Physical Map of the *Bartonella bacilliformis* Genome

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The genome of *Bartonella bacilliformis* was shown to be a single circular DNA molecule of about 1,600 kbp having six *Not*I, four *Sfi*I, and two *Ceu*I sites. A physical map of the DNA was constructed by contour-clamped homogeneous electric field pulsed-field gel electrophoresis of DNA restriction fragments. RNA operons, the invasion-associated locus, and a flagellin gene were located on the map by hybridization.

*Bartonella bacilliformis* is a flagellated, highly motile, hemotrophic, gram-negative bacterium (6, 13) which is transmitted to humans by sand fly bites (4, 6, 7, 13). It is the causative agent of Carrió’s disease, an endemic disease in certain areas of Columbia, Ecuador, Peru, Bolivia, and Chile (4). To facilitate study of this interesting bacterium, we have constructed an initial physical map of its DNA by pulsed-field gel electrophoresis (PFGE) of *Not*I, *Sfi*I, and *Ceu*I restriction fragments.

**Number and sizes of restriction fragments.** *B. bacilliformis*, grown to $1 \times 10^8$ to $2 \times 10^8$ bacteria per ml as previously described (9), was washed with phosphate-buffered saline (PBS), resuspended in 1 ml of PBS, mixed with 1 ml of molten 2% pulsed-field agarose (Bio-Rad Laboratories, Richmond, Calif.), and poured into a commercial plastic mold (Bio-Rad, Richmond, Calif.) designed to form thin agarose slabs. The bacteria were lysed within the agarose slabs by soaking the slabs in 1% sodium dodecyl sulfate–proteinase K (1 mg/ml)–TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA [pH 8.0]) for 48 h at 37°C, rinsing them with 10 changes of TBE, soaking them in 100% ice-cold acetone for 20 min, and rinsing them with 5 changes of TBE. The processed slabs were stored in 0.5 M EDTA at 4°C. Digestions were performed on small agarose plugs (9 by 1 by 1 mm) cut from the slabs. The plugs were washed and placed into microcentrifuge tubes. Restriction endonuclease digestions were performed under conditions recommended by New England Biolabs (Beverly, Mass.). After electrophoresis, the DNA was initially visualized with ethidium bromide after PFGE; the fragments and therefore is located entirely within N2. N2 contained additional segments common to S1 and S2. Therefore, the order of these *Sfi*I fragments must be S1-S3-S2 or the reverse. S4 (DD8) was found only in N5, and N5 contained additional segments common to S1 and S2. Therefore, the order of fragments S1, S2, and S4 must be S1-S4-S2 or the reverse. These two orders are possible only if the map is circular.

Similarly, N1 (DD1) and N6 (DD7) are common only to S2. S2 also contained segments common to N2 and N5, so the order must be N2-(N1, N6)-N5 or the reverse, where the order of N1 and N6 is not determined. N3 (DD2) and N4 (DD5) are common only to S1. S1 also contained segments common to N2 and N5, so the order must be N2-(N3, N4)-N5 or the reverse. Again, these results are possible only if the map is circular.

To determine the correct order for the pairs N1-N6 and N3-N4, DNA fragments (19 bands [N1 to N6 and PN1 to PN13]) resulting from partial *Not*I cleavage (PN) with 1 U of *Not*I for 1 to 2 h were separated by electrophoresis in one dimension and then completely digested with *Not*I and $^{32}$P labeled within the agarose. The completely digested fragments were then separated by electrophoresis conducted at right angles to the original direction (data not shown). PN1, the largest fragment (1,550 kbp), resolved into N1, N2, N3, N4, N5, and N6 (combined size of 1,617 kbp), resolved into N5 (109 kbp) and

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N6 (50 kbp), so N5 and N6 are adjacent. The second-smallest fragment, PN12 (250 kbp), resolved into N5 (109 kbp) and N4 (116 kbp), so N5 and N4 are adjacent. Confirming the order N4-N5-N6, PN11 (285 kbp) resolved into N4, N5, and N6. The next-largest partial cleavage fragment, PN10, was 664 kbp long, so no fragments were found at sizes consistent with the alternative arrangements of neighbors, N5-N3 (predicted size, 463 kbp) and N2-N6 (predicted size, 494 kbp).

The order of the four SfiI fragments in the B. bacilliformis genome is S1-S3-S2-S4-S1. The order of the 6 NotI fragments is N1-N2-N3-N4-N5-N6-N1. That the genomic order of NotI digestion fragments corresponds to the order of their sizes is presumably accidental. The restriction map is diagrammed in Fig. 2.

Locations of rRNA genes and CeuI sites. By hybridization, the B. bacilliformis rRNA genes were localized to DD4 and DD8. The 16S rRNA oligonucleotides used were from the 5′ region (nucleotides 1 to 14 and 4 to 28) and the middle region (nucleotides 726 to 750 and 727 to 746) of the B. bacilliformis 16S rRNA gene (GenBank accession number X60042) (3, 5, 12). The 5S rRNA oligonucleotide consisted of nucleotides 1 to 19 of the B. bacilliformis 5S rRNA gene (GenBank accession number L08503) (10).

CeuI cuts within a 26-bp DNA recognition sequence which is found in a highly conserved region of bacterial 23S rRNA genes and which has not been found elsewhere in bacterial DNA (8). CeuI would be expected to cleave the B. bacilliformis 23S rRNA gene (GenBank accession number L39095) once within positions 1836 to 1861.

Cleavage with CeuI alone yielded two fragments. Cleavage with CeuI and then with SfiI resulted in the disappearance of fragments S1, S4, and DD4 (194 kbp) region (nucleotides 1 to 14 and 4 to 28) and the middle region (nucleotides 726 to 750 and 727 to 746) of the B. bacilliformis 16S rRNA gene (GenBank accession number L39095) once within positions 1836 to 1861.

