**Tropheryma whipplei** Twist: A Human Pathogenic Actinobacteria With a Reduced Genome

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The human pathogen *Tropheryma whipplei* is the only known reduced genome species (<1 Mb) within the Actinobacteria [high G+C Gram-positive bacteria]. We present the sequence of the 927,303-bp circular genome of *T. whipplei* Twist strain, encoding 808 predicted protein-coding genes. Specific genome features include deficiencies in amino acid metabolisms, the lack of clear thioesterases and thioredoxin reductase homologs, and a mutation in DNA gyrase predicting a resistance to quinolone antibiotics. Moreover, the alignment of the two available *T. whipplei* genome sequences (Twist vs. TW08/27) revealed a large chromosomal inversion the extremities of which are located within two paralogous genes. These genes belong to a large cell-surface protein family defined by the presence of a common repeat highly conserved at the nucleotide level. The repeats appear to trigger frequent genome rearrangements in *T. whipplei*, potentially resulting in the expression of different subsets of cell surface proteins. This might represent a new mechanism for evading host defenses. The *T. whipplei* genome sequence was also compared to other reduced bacterial genomes to examine the generality of previously detected features. The analysis of the genome sequence of this previously largely unknown human pathogen is now guiding the development of molecular diagnostic tools and more convenient culture conditions.

[Supplemental material is available online at www.genome.org. The sequence data from this study have been submitted to GenBank under accession no. AE041844. Genome sequence and annotation are also available at http://igs-server.cnrs-mrs.fr/]

*Tropheryma whipplei* is the bacterial agent of Whipple’s disease, a spectacular chronic disease described in 1907 by Nobel laureate George Whipple (Whipple 1907). Whipple’s disease is characterized by intestinal malabsorption leading to cachexia and death if untreated. The causative agent of Whipple’s disease, *Tropheryma whipplei*, is the bacterial agent of *T. whipplei* within the high G+C content Gram-positive bacteria. *T. whipplei* is relatively well equipped, with different biological functions compared to other bacteria with reduced genomes (<1 Mb).

**RESULTS AND DISCUSSION**

**Genome Sequence and Predicted Functions**

**General Features**

The 927,303-base pair (bp) circular genome of *T. whipplei* Twist exhibits 808 predicted protein coding genes and 54 RNA genes (Fig. 1A,B; Table 1). The average G+C content is 46%, by far the lowest among the genome sequences available for other high G+C content Gram-positive bacteria. Coding content is 85.6%. The number of ORFs is 6421, which is lower than the number estimated by genome sequencing (7897 ORFs; Supplementary Fig. S1, available online at www.genome.org). Predicted gene functions (Fig. 2) indicate that *T. whipplei* is relatively well equipped, with different biological functions compared to other bacteria with reduced genomes (<1 Mb).
Information Processing

*T. whipplei* exhibits a complement of information processing genes comparable to that found in other small parasitic bacteria (Fig. 2). The DNA polymerase III complex—the primary replication machinery—is composed of the alpha (*dnaE*), beta (*dnaN*), gamma-tau (*dnaZX*), and putative delta subunits, as found in *Mycoplasma genitalium*. *T. whipplei* lacks homologs for the DNA polymerase polC, the second and essential polymerase in *Bacillus subtilis* (Dervyn et al. 2001). In contrast, *T. whipplei* appears to have two DNA gyrase subunits, each made of two subunits, as found in *S. coelicolor*, another Actinobacteria. *T. whipplei*'s TWT006/TWT005 gene pair appears to be orthologous to the gyrase subunits found in Actinobacteria; their role is to eliminate positive supercoils at the replication fork. The second gyrase-like copy, TWT491/TWT494, are orthologous to *S. coelicolor* SCO5836/SCO5822 genes that probably encode topoisomerase IV (ParC/ParE); this system is involved in chromosome segregation. Interestingly, an alanine residue was found at position 81 of GyrA and at position 96 of ParC, at which serine residues are usually found. In *Escherichia coli*, mutations at these positions are associated with the acquisition of resistance to quinolone antibiotics (Drlica and Zhao 1997; Drlica 1999). Based on these data, *T. whipplei* is predicted to be resistant to quinolones. This was recently confirmed experimentally (Masselot et al. 2003).

