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Unconventional Genomic Organization in the Alpha Subgroup of the *Proteobacteria*

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Pulsed-field gel electrophoresis was used to analyze the genomic organization of 16 bacteria belonging or related to the family *Rhizobiaceae* of the alpha subgroup of the class *Proteobacteria*. The number and sizes of replicons were determined by separating nondigested DNA. Hybridization of an *rrn* gene probe was used to distinguish between chromosomes and plasmids. Members of the genus *Agrobacterium* all possess two chromosomes, and each biovar has a specific genome size. As previously demonstrated for *Agrobacterium tumefaciens* C58, the smaller chromosomes of *Agrobacterium* biovar 1 and *Agrobacterium rubi* strains appear to be linear. The genomes of *Rhizobium* strains were all of similar sizes but were seen to contain either one, two, or three megareplicons. Only one chromosome was present in the member of the related genus *Phyllobacterium*. We found one or two chromosomes in *Rhodobacter* and *Brucella* species, two chromosomes in *Ochrobactrum anthropi*, and one chromosome in *Mycoplana dimorpha* and *Bartonella quintana*; all of these genera are related to the *Rhizobiaceae*. The presence of multiple chromosomes is discussed from a phylogenetic and taxonomic point of view.

Bacterial genomes were long considered to consist of a single circular chromosome. With the discovery of the existence of multiple circular chromosomes or a linear chromosome in some bacteria, this paradigm is no longer valid. Two different circular chromosomes were reported for *Rhodobacter sphaeroides* (39), *Brucella melitensis* 16M (27), and *Leptospira interrogans* (45), while three chromosomes are present in the genomes of *Rhizobium meliloti* (38), *Burkholderia cepacia* (7), and related species (33). A linear chromosome was reported first for the spirochete *Borrelia burgdorferi* (3, 11) and then for the gram-positive organisms *Streptomyces lividans* (25) and *Rhodococcus fascians* (8). We subsequently demonstrated that the genome of the gram-negative bacterium *Agrobacterium tumefaciens* C58 consisted of two chromosomes, one circular and the other linear (1). Most of the organisms presenting a multipartite genomic organization are confined to certain species within the purple bacteria (or *Proteobacteriaceae*), a phylum of the *Bacteria*, and perhaps this feature is correlated with the phylogeny of these bacteria. In the present study, we have investigated the genomic organization of organisms belonging to the alpha subgroup of the class *Proteobacteria*, particularly members of the genera *Mycoplana*, *Ochrobactrum*, *Rhodobacter*, *Phyllobacterium*, *Rhizobium*, and *Agrobacterium*. Although the first three genera do not belong to the family *Rhizobiaceae*, 16S rRNA sequence comparisons suggest that they belong to a tight phylogenetic group which also includes the genera *Brucella* and *Bartonella* (*Rochalimaea*) (9, 43).

MATERIALS AND METHODS

Strains and growth conditions. The strains used in this study are listed in Tables 1 and 2. Three well-studied laboratory strains, *Agrobacterium tumefaciens* C58, *Agrobacterium rhizogenes* K84, and *Rhizobium meliloti* 2011, were gifts from X. Nesmes and M. Fernandez (Laboratoire d'Ecologie Microbienne du Sol, Université Claude Bernard Lyon I, Villeurbanne, France). *Brucella melitensis* 16M is from our laboratory collection. These strains were grown as previously described (1, 27, 38). Strains originating from the American Type Culture Collection (ATCC) or the Collection Française des Bactéries Phytopathogènes (CFBP) were grown as recommended by the suppliers.

Preparation of the rRNA probe. This probe was prepared by PCR amplification as previously described (1).

Preparation of high-molecular-weight genomic DNAs. Intact genomic DNAs were prepared in agarose plugs as usually described for gram-negative bacteria (1) except for those of some strains, which were better lysed by proteinase K.

PFGE of intact DNAs. Pulsed-field gel electrophoresis (PFGE) was performed in a contour-clamped homogeneous electric field apparatus in 0.5× TBE (36), using the Gene Navigator system from Pharmacia (Saint-Quentin-Yvelines, France). *Saccharomyces cerevisiae*, *Shizosaccharomyces pombe*, *Henselae wingei* (Bio-Rad), *A. tumefaciens* C58, and/or *R. meliloti* DNA (38) and multimers of phage λ DNA were used as molecular size markers. Different pulsing conditions were used to separate either the larger molecules (above 1 Mb) or the smaller ones (below 1 Mb) (1). Gels were stained with ethidium bromide and photographed under short-wavelength UV light. The sizes of replicons were determined by averaging the measurements from several gels.

