Supporting Information

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SI Methods

Construction of *bmtA* Mutants. *bmtA* was inactivated through homologous recombination by using a suicide vector pOY04. All constructs were confirmed by using PCR amplification, restriction digestion, and sequence analysis. The 1,100-bp 5′ arm for creating pOY04 was PCR-amplified by using primers ZM010F and ZM010R, whereas the 1042-bp 3′ arm was amplified by using ZM011F and ZM011R (Table S1). The 5′ DNA fragment was cloned into pGEM-Teasy vector (Promega), yielding pOY01. After digestion with BssHII, the 3′ arm was ligated into pOY01 that was digested with AscI, creating pOY03. The P*flgB*-Kan cassette, excised from pJD55 (1, 2) by using AscI, was then ligated into pOY03 at the AscI site. The resulting construct, pOY04, was transformed into *Bb* 297 as previously described (3, 4). Transformants were selected by using kanamycin (160 μg/mL) and confirmed by PCR amplification.

Complementation of the *bmtA* Mutant. To complement the *bmt*A mutation in *trans*, plasmid pOY15 was constructed based on the pJD54 shuttle vector, a derivative of pJD44 in which the original *aph* [3′]-*IIIa* was replaced with the P*flgB*-aadA cassette of pKFSS1 (1, 2, 5). Briefly, the *bmtA* region was PCR-amplified by using primers ZM016F and ZM016R, digested with EcoRI and HindIII, and ligated into pPROEX-HTA (Invitrogen), which yielded pOY11. Next, the promoter of the *Bb flaB* gene, designated P*flaB*, was PCR-amplified by using primers ZM21.2F and ZM21R. After digestion with BamHI and EcoRI, the P*flaB* fragment was ligated into pOY11. The resulting construct was designated pOY13. The P*flaB-bmtA* cassette then was excised from pOY13 by using BamHI and HindIII, and it was ligated into pJD54, creating pOY15. The resulting construct, pOY15, was electroporated into *Bb* OY04, which created OY06. A mock-complemented strain was constructed similarly by transforming pJD54 into *Bb* OY04. All transformants were selected by using 160 μg/mL kanamycin and 150 μg/mL streptomycin, and all transformants and constructs were confirmed by PCR.

Determination of Plasmid Profiles Among Strains and Recovery of the Shuttle Plasmid from Complemented Strains. *Bb* genomic DNA was isolated by using the Wizard Genomic DNA Purification Kit (Promega) according to manufacturer's instructions. As previously described, plasmid contents for all *Bb* strains were determined by PCR using primers specific for each of the known endogenous *Bb* strain 297 plasmids (6). To determine the presence of the complementation plasmid pOY15 in strain OY06, DNA isolated from this strain was transformed into *E. coli* cells, and spectinomycin-resistant clones were screened by PCR using primers a5 and a6 specific for *bmtA*. Sequence analysis also was used to confirm the presence of the shuttle plasmid.

RT-PCR. Total RNA was extracted from *Bb* strains by using TRIzol (Invitrogen), further purified by using the RNeasy Mini Kit (Qiagen), and quantified by using a Nanodrop ND-100 spectrophotometer (NanoDrop Technologies). Genomic DNA in the RNA samples was removed by using RNase-free DNase I (GenHunter Technology). For RT-PCR, cDNA was generated from 1 μg of *Bb* RNA by using the SuperScript III reverse transcriptase (Invitrogen) according to the manufacturer's protocol.

Intracellular Metal Content Measurement. Intracellular metal contents of spirochetes were measured as described previously (7). Briefly, 200 mL of bacterial culture grown to stationary phase was harvested by centrifugation at 4,000 × *g* (4 °C) for 30 min. The pellet was washed twice with 200 mL of ice-cold 0.1 M Tris buffer (pH 7.0) and once with sterilized Milli-Q (Millipore Corp.) water. To lyse cells, the pellet was suspended in 1 mL of 65% HNO_3 (TraceMetal grade; Sigma), gently vortexed, and then heated to 78 °C for 15 min. A total of 9 mL of sterilized Milli-Q water was added to the lysed cells. Samples were centrifuged at 13,000 × *g* for 5 min, and the supernatant was analyzed for metal content by using inductively coupled plasma atomic emission spectrometry by the Research Analytical Laboratory, University of Minnesota (St. Paul, MN). Yttrium (1 ppm) was used as an internal standard. The data were normalized according to spirochete numbers, which were determined by darkfield microscopy when the cultures were collected. Three independent tests were performed, and the results were analyzed by using Student's *t* test.