*T. whipplei* contains two paralogous genes for chromosome partitioning protein ParA. One (parA) is apparently orthologous to those of *Mycobacterium* and *Streptomyces*. The other (parA2) is rather similar to plasmid-encoded parA in the actinomycete *Rhodococcus erythropolis*. In addition, *T. whipplei* exhibits two replicative DNA helicases, dnaC and pcrA; the latter is involved in plasmid rolling-circle replication and ultraviolet-induced damage repair in *B. subtilis* (Petit et al. 1998). *T. whipplei* contains three...
Figure 1  (A) Circular representation of the *T. whipplei* Twist genome (upper panel), and the alignment of the Twist and TW08/27 genomes (lower panel). The origin of replication was predicted to be near the dnaA gene, based on the conservation of dnaA-dnaN-recF gene cluster and the change of AT-skew signal. One leading strand displays a pronounced excess in T vs. A and a slight excess of G vs. C. The location of the oriC was verified by Southern-blot hybridization with a digoxigenin-labeled oriC probe onto the NotI restriction profile. In the upper panel, the outermost (1st) circle indicates the nucleotide positions. 2nd circle: The two chromosomal segments, one of which exhibits an inversion (see text). 3rd and 4th circles: The ORF locations on the plus and minus strands, respectively. Functional categories are color-coded (see Fig. 1B). 5th and 6th circles: tRNAs. 7th circle: The locations of three rRNAs are indicated by black arrows. 8th circle: The repeat locations for the two largest families. The most internal circle shows the AT-skew (A−T/A+T) computed with a sliding window size of 10 kb. The lower panel shows the detailed structure of the chromosomal inversion (red arrows), flanked by repeated sequences coding for WND-domains (green arrows). Black arrows: WiSP protein-coding genes at the extremities of the inverted segments. (B) Linear view of the complete circular genome of *T. whipplei*. Arrows = genes, with color-coded functional categories; small red flags = tRNAs; open arrows = other RNAs; open boxes = repeats belonging to the two major categories.
amino acids are represented among these aaRSs, except for glutamine (GlnRS) and asparagine synthetase (AsnRS), as in *T. whipplei*.

In prep.)

Both *T. whipplei* and *Buchnera* (Shigenobu et al. 2000) have retained approximately half of the amino-acid biosynthetic pathways. It is thought that *Buchnera* species specifically retained the pathways corresponding to amino acids essential for their insect hosts. As no such symbiotic association is known for *T. whipplei*, its residual biosynthetic capacity might point out the amino acids that are in the most limited supply from its environment or host. *T. whipplei* lacks clear orthologs for thioredoxins (Trx) and thioredoxin reductase (TrxR) of *M. tuberculosis*, whose genome encodes three Trx (Rv3914, Rv1470, Rv1471) and one TrxR (Rv3913) in two operons. TrxR is a ubiquitous enzyme that reduces Trx, which in turn acts as electron donor in various essential redox reactions in the cell. TrxR- and Trx-encoding genes have been found in all bacterial genomes sequenced so far (Fig. 4). This includes *Coxiella burnetii*, which shares the same intracellular acidic vacuoles niche as *T. whipplei*. Because this would represent the first case of a bacterium without a functional thioredoxin system, this matter was investigated in greater detail. Protein motif searches using the PROSITE database entries (PS500194 for Trx; PS500573 for TrxR) failed to identify any ORFs with the sequence signatures in the *T. whipplei* genome. Another search using the TIGRFAM database (TIGR01068 for Trx; TIGR01292 for TrxR) again failed to identify significantly similar sequences (above the noise cutoff). However, we further analyzed some candidates listed below this threshold. One was ORF TWT756, similar to *B. subtilis* thioredoxin-like protein gene *resA*. Another was ORF TWT210, exhibiting a significant sequence similarity to proteins of the pyridine nucleotide-disulfide oxidoreductases class II family. TWT210 might thus encode the thioredoxin reductase function, despite its weak similarity to known thioredoxin reductases. The glutaredoxin system serves similar roles as the thioredoxin system. *T. whipplei* has one copy of glutathione reductase gene (Rv2855), but the glutaredoxin system appears incomplete because of the lack of glutaredoxin genes. *T. whipplei* exhibits a distant homolog TW7629 to glutathione reductase, but shows no evidence of glutaredoxin genes. In conclusion, experiments are necessary to confirm that *T. whipplei* might be the first example of a bacterium without the—usually—essential thioredoxin pathway.