RESULTS AND DISCUSSION

To examine the diversity in replicon number and size and to distinguish between the linear and circular forms, we employed PFGE. Except for some randomly linearized forms (originating from the preparation of DNA), which generate faint bands in PFGE, circular molecules do not enter the gel (24, 38). In contrast, linear molecules give rise to a marked increase in the thickness and intensity of ethidium bromide-stained bands.

The sizes of entire replicons can be estimated by comparing their migration to that of different high-molecular-weight markers, although for very large fragments the degree of accuracy is rather low. The migration distance in the gel depends on not only the pulse time but also the G+C content of the molecule (23, 30). Nondigested DNAs were submitted to PFGE to investigate the genomic organization of 16 organisms belonging to six genera (Tables 1 and 2).

Bacteria belonging to the *Rhizobiaceae*. In the family *Rhizobiaceae* are found bacteria which live in association with plant cells. The classification of *Agrobacterium* and *Rhizobium* spe-

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TABLE 1. Strain designations, number and sizes of replicons, and estimated genome sizes for organisms belonging to the family *Rhizobiaceae*

Bacterium	Strain and origin	No. of replicons[a]	Sizes (kb) of replicons[b]	Estimated genome size (kb)	Reference(s)
A. radiobacter bv. 1	CFBP 2414^T	3 (2)	3,000*, 2,100* (L), 680	5,780	This study
A. tumefaciens bv. 1	ATCC 23308^T	4 (2)	3,000*, 2,100* (L), 550, 250	5,900	This study
	C58[c]	4 (2)	3000* 2100* (L), 550, 250	5,900	1
A. tumefaciens bv. 3 (*vitis*)	CFBP 2721	4 (2)	3,500*, 1,100*, 500, 250, 200	5,550	This study
	CFPB 2607	4 (2)	3,500*, 1,050*, 250, 180	4,980	This study
A. rhizogenes bv. 2	ATCC 11325^T	4 (1)	4,000*, 2,700, 285 (NS), 250 (NS)	7,235	This study
	K84[c]	4 (1)	4,000*, 2,700, 365, 200	7,265	This study
A. rubi	ATCC 13335^T	4 (2)	3,100*, 1,800* (L), 550, 285	5,735	This study
Rhizobium meliloti	1021 and 2011[c]	3 (1)	3,500*, 1,700, 1,400	6,600	38 and this study
Rhizobium fredii	ATCC 35423^T	3 (1)	4,000*, 2,200, 450	6,650	This study
Rhizobium leguminosarum bv. trifolii	ATCC 14480^T	4 (1)	4,600*, 1,100, 650, 450	6,800	This study
Rhizobium leguminosarum bv. phaseoli	ATCC 14482^T	4 (NE)	4,600, 1,100, 450, 285	6,435	This study
Bradyrhizobium japonicum	110	1 (1)	8,700*	8,700	22
P. myrsinacearum	ATCC 43590^T	5 (1)	3,500*, 680, 500, 365, 285	5,330	This study

[a] The numbers in parentheses are the numbers of replicons labeled by the 16S rRNA probe; NE, not examined.
[b] Asterisks indicate replicons labeled by 16S rRNA probe; L, linear replicon; NS, not shown.
[c] Strain from Laboratoire d'Ecologie Microbienne du Sol, Lyon, France.

cies is based on both phenotypic traits and plasmid-encoded characteristics inducing symbiosis or tumorigenesis (44), rather than chromosomal genes. Moreover, in these genera, extrachromosomal elements represent a major part of the genome (26).

(i) The genus *Agrobacterium*. The genus *Agrobacterium* consists of several genetically and phenotypically different groups or clusters (21). Differences in 16S rRNA sequences clearly separated strains of biovar 1, biovar 2, biovar 3 (*Agrobacterium vitis*), and *Agrobacterium rubi* (37).

We have previously shown that the biovar 1 strain *A. tumefaciens* C58 contains four replicons: two megabase-sized chromosomes (one of which is linear) and two plasmids (1). Two other strains belonging to this biovar, *Agrobacterium radiobacter* CFBP 2414^T and *A. tumefaciens* ATCC 23308^T, were tested. Two megabase-sized replicons, both hybridizing with the rRNA probe, were seen in these strains (Fig. 1 and 2 and Table 1). The smaller band was more intense and more diffuse than the larger one, suggesting that, as in *A. tumefaciens* C58, this replicon is linear. Electrophoresis under different pulsing conditions revealed the presence of small replicons corresponding to plasmids; there were two in *A. tumefaciens* ATCC 23308^T but only one in *A. radiobacter* CFBP 2414^T, which is not a pathogen (Fig. 3 and Table 1).

