomes are known to occur in the prokaryotes *Brucella melitensis* (27), *Leptospira interrogans* (41), and *Rhodobacter sphaeroides* (38). *Agrobacterium tumefaciens* C58 has one circular chromosome plus a linear chromosome (2). Also, the two smaller bands are in the range proposed for the minimal genome size required for life of 318 to 562 kb (16); 318 kb corresponds closely to the size for a set of 256 genes proposed by Mushegian and Koonin (28) to be necessary to sustain a cell. Thus, an additional possibility is that one or more minimal chromosomes from another organism may be present in these preparations. These various possibilities can be examined experimentally by using specific probes for ribosomal protein (13), *tuf* (33), and other conserved genes.

In preparations from several isolates, especially AY group phytoplasmas, we observed one or several bands with sizes mostly between 400 and 50 kb that did not hybridize or hybridized only weakly with the rDNA probe (Table 1). Large DNA bands that did not hybridize with a 16S rDNA probe have also been reported to occur in other AY phytoplasmas; however, these bands did hybridize with a cloned chromosomal fragment from severe aster yellows (29). When we examined different DNA preparations from plants infected with the same strains, these bands were quite variable in different preparations with respect to size and occurrence.

Concluding remarks. The values reported here extend the known range of phytoplasma chromosome sizes to 1,350 to 530 kb and nearly fill in much of a continuous span. The width of this span is more than twofold in extent and shows that phytoplasma chromosomes have a size range essentially as broad as that of the genus *Mycoplasma*, the genus with the largest number of species. However, the species composing the genus *Mycoplasma* appear to be much more diverse than the organisms in the species *candidatus* genus *Phytoplasma*, and while the genus *Mycoplasma* contains more than 100 species (30), currently there are only about 20 taxonomic groups within the phytoplasmas that can be distinguished at the putative species level (35). Also, species in the genus *Mycoplasma* differ in their 16S rDNA sequences by as much as 25.5% (40), whereas the maximum 16S rDNA sequence dissimilarity within the phytoplasmas is 14% (35).

Genome sizes among the phytoplasma groups vary. Although these groups may undergo further refinement and subdivision, the varying sizes indicate that different amounts of genomic material have been lost by the phytoplasmas composing the various phylogenetic groups. Accordingly, it should be recognized that an isolate containing a distinctly smaller chromosome may be discovered in any group, because mycoplasma chromosome sizes result from differential losses of genomic material. The small genomes observed in several phytoplasma groups, such as the similar-sized small chromosomes observed in the genus *Mycoplasma*, probably have evolved to near the minimum size limit. In the case of phytoplasmas, survival requires a genome that permits both insect transmission and ability to grow in a plant.

To date, all attempts by investigators to culture phytoplasmas have failed. The genome sizes of many phytoplasmas are larger than those of many culturable mycoplasmas; therefore, small genome size alone cannot explain the inability to culture phytoplasmas. Although genes required for axenic growth may be disabled or absent from either large or small chromosomes, phytoplasmas possessing larger chromosomes may be more likely to have retained a complement of the genes necessary for independent growth. Thus, phytoplasmas containing large genomes should be included in future cultivation studies (29).

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