### Interaction With the Environment

We identified 40 genes for transporters, which probably comprise 10 to 15 different transport systems. These include two systems for amino acids transport, one for L-arabinose, two for iron, and one for phosphate. We identified two putative membrane pro-

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### Table 1. General Features of the *T. whipplei* Genome

| Feature                     | Value                  |
|-----------------------------|------------------------|
| Genome size                 | 927,303 bp             |
| G + C content               | 46.3%                  |
| Coding content              | 85.6%                  |
| ORFs                        | 808                    |
| Median size                 | 287 aa                 |
| Annotated                   | 535 (66%)              |
| Best database match         | 678 (84%)              |
| High G + C gram-positive    | 552                    |
| Streptomyces coelicolor     | 292                    |
| Corynebacterium glutamicum  | 72                     |
| Mycobacterium leprae        | 36                     |
| Other species               | 57                     |
| Low G + C Gram-positive     | 39                     |
| Proteobacteria              | 47                     |
| Other bacteria              | 19                     |
| Archaea                     | 13                     |
| Eucarya                     | 7                      |
| Viruses (Equine herpesvirus) | 1                      |
| ORFans                      | 130 (16%)              |
| tRNA                        | 54                     |
| tmRNA                       | 49 (20 species)        |
| M1 RNA (RNase P)            | 1                      |

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The thioredoxin reductase (TrxR) of *M. tuberculosis*, whose genome encodes three Trx (Rv3914, Rv1470, Rv1471) and one TrxR (Rv3913) in two operons. TrxR is a ubiquitous enzyme that reduces Trx, which in turn acts as electron donor in various essential redox reactions in the cell. TrxR- and Trx-encoding genes have been found in all bacterial genomes sequenced so far (Fig. 4). This includes *Coxiella burnetii*, which shares the same intracellular acidic vacuoles niche as *T. whipplei*. Because this would represent the first case of a bacterium without a functional thioredoxin system, this matter was investigated in greater detail. Protein motif searches using the PROSITE database entries (PS500194 for Trx; PS500573 for TrxR) failed to identify any ORFs with the sequence signatures in the *T. whipplei* genome. Another search using the TIGRFAM database (TIGR01068 for Trx; TIGR01292 for TrxR) again failed to identify significantly similar sequences (above the noise cutoff). However, we further analyzed some candidates listed below this threshold. One was ORF TWT756, similar to *B. subtilis* thioredoxin-like protein gene *resA*. Another was ORF TWT210, exhibiting a significant sequence similarity to proteins of the pyridine nucleotide-disulfide oxidoreductases class II family. TWT210 might thus encode the thioredoxin reductase function, despite its weak similarity to known thioredoxin reductases. The glutaredoxin system serves similar roles as the thioredoxin system. *M. tuberculosis* has one copy of glutathione reductase gene (Rv2855), but the glutaredoxin system appears incomplete because of the lack of glutaredoxin genes. *T. whipplei* exhibits a distant homolog TW7629 to glutathione reductase, but shows no evidence of glutaredoxin genes. In conclusion, experiments are necessary to confirm that *T. whipplei* might be the first example of a bacterium without the—usually—essential thioredoxin pathway.
Figure 2  Comparative analysis of the number of genes present in each functional category as defined by the COG database. For the sake of comparing bacterial genomes of largely different sizes, a percentage graph is provided in Suppl. Fig. S2.
proteins related to drug efflux proteins that may play a role in modulating antibiotic susceptibility. *T. whipplei* also exhibits a gene for dimethyladenosine transferase (ksgA), known to be related to kasugamycin resistance in *E. coli*. Those genes may help *T. whipplei* resist various antibiotics. A cold-shock protein gene *capB* was found, suggesting that the lifestyle of *T. whipplei* may include some cold periods and arguing against obligate human parasitism. The genes for two sensor histidine kinase/response regulator systems were also identified. Two protein translocation gene sets were identified, a Sec system and a twin-arginine translocase (TAT).