The results for *A. rubi* ATCC 13335^T, which is one of the three strains forming this cluster (21), were similar to those for biovar 1 strains: it contained one circular and one apparently linear chromosome plus two plasmids (Fig. 1 to 3 and Table 1).

Two strains belonging to biovar 2, *A. rhizogenes* ATCC 11325^T and *A. rhizogenes* K84, were studied. In both strains, two megareplicons, both apparently circular, were separated, but only the larger one hybridized with the rRNA probe (Fig. 1 and 2 and Table 1). Also present in the ATCC 11325^T and the K84 strains were two smaller replicons which correspond to the previously reported agrocine and tumor-inducing plasmids of the latter strain (21) (Fig. 3 and Table 1).

Strains of biovar 3 are tumorigenic for grape vines (21). Two of them, *A. tumefaciens* CFBP 2721 and CFBP 2607, were analyzed. Two megareplicons, both hybridizing with the rRNA probe and both apparently circular, could be seen, together with three plasmids for the former strain and two plasmids for the latter (Fig. 1 and 3 and Table 1).

The unusual genomic organization of *A. tumefaciens* C58 was first suspected because of the greater intensity and the diffuse aspect of one of the two megabase-sized bands, the one corresponding to all of the linear molecules when, under the same conditions, only some randomly linearized forms of the circular replicons enter the gel (38). Further evidence was established by insertion of a unique restriction site into each of the chromosomes that led, after enzymatic digestion, to the linearization of the circular molecules and the generation of two fragments from the linear molecules (18). In the other biovar 1 strains and *A. rubi*, two megabase-sized replicons were present, with the smaller molecule probably being linear. Thus, this multipartite genome with different topologies appears to be a common feature of strains of biovar 1 and *A. rubi*.

TABLE 2. Strain designation, number and sizes of replicons, and estimated genome sizes for organisms related to the family *Rhizobiaceae*

Bacterium	Strain	No. of replicons[a]	Sizes (kb) of replicons[b]	Genome size estimate (kb)	Reference
M. dimorpha	ATCC 4279^T	3 (NE)	3,200, 300, 150	3,150	This study
Rhodobacter capsulatus	ATCC 11166	2 (1)	3,800*, 150	3,950	This study
	SB1003	2 (1)	3,800*, 134	3,934	12
Rhodobacter spheroides	2.4.1	7 (2)	3,000*, 900*; 5 plasmids	4,350	39
Brucella melitensis	16M	2 (2)	2,050*, 1,150*	3,200	27
O. anthropi	ATCC 49188^T	4 (2)	2,700*, 1,900*, 150, 100	4,850	This study
	LMG 3301	4 (NE)	2,700, 1,900, 50, <50	4,700	This study
Bartonella quintana	VR960	1	1,700	1,700	32

[a] The numbers in parentheses are the numbers of replicons labeled with the 16S rRNA probe; NE, not examined.
[b] Asterisks indicate replicons labeled by the 16S rRNA probe.