Cleavage with CeuI alone yielded two fragments. Cleavage with CeuI and then with SfiI resulted in the disappearance of fragments S1 and S4 (DD8), so at least one CeuI site must be located in each of these fragments. CeuI digestion followed by NotI digestion resulted in the disappearance of N2 and N5. The DNA segment common to S1 and N2 is DD4 (194 kbp)
and the DNA segment common to S4 and N5 is DD8 (15 kbp), so the location of the CeuI sites is in agreement with the hybridization results.

The new DNA fragments with lengths of 644 and 89 kbp found by CeuI-SfiI digestion must be derived from S1 (753 kbp), indicating that the CeuI site in S1 (and DD4) is about 89 (89 to 109) kbp from the SfiI site that marks the boundary between S3 and S1. The data from the CeuI-NotI digestion indicate that the CeuI cleavage site is located about 105 to 142 kbp from the same SfiI site. In the CeuI-NotI digestion, N2 (444 kbp) and N5 (109 kbp) are replaced by new DNA fragments of 392 kbp, 89 kbp (which because of its width and brightness appears to be a double band), and 12 kbp in length. On the basis of the 392-kbp value, the CeuI site would be located about 142 kbp from the SfiI site, and on the basis of the 89-kbp value, the CeuI site would be 105 kbp from the SfiI site.

The 12-kbp fragment must be derived from N5 (109 kbp), indicating that this CeuI site lies about in the middle of DD8. Locating the CeuI site near the middle of DD8 is consistent with the results of a CeuI-SfiI digestion run on a standard gel in which new fragments with lengths of 8 and 7 kbp were found. These fragments must be derived from S4 (15 kbp), also indicating that the CeuI site is near the center of S4 (DD8).

**Location of invasion-associated locus and flagellin genes.** By hybridization, the *B. bacilliformis* invasion-associated locus genes studied by Mitchell and Minnick (11) were localized to DD4. Invasion-associated locus oligonucleotides corresponded to sequences from positions 202 to 181 (on the negative strand of *ialA*) and positions 463 to 484 (on the positive strand of *ialB*) (GenBank accession number L25276) (11). $^{32}$P-labeled oligonucleotide probes were prepared with T4 polynucleotide ki-

<table>
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<th>Fragment size (kbp)</th>
<th>SfiI fragment</th>
<th>NotI fragment</th>
<th>Double-digestion fragment(s)</th>
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* The multiple double-digestion fragments are components of the single-digestion fragment indicated in the same row.

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**FIG. 2. Physical map of the *B. bacilliformis* genome.** Locations (with positions in kilobase pairs) of two CeuI (C), four SfiI (S), and six NotI (N) sites are shown on a circular *B. bacilliformis* DNA molecule of 1,617 kbp. The locations of genomic DNA fragments DD1 to DD10, N1 to N6, and S1 to S4 are indicated in the inner circles.
our laboratory (GenBank accession number L20677) was located to DD7. The flagellin gene was available on a plasmid clone (1), which was linearized and labeled with $^{32}$P with a random priming Ready-To-Go DNA labeling kit (Pharmacia, Piscataway, N.J.) according to the manufacturer’s instructions.

For hybridization, nonradioactive DNA fragments of $^{32}$P-, end-labeled fragments resulting from NotI-SfiI digestion of the B. bacilliformis genome were separated in alternate lanes of pulsed-field gels. The gels were then stained with ethidium bromide and photographed. The DNA was nicked and vacuum transferred to a nylon membrane. The resulting blots were cut into strips each containing two lanes, one lane containing $^{32}$P-, end-labeled reference fragments and the other lane containing the corresponding nonradioactive fragments to be probed. The individual strips were then hybridized to various probes and analyzed by autoradiography. DNA which hybridized to a probe could be unambiguously assigned to 1 of the 10 double-digest fragments.

**Genome size estimation.** Time course reassociation of denatured B. bacilliformis genomic DNA was previously used to estimate the size of the B. bacilliformis genome to be one-sixth of that of Escherichia coli, or about 752 kbp (4). That B. bacilliformis would have a genome smaller than those of some Mycoplasma spp. while retaining genetically complex features such as flagella and a gram-negative cell wall was surprising.

PFGE permits a more accurate and direct physical measurement of the size of the genome than reassociation kinetics does. The sum of restriction fragment lengths showed that the size of the B. bacilliformis genome is twice that determined by time course reassociation techniques; the PFGE-based measurement seems to be in better agreement with the biological complexity of B. bacilliformis. The order of restriction fragments showed that they must have been cut from a circular DNA molecule. Additional evidence that the B. bacilliformis genome is circular is provided by the observations that cutting at the two CeuI sites produced only two fragments and that uncleaved B. bacilliformis DNA did not enter the gel, but DNA incubated with S1 nuclease, which is believed to introduce only one break in circular DNA (1a, 2), produced a single, discrete DNA species (presumably a linearized form of the entire intact genome), migrating with an apparent size of 1,550 kbp. Also, the largest NotI partial cleavage fragment, PN1, which contains all six NotI complete cleavage fragments, entered the gel. PN1 must be the entire genome in linear form, resulting from a single NotI cleavage in the circular DNA. Both PN1 and S1 nuclease-treated genomic DNA, linearized but apparently complete DNA molecules, migrated with an apparent size of 1,550 kbp. The sum of the sizes of restriction fragments, which might contain multiple systematic errors, indicated that the size of the intact B. bacilliformis chromosome is 1,617 kbp for both NotI and SfiI. We take the current best estimate for the genome size to be about 1,600 kbp.

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**REFERENCES**