Finally, *T. whipplei* possesses *whiA* and *whiB*, two regulatory (possibly transcriptional) factors essential for the sporulation of *S. coelicolor* (Molle et al. 2000), and another *whiB* homolog (whiB). Spores have not yet been observed for *T. whipplei*, but the presence of these genes suggests that they may arise in environments not yet mimicked in laboratory culture conditions.

**Gene Families**

We identified 38 families of predicted gene paralogs (Suppl. Table S1). Of these, eight are lineage-specific families (exhibiting higher similarities within the family than to any other genes from other bacteria). They include WiSP membrane proteins (Bentley et al. 2003), other predicted membrane proteins, exodeoxyribonuclease III, inorganic pyrophosphatases, and iron ABC transporters.

**Comparative Genome Analysis**

The genome sequence of another *T. whipplei* isolate (strain TW08/27; GenBank: BX072543) recently became available (Bentley et al. 2003). The two genomic sequences of the different *T. whipplei* strains are mostly identical (>99% identity at the nucleotide sequence level), and encode quasi-identical gene complements. However, the Twist and TW08/27 genome organizations differ by the inversion of a large chromosomal segment (Twist coordinates: 182333–713982; Fig. 1A). Such inversions, approximately symmetrical to the origin of replication, are frequently observed in interspecific bacterial genome comparisons (Eisen et al. 2000; Hughes 2000; Makino and Suzuki 2001). However, such a difference between otherwise almost identical strains is an indication of a very active genome rearrangement process in *T. whipplei*. As already suggested for several Gram-negative species (*Salmonella*, *Neisseria*, *Pseudomonas*, and *Bordetella*), such genome rearrangement might be the consequence of the host-bacteria interaction (Hughes 2000). Interestingly, the boundaries of *T. whipplei* genome inversion are within the coding regions of two genes of

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**Figure 3** Predicted amino acid metabolisms of *T. whipplei* based on the *M. tuberculosis* metabolisms described in KEGG. Metabolic pathways were predicted to be lost for nine amino acids (pink ovals). Additional deficiencies were predicted for other seven amino acids (blue ovals). Metabolic pathways for three amino acids (green ovals) were retained. Enzymes for the alanine (yellow oval) biosynthesis were not identified in both *M. tuberculosis* and *T. whipplei*. Enzymatic steps predicted to be present in both *M. tuberculosis* and *T. whipplei* are shown in black. Enzymatic steps present only in *M. tuberculosis* or in *T. whipplei* are shown in red and green, respectively. Enzymatic steps absent in both *M. tuberculosis* and *T. whipplei* are shown with dashed black lines.
the WISP membrane protein family. More precisely, they involve two virtually identical nucleotide sequences, corresponding to the N-terminal WND-domains of the WISP proteins. In the recombination process, the WISP genes in the TW08/27 isolate (TW157, TW625) were significantly altered in the Twist isolate. The TW08/27 genome exhibits eight copies of WND-domain sequences that are up to 99% identical over an 800-nucleotide span. In contrast, the rest of these WISP genes are all quite different. This strongly suggests that WND-domain sequences act both as coding regions and as DNA repeats promoting genome recombination. 

PCR experiments were performed to validate the genome organization of our Twist isolate, using primers precisely defining the boundaries of the inversion. While the experiments confirmed the Twist genome organization for both extremities of the inverted segment, a PCR product compatible with one extremity of the TW08/27-like arrangement was also observed. This intra-isolate variation, as well as the differences between the Twist and TW08/27 genomes, suggests that T. whipplei exhibits a large genomic plasticity mediated by the highly conserved WND-domain repeats. We also found a paralogous gene family for another type of predicted membrane spanning protein (TW0759, TW0780, TW0653) that exhibits highly conserved bipartite segments separated by a variable sequence.

It is tempting to speculate that the frequent genome rearrangements mediated by such “coding” repeats lead to significant changes in the set of proteins exposed at the surface of the bacteria, and might constitute an adaptive response to the host defense or various environmental conditions. 