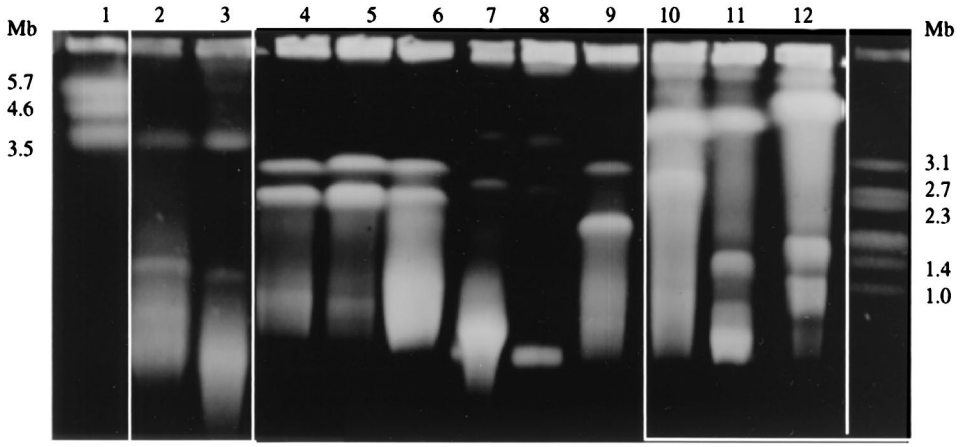

FIG. 1. PFGE of intact DNAs of bacterial species belonging to the family *Rhizobiaceae*: separation of large replicons. Lanes: 1, *Saccharomyces pombe*; 2, *A. vitis* CFBP 2721; 3, *A. vitis* CFBP 2607; 4, *A. tumefaciens* C58; 5, *A. tumefaciens* ATCC 23308^T; 6, *A. radiobacter* CFBP 2414^T; 7, *A. rhizogenes* ATCC 11325^T; 8, *A. rhizogenes* K84; 9, *A. rubi* ATCC 13335^T; 10, *Rhizobium fredii* ATCC 35423^T; 11, *Rhizobium leguminosarum* bv. phaseoli ATCC 14482^T; 12, *Rhizobium leguminosarum* bv. trifolii ATCC 14480^T; far right, *H. wingei*. The positions of molecular size markers are indicated on both sides of the gel.

Biovar 2 and 3 strains also possess two megareplicons; however, their intensity in the PFGE gel suggests that they are both circular. Interestingly, while both megareplicons of biovar 3 hybridized with the rRNA probe, only the larger one of biovar 2 hybridized with it. Nevertheless, the presence of other essential housekeeping genes on the second molecule is possible, as was shown for *Rhizobium meliloti* (35). The sizes of the biovar 2 strain genomes (>7,200 kb) are larger than those of the biovar 1 and *A. rubi* strains (5,900 to 5,735 kb) as well as those of biovar 3 strains (5,500 or 4,980 kb).

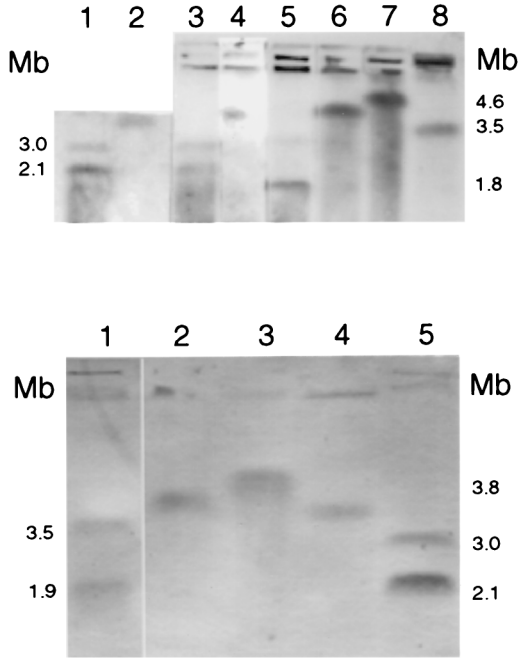

FIG. 2. Hybridization of large replicons with the 16S rRNA probe. (Upper panel) 1, *A. radiobacter* CFBP 2414^T; 2, *A. rhizogenes* K84; 3, *A. tumefaciens* C58; 4, *A. rhizogenes* ATCC 11325^T; 5, *A. rubi* ATCC 13335^T; 6, *Rhizobium fredii* ATCC 35423^T; 7, *Rhizobium leguminosarum* bv. trifolii ATCC 14480^T; 8, *Rhizobium meliloti* 2011. (Lower panel) Lanes: 1, *O. anthropi* ATCC 49188^T; 2, *P. myrsinacearum* ATCC 43590^T; 3, *Rhodobacter capsulatus* ATCC 11166; 4, *Rhizobium meliloti* 2011; 5, *A. tumefaciens* C58. The positions of molecular size markers are shown on both sides of the two panels.

(ii) The genus *Rhizobium*. Sobral et al. have described the presence of three megabase-sized replicons in *Rhizobium meliloti* 1021, one chromosome of 3.5 Mb and two megaplasmids of 1.7 and 1.3 Mb, so called because they did not hybridized with an rRNA probe (38). Nevertheless, essential housekeeping genes such as the GroEL chaperonin-encoding genes are present on these molecules, thus raising questions about the chromosomal status of these replicons (35). We tested a second strain, *R. meliloti* 2011, and found three replicons of sizes similar to those of strain 1021, again with only the larger one hybridizing with the rRNA probe (Fig. 2 and 3, and Table 1).

For *Rhizobium fredii* ATCC 35423^T, two megabase-sized replicons and a small replicon were separated. Only the largest one was shown to hybridize with the rRNA probe (Fig. 1 to 3 and Table 1).