T. whipplei gene content was classified into functional categories (Fig. 2) and compared with the other bacteria with reduced (<1Mb) genomes (Mycoplasma species, Ureaplasma, Buchnera, and Wigglesworthia). Overall, T. whipplei exhibits a larger complement of genes for most functional categories compared to these other bacteria. However, Wigglesworthia exhibits more genes relevant to lipid metabolism, cell envelope biogenesis, the outer membrane, and coenzyme metabolism. For the categories of carbohydrate transport and metabolism and DNA replication and repair, Mycoplasma pulmonis is better equipped. Finally, Buchnera exhibits a larger gene complement for energy production and conversion. This variability indicates that small bacterial genomes are not the result of a universal and unique reductive evolution pathway.

Genomic Features of Reduced Genomes

A certain number of genome reduction “rules” have been enunciated, following the analysis of the available genomes of parasitic/intracellular bacteria. Characteristic features of reduced genomes may be associated with the adaptive strategies linked to their strict or facultative association with hosts (Koonin et al. 2001; Doolittle 2002). The genome sequence of T. whipplei provides the first opportunity to examine these rules for a high G+C Gram-positive bacteria.

**G+C Content**

Within each clade, reduced genome bacteria tend to exhibit the lowest G+C content. Rickettsia, Buchnera, Wigglesworthia, Mycoplasma, and Ureaplasma species are all in the range of 22%–33% except for Mycoplasma pneumoniae (40%). At 46%, the G+C content of T. whipplei is by far the lowest of the Actinobacteria, including M. leprae (58%), M. tuberculosis (66%), and S. coelicolor (72%). The cause for this general trend remains unknown, although it has been linked to a mutational bias due to the loss of repair and recombination functions (Moran 1996; Moran and Wernegreen 2000). Consistently, the genome of T. whipplei is lacking most of the base excision repair genes found in M. tuberculosis. We also noticed that the genes most conserved (ubiquitous) across distant clades tend to resist this trend. For example, the average G+C content of the T. whipplei ORFs for S3 ribosomal proteins is 50%, whereas for all of the ORFs is 47%.

**Horizontal Gene Transfer**

Gene acquisition by horizontal transfer appears less frequent for small genome parasitic bacteria such as M. genitalium and Chlamidia (Ochman et al. 2000; Brinkman et al. 2002) than for free-living bacteria. One trivial explanation might be found in their less promiscuous lifestyles, offering much fewer opportunities to exchange DNA with other microorganisms. The analysis of two Rickettsia genomes (Ogata et al. 2001) confirmed this tendency by revealing the lack of horizontal gene transfer over a period of 40–80 million years. The reduced T. whipplei genome appears to follow the same rule. Potentially foreign genes can be identified by their atypical G+C content (Lawrence and Ochman 1998). In the T. whipplei genome, only two such ORFs are identified (TWT313 and TWT613). Indeed, the G+C content of the T. whipplei ORFs varies little (the standard deviation is 3%), as in other obligate intracellular bacteria (Brinkman et al. 2002). Horizontally transferred genes are also pointed out by inconsistencies in phylogenetic trees. This approach identified nine candidate genes likely to have been acquired by horizontal gene transfer (Suppl. Table S2), thus amounting to about 1% of the genome. Of these nine, five are aminoacyl-tRNA synthetases, including a valyl-tRNA synthetase of the archaean-type, also observed in Rickettsia (Woese et al. 2000). Four of these anomalous phylogenies with different topologies are shown in Figure 5, for two aminoacyl-tRNA synthetases (AspRS, ValRS), and two enzymes (PurB, PyrB) of the nucleotide metabolism. It should be noted that some of those anomalous phylogenies might also originate from ancient gene duplication followed by lineage-specific loss of paralogs.

**Repeated Sequences**

Another proposed rule is that repeated sequences should be less frequent in the reduced genomes of parasitic bacteria (Frank et al. 2002). A possible cause is again that the sequenced lifestyle of these bacteria diminishes their exposure to foreign selfish DNA elements (bacteriophages and transposons). Concurrently, even-
tual repeated sequences are eliminated from these genomes by recombination. However, there are already some strong exceptions to that rule. *M. pneumoniae* exhibits a large number of repeats (Rocha et al. 1999). We observed many repeated sequences in an obligate intracellular parasite *Rickettsia conorii* (Ogata et al. 2002; Claverie and Ogata 2003). *T. whipplei* is another counter-example, with a high frequency of repeats. Thirty-eight repeat families were identified representing 6% of the *T. whipplei* genome. The two largest families account for 29,850 nt (3.22%) and 11,137 nt (1.20%) respectively (Fig. 1A,B). The family of the longest repeat consists of a pair of nearly identical sequences (99.91% identity over 4692 nucleotides). The positions of the repeats frequently correspond to abrupt changes in the AT-skew profile (Fig. 1A), suggesting their involvement in frequent local genome rearrangements (Petes and Hill 1988).

**Genome Degradation**

Finally, “on-going genome degradation” (Andersson and Andersson 2001; Lawrence et al. 2001) has been proposed to be induced by the sheltered and isolated lifestyle of parasitic bacteria. Accordingly, *R. prowazekii* (Andersson et al. 1998) and *M. leprae* (Cole et al. 2001) both exhibit a number of pseudogenes in their genomes, which appear under the process of elimination. However, this tendency is not so clear for other reduced genome bacteria such as *Chlamydia*. Moreover, two closely related *Rickettsia* exhibit very different levels of genome degradation (Ogata et al. 2001). Here, the *T. whipplei* genome exhibits a coding content (86%) comparable to the one of free-living bacteria, and shows few pseudogenes and little sign of on-going degradation.

**Conclusion**

An important finding derived from *T. whipplei* Twist (this work) and TW08/27 (Bentley et al. 2003) genome sequences is the existence of frequent genomic instability mediated by protein-coding repeats within genes of membrane proteins. This active recombination process probably causes the bacteria to expose different sets of proteins at their surface, in response to the host defense or various environmental conditions. At the same time,
frequent recombinations homogenize the repeat sequences and maintain or amplify their capacity to be involved in forthcoming genome rearrangements.

A related phenomenon has been reported to explain the variation of the MSP2 outer membrane protein of *Anaplasma marginale*, a rickettsial pathogen. Gene conversions between functional *msp2* genes and their pseudogenes (Brayton et al. 2001) have been invoked as the most likely mechanism. The *msp2* genes also exhibit highly conserved DNA segments, as found in *T. whipplei* WISP genes. UV-induced genomic inversions mediated by highly conserved lipoprotein-like ORF sequences were also recently reported (Uchida et al. 2003) in *Streptomyces griseus*, an environmental actinobacterium. Intra-ORF genome inversions might thus be a process generally used by Actinobacteria to modulate the expression of their surface proteins in response to their environment.

The *T. whipplei* genome sequence now provides important and practical information on a poorly characterized bacterium, isolated only three years ago. In an attempt to improve the current molecular diagnosis for Whipple’s disease, new PCR primers were designed according to the sequence of the highly conserved WND-domain repeats. The primers (Tw53–3F: 5’-TGT GTC TGT GGT TGG GGT AA-3’ / Tw53–3R: 5’-CCT CCT GCT CTA TCC CTC CT-3’) were tested against diluted *T. whipplei* cultures, and detected 10 to 100 more cells than *rpoB*-based primers (Drancourt et al. 2001).

The prediction of the *T. whipplei* resistance to quinolone antibiotics, which was later confirmed (Masselot et al. 2003), suggested the avoidance of the use of these compounds in the treatment of Whipple’s disease.

Finally, the detailed analysis of the predicted metabolism of *T. whipplei* suggests useful clues on how to rationally modify the current culture conditions and improve our capacity to grow and study this extremely fastidious bacterium in the laboratory (P. Renesto, N. Crapoulet, H. Ogata, B. La Scola, G. Vestris, J.-M. Claverie, and D. Raoult, in prep.).