In *Rhizobium leguminosarum* ATCC 14480^T and ATCC 14482^T (corresponding to the biovars trifolii and phaseoli), we found two circular megareplicons plus the two large plasmids previously described for this species (29). We hybridized an rRNA probe with separated replicons of *R. leguminosarum* bv. trifolii. Again, only one (the largest) contains rRNA genes (Fig. 1 to 3 and Table 1).

The organization of the genome of *Bradyrhizobium japonicum* is different; this genome has a single chromosome that is larger (8,700 kb) than that of the other *Rhizobium* species (6,400 to 6,700 kb) (6, 22).

(iii)The genus *Phyllobacterium*. For *Phyllobacterium myrsinacearum* ATCC 43590^T, five replicons were separated, with the larger (megabase-sized) one hybridizing with the rRNA probe (Fig. 2, 4, and 5 and Table 1). The genomic organization for the genus *Phyllobacterium* also seems different from that of the other genera of the *Rhizobiaceae*, with there being only one megareplicon (but several large plasmids) and a smaller total genome size (5,330 kb).

Related bacteria belonging to others genera and families. Members of the *Rhizobiaceae* are also related to taxonomically different organisms (9, 43). *Rhodobacter sphaeroides* is found on a distant branch of rRNA superfamily IV, which comprises *Agrobacterium* species, *Rhizobium* species, and *Brucella abortus* (9). Other bacteria that are closely related to rRNA superfam-

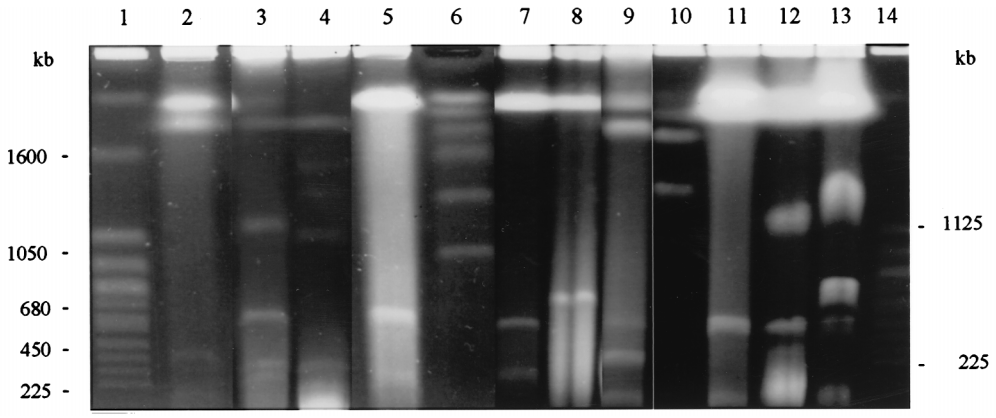

FIG. 3. PFGE of intact DNAs of bacterial species belonging to the family *Rhizobiaceae*: separation of small replicons. Lanes: 1, *Saccharomyces cerevisiae*; 2, *A. rhizogenes* K84; 3, *A. vitis* CFBP 2721; 4, *A. vitis* CFBP 2607; 5, *A. tumefaciens* C58; 6, *H. wingei*; 7, *A. tumefaciens* ATCC 23308^T; 8, *A. radiobacter* CFBP 2414^T; 9, *A. rubi* ATCC 13335^T; 10, *Rhizobium meliloti* 2011; 11, *Rhizobium fredii* ATCC 35423^T; 12, *Rhizobium leguminosarum* bv. phaseoli ATCC 14482^T; 13, *R. leguminosarum* bv. trifolii ATCC 14480^T; 14, *Saccharomyces cerevisiae*. The positions of molecular size markers are shown on both sides of the gel.

ily IV are *Mycoplana dimorpha*, *Ochrobactrum anthropi*, and *Bartonella quintana* (37, 40, 43). These related bacteria represent a heterogeneous group whose members have few common features; *Rhodobacter sphaeroides* is a facultative photosynthetic bacterium (39), *M. dimorpha* is a soil-living organism (43), *Brucella* and *Bartonella* species are animal pathogens (42, 43), and *O. anthropi* is a rare opportunistic pathogen of immunocompromised patients (2).

(i)The genus *Rhodobacter*. Suwanto and Kaplan have shown that *Rhodobacter spheroides* 2.4.1 possesses two chromosomes, one of 3,000 kb and the other of 900 kb (39). However, the chromosomal structure of *Rhodobacter capsulatus* SB1003 is quite different, consisting of a unique 3,800-kb chromosome and a 134-kb plasmid (12). We investigated another strain of *Rhodobacter capsulatus* (ATCC 11166) and found only one megabase-sized replicon, which hybridized to the rRNA probe, and a small replicon (Fig. 2, 4, and 5 and Table 2).

(ii) The genera *Ochrobactrum and Brucella*. Two strains of *O. anthropi* were studied. Two megareplicons of similar sizes were found in both strains ATCC 49188^T and LMB 3301, and two small replicons of different sizes were found in each of these strains (Fig. 2, 4, and 5 and Table 2). Only the two larger bands hybridized with the rRNA probe. The physical map of

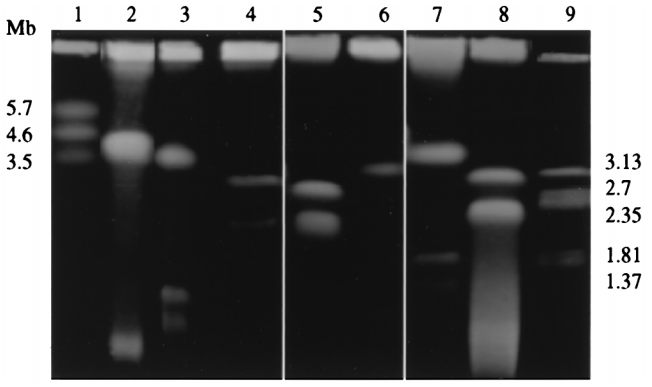

FIG. 4. PFGE of intact DNAs of bacterial species related to the *Rhizobiaceae*: separation of large replicons. Lanes: 1, *Schizosaccharomyces pombe*; 2, *Rhodobacter capsulatus* ATCC 11166; 3, *P. myrsinacearum* ATCC 43590^T; 4, *O. anthropi* ATCC 49188^T; 5, *O. anthropi* LMG 3301; 6, *M. dimorpha* ATCC 4279^T; 7, *Rhizobium meliloti* 2011; 8, *A. tumefaciens* C58; 9, *H. wingei*. The positions of molecular size markers are shown on both sides of the gel.

Brucella melitensis has been constructed, and it demonstrated the presence of two circular chromosomes (27). These two chromosomes are also present in the other species of this genus (28), with the exception of one biovar (see below).

(iii) The genera *Mycoplana* and *Bartonella*. The type strain *M. dimorpha* ATCC 4279^T had only one (megabase-sized) replicon and two plasmids (Fig. 4 and 5 and Table 2). *Bartonella quintana* was shown to possess only one chromosome (reference 32 and unpublished data).

Does genomic organization have phylogenetic significance? Using highly conserved sequences such as the rRNA genes or housekeeping proteins such as the GroEL chaperonin and RecA, phylogenetic trees have been constructed which have allowed the definition of the alpha subgroup of the *Proteobacteria* (9, 10, 40–42). The genomic organization of bacteria belonging to this group has been studied to see if a correlation with the phylogeny could be demonstrated.

Genome size differences, increasing with the evolutionary genetic distance between lineages, were shown to exist for the major subgroups of *Escherichia coli*, which suggests that there is a phylogenic component to this variation (4). The genome of *A. rhizogenes* K84 (7,265 kb) is 1.45 times larger than that of *A. vitis* CFBP 2607 (4,980 kb). This degree of variation is comparable to that seen for different strains of *Burkholderia cepacia* (13). Strains of *Agrobacterium* biovars 1 and 2 exhibit only 15% DNA homology (21). Our results show that their genome sizes and organizations are also very different, thus providing further evidence that they are genetically distinct. Sawada et al. place *Agrobacterium* biovar 2 closer to *Rhizobium fredii*, and this is again supported by the genomic organization (37).

Most of the organisms possessing several megabase-sized replicons belong to the alpha subgroup of the *Proteobacteria* (Fig. 6); the exceptions are *Burkholderia (Pseudomonas) cepacia*, which is classified in the β2 subgroup (42), and *L. interrogans*, which is a spirochete (45). The members of subgroup α2 form a tight cluster, while the β subgroup constitutes a quite phylogenetically diverse class (20, 42). The existence of a more complex genomic architecture (with two or three chromosomes) may have phylogenetic significance if this trait is also found in other organisms of the same lineage. Among the alpha-subgroup genera that we have investigated, this feature is present in all of the species of *Agrobacterium*, *Rhizobium*, *Brucella* (except one [see below]), and *Ochrobactrum*. On the contrary, *Bradyrhizobium*, *Phyllobacterium*, *Mycoplana*, and