**METHODS**

**Source and Preparation of DNA**

*T. whipplei* Twist strain, a type 2A, was cocultivated with HEL cells in 150-cm² flasks as described (Raoult et al. 2000) to the 20th passage. Purified bacteria (analyzed using electron microscopy) were submitted to pressure shock using a French-pressure device (Bioritech), and lysed bacteria were mixed with 1% low melting point agarose (FMC Bio Products) in TE to form plugs in a mold (Bioritech), and lysed bacteria were mixed with 1% low melting point agarose (FMC Bio Products) in TE to form plugs in a mold. The plugs were subjected to pressure shock using a French-pressure device (Bioritech), and lysed bacteria were mixed with 1% low melting point agarose (FMC Bio Products) in TE to form plugs in a mold. The plugs were subjected to pressure shock using a French-pressure device. The plugs were lysed in a sodium lauryl sarcosine solution of EDTA-sarcosine-proteinase (30 mL 0.5 M EDTA, pH 8.5, 5.2 mL 10% sodium lauryl sarcosine solution (Sigma) to which 30 mg lysozyme (Boehringer Mannheim) was added and incubated at 37°C for 16 h on a roller. After two gentle washings in TE, the plugs were incubated overnight in a solution of EDTA-sarcosine-proteinase (30 mL 0.5 M EDTA, pH 8.5, 1.5 mL 10% sodium lauryl sarcosine, 60 mg proteinase K [Euromedex]) at 50°C. This operation was repeated three times, and the plugs were finally washed gently three times. Digested plugs were stored in EDTA 0.2 M, pH 8.0 at 4°C until used for cloning.

**Genome Sequence Analysis**

A first library A (5-kb inserts obtained by mechanic shearing, cloned in pcDNA 2.1 with Bst XI adaptors) was constructed, and a preliminary analysis of 96 clones disclosed <10⁻¹ HEI cell DNA contamination. Plasmid clones were sequenced at both ends of the insert with flanking vector sequences as primers. Dye primer reactions were analyzed on an LI-COR 4200L, and six genomic equivalents were sequenced. We generated a second library B by cloning 20-kb fragments in pCNS with BstXI adaptors. Dye primer reactions were analyzed on an LI-COR 4200L, and six genomic equivalents were sequenced. We generated a second library B by cloning 20-kb fragments in pCNS with BstXI adaptors. The whole-genome assembly was performed by means of the PHRED, PHRAP, and CONSED 11.0 software packages (Ewing and Green 1998; Ewing et al. 1998; Gordon et al. 1998). A total of 6340 (6.34 X) and 5286 (2.93 X) end sequences (X’s indicate genome equivalents) respectively, from libraries A and B were incorporated into contigs. Cloning gaps and several sensitive regions were resolved and confirmed by sequencing-duplicated PCR products. In addition, regions with lower quality were optimized using dye terminator reaction analyzed on a capillary ABI3100. The final sequence includes 99.9% positions with PHRED scores over 40. The coherence of the assembly was verified by comparison of the Spe I and Not I restriction site pattern previously obtained by pulsed-field gel electrophoresis. Also, the assembly was confirmed by sequencing PCR products obtained by incorporating primers designed on the basis of the scaffold of the molecule.

**Informatics**

Nucleotides in the *T. whipplei* genome were numbered according to the *M. tuberculosis* genome, where position one corresponds to the predicted origin of replication. Genes for rRNA were identified with the use of RNAscan-SE (Lowe and Eddy 1997). Other RNAs were identified using BLAST (Altschul et al. 1997). ORFs at least 50 amino acid residues-long were predicted using Sift (Audic and Claverie 1998). Additional ORFs shorter than 50 aa residues were identified by homology searches. ORFs overlapping with other ORFs were removed retrospectively. Repeated sequences were delineated using RepeatFinder (Volfkovsky et al. 2001). ORF functions were predicted based on the homology searches by BLAST against the SWISS-PROT and TreMBL sequence databases (Boeckmann et al. 2003). Metabolic pathways were analyzed using KEGG (Kanehisa et al. 2000). Preliminary sequence data for *B. burnetii* were obtained from The Institute for Genomic Research (http://www.tigr.org/). The functional classifications of the genes were performed in reference to the COG database (Tatusov et al. 2001) except for *T. whipplei*. COG categories for *T. whipplei* were determined by homology to COG database sequences.

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