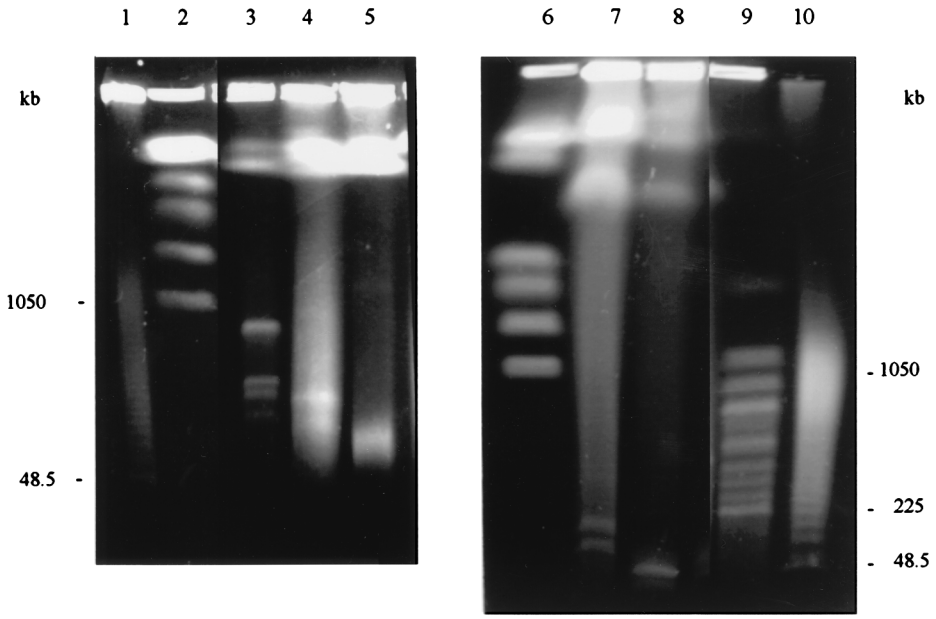

FIG. 5. PFGE of intact DNAs of bacterial species related to the *Rhizobiaceae*: separation of small replicons. Lanes: 1, lambda DNA ladder; 2, *H. wingei*; 3, *P. myrsinacearum* ATCC 43590^T; 4, *M. dimorpha* ATCC 4279^T; 5, *Rhodobacter capsulatus* ATCC 11166; 6, *H. wingei*; 7, *O. anthropi* ATCC 49188^T; 8, *O. anthropi* LMG 3301; 9, *Saccharomyces cerevisiae*; 10, lambda DNA ladder. The positions of molecular size markers are indicated to the left and right.

Bartonella species have only one chromosome. Finally, in the genus *Rhodobacter*, *R. sphaeroides* has two chromosomes while *R. capsulatus* has only one.

The *Rhizobiaceae* can be divided into two groups. The fast-growing strains (*Rhizobium meliloti*, *Rhizobium fredii*, and *Rhizobium leguminosarum*) all have complex genomes, while the slow-growing species *Bradyrhizobium japonicum* has a single, very large chromosome (22). The genus *Bradyrhizobium*, however, is only remotely related to the other genera of the *Rhizobiaceae* (41). The deeper branching found for *Bradyrhizobium japonicum* with both the 16S rRNA and the GroEL sequences (9, 40, 43) could mean that the origin of this lineage is close to the single-chromosome ancestor. This taxonomically different genus (17) represents a separate line of descent in the α2 subgroup of the *Proteobacteria* (41), one which is also remote from the *Agrobacterium* rRNA branch in rRNA superfamily IV (16). In contrast, *Rhodobacter capsulatus* and *Rhodobacter sphaeroides*, with one and two chromosomes, respectively, branch together on the phylogenetic tree (10). In this case of two species belonging to the same lineage, it is difficult to explain how two organisms with such different genomic organizations could have a common ancestor unless this feature is not linked with the phylogeny. Moreover, within the same species—*Brucella suis*—the genome of the biovar 3 reference

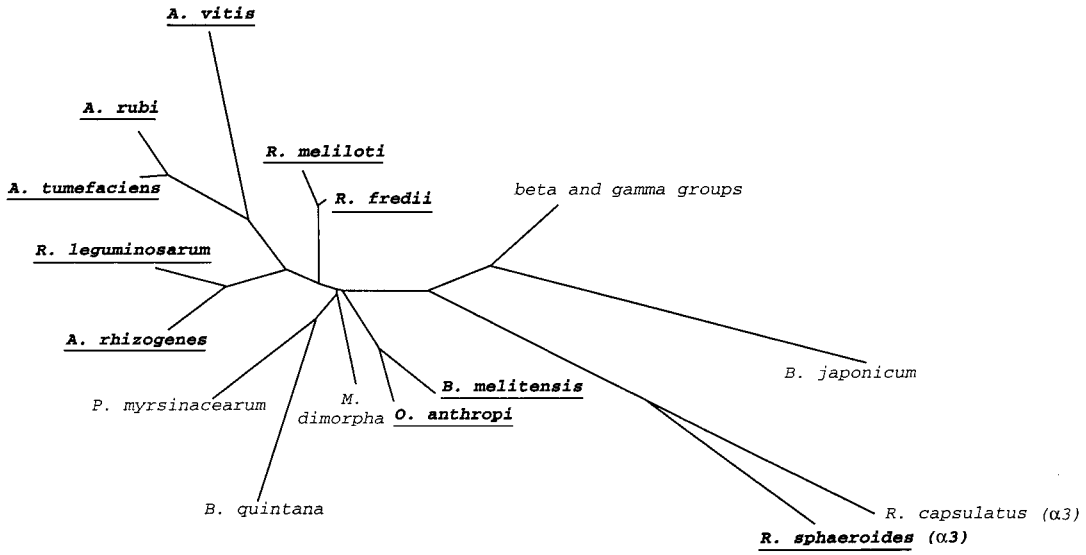

FIG. 6. Phylogenetic tree showing the genomic organization of organisms belonging to the α2 subgroup of the *Proteobacteria* (plus *Rhodobacter* species, which belong to the α3 subgroup) (redrawn from reference 43). Organisms with complex genomes are indicated in boldface and underlined.

strain is composed of a single chromosome of 3.2 Mb while biovar 1 members each possess two chromosomes, of 2.1 and 1.15 Mb, and biovar 2 and 4 members each have two chromosomes, of 1.85 and 1.35 Mb. The four biovars are phenotypically very similar, and the restriction maps of their genomes are also very similar except for the distribution of the same sequences on different linkage groups (19). Other evidence is from outside of the α-proteobacteria, for the gram-positive bacterium *Bacillus cereus*, whose different strains vary with respect to their chromosome sizes and genome organizations. Within this species, the genome may exist either as one large chromosome with small plasmids or as a small chromosome with stably maintained large extrachromosomal elements which may be considered as fragments of a secondary chromosome (5).

Thus, the presence of multiple chromosomes in α-proteobacterial genomes is not related to a common phylogeny, since it is not always shared either by all of the members of a same clade (e.g., *Rhodobacter* genus) or even by all of the strains of the same species (e.g., *Brucella suis*). This trait, found inconstantly among different bacterial lineages, rather seems to have been acquired independently. Where does this complex organization originate?

The classical model of genome evolution involves gene duplication followed by divergence. This can occur via a tandem duplication in the genome, achieved by recombination between repeated sequences (34). Such repeats could be rRNA operons. Following this, a second intrachromosomal recombination event, occurring anywhere within the duplicated region, will result in the formation of two stable replicons if both molecules have an origin of replication; alternatively, the second origin of replication could be acquired by lateral transfer from a different organism. A comparison of the sequences of these molecules will distinguish between these two possibilities. Nevertheless, there is no known environment shared by these different organisms which could explain their "infection" by a new origin. In the case of the genus *Brucella*, we have shown that the different species exhibit differences in genomic organization. The differences in chromosome size and number can be explained by the occurrence of rearrangements at chromosomal regions containing the three *rrn* genes. The location and orientation of these genes confirmed that these rearrangements are due to homologous recombination at the *rrn* loci (19). This phenomenon occurred naturally in the genus *Brucella*; however, recently the 4,188-kb circular genome of *Bacillus subtilis* was artificially dissected into two stable circular chromosomes in vivo by such a mechanism (15).

The coexistence of linear and circular chromosomes in the same bacterial cell raises another question. The chromosome of *Streptomyces lividans* probably oscillates between linear and circular forms, and this may also occur in other bacteria (31). It has been suggested by Hinnebusch and Tilly (14) that one of the origins of linear DNA in bacteria could be genetic exchange between procaryotes and eucaryotes. These authors also add that the most evident example of this gene exchange is the transfer of DNA from the phytopathogen *A. tumefaciens* into a plant cell to induce the formation of a crown gall tumor. It is perhaps not a coincidence that in this species the chromosomes exhibit both types of structures.

The reason for the presence of a complex genomic organization in many members of the α-proteobacteria remains to be determined. While we have shown that the possession of a complex genome does not have a clear phylogenetic significance, we can speculate that there are structures in or functions of the genome of the alpha subgroup which favor their appearance.

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