Microinjection of *Bb* into Nymphal Ticks. *I. scapularis* nymphs were obtained from the Tick Rearing Facility at Oklahoma State University (Stillwater, OK). Microinjection was used to introduce spirochetes into the gut of *I. scapularis* nymphs as previously described (8, 9). Briefly, each *Bb* variant was cultivated under normal conditions in BSK-H medium (Sigma) (10) in the presence or absence of selective antibiotics. Bacteria were harvested by centrifugation and concentrated in BSK-H to a density of 10^8 spirochetes per milliliter. A total of 10 μL of the cell suspension was then loaded into a 1-mm diameter glass capillary needle (World Precision Instruments Inc.) by using a microloader (Eppendorf AG). The bacterial suspension was then injected into the rectal aperture of unfed nymphal ticks by using a femtojet microinjector system (Eppendorf AG). The parameters for injection were a pressure of 1,000 hPa, injection time of 0.1 s, and a compensation pressure of 0 hPa, which delivered an average volume of 0.15 μL (1–2 × 10^4 spirochetes). After microinjection, ticks were placed on adult C3H/HeN mice (10 ticks per mouse), allowed to feed to repletion (4–5 days), and then collected.

Larval-Tick Infection by Immersion. Artificial infection of larval ticks with various *Bb* strains was carried out by using a previously described immersion method (11). Briefly, *I. scapularis* larval ticks were first equilibrated to a lower relative humidity to enhance spirochete uptake. Ticks were then suspended in 0.5 mL of 1 × 10^7 spirochetes per milliliter of various strains of *Bb* in BSK-H and incubated at 34 °C for 90 min. After immersion, ticks were washed with sterile PBS, recovered in 98% relative humidity, and fed to repletion on naïve C3H/HeN mice. Subsets of larvae were dissected immediately and analyzed by immunofluorescence assay (IFA) to confirm the presence of spirochetes.

IFAs. The entire contents of the infected ticks were expressed onto silylated microscope slides (CEL Associates). Slides were allowed to air dry before being placed on a 65 °C heating block for 25 min, followed by fixation in acetone for 5 min. Slides were incubated at 37 °C for 1 h with blocking solution (PBS/0.1% Tween 20 with 5% goat serum) in a humidified chamber. The blocking solution was replaced with a 1:100 dilution of BacTrace FITC-conjugated goat anti-*Bb* antibody (Kirkegaard and Perry Laboratories). The slides then were incubated for 1 h at 37 °C in

a dark, humidified chamber, and they were washed twice in PBS/0.1% Tween 20. Slides were then counterstained with 20 mg/mL propidium iodide in PBS for 3 min, washed twice with PBS/0.1% Tween 20, and then treated with Antifade light mounting medium (Molecular Probes). Samples were observed for *Bb* by using an Olympus BX50 fluorescence microscope with a 40× objective equipped with a CCD camera (CCD-100S; DAGE-MTI) and Olympus DP Controller software.

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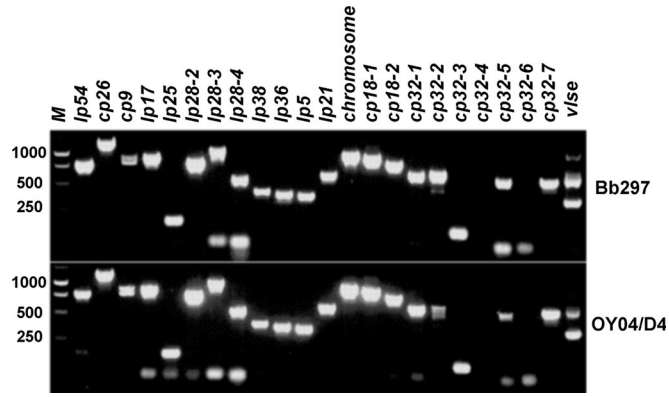

Fig. S1. Assessment of the plasmid contents of *B. burgdorferi* parental strain 297 (Bb297) and the *bmtA* mutant clone OY04/D4 via PCR amplification. Each plasmid for detection is designated above each gel lane. DNA size standards (M) are indicated at the left in base pairs.

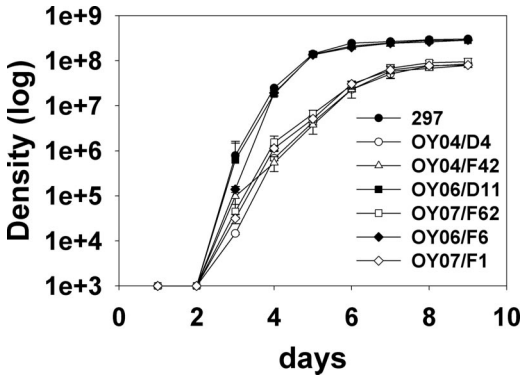

Fig. S2. In vitro growth of *bb0219* mutants. *Bb* was inoculated into BSK-II medium at 1,000 spirochetes per mL. Spirochetes were enumerated by using darkfield microscopy. Values are the means from 3 independent experiments. Error bars indicate standard deviations ($n = 3$). *Bb* strain designations are: WT 297; *bb0219* mutants OY04/D4 and OY04/F42; complemented strains OY06/D11 and OY06/F6; and mock-complemented strains OY07/F1 and OY07/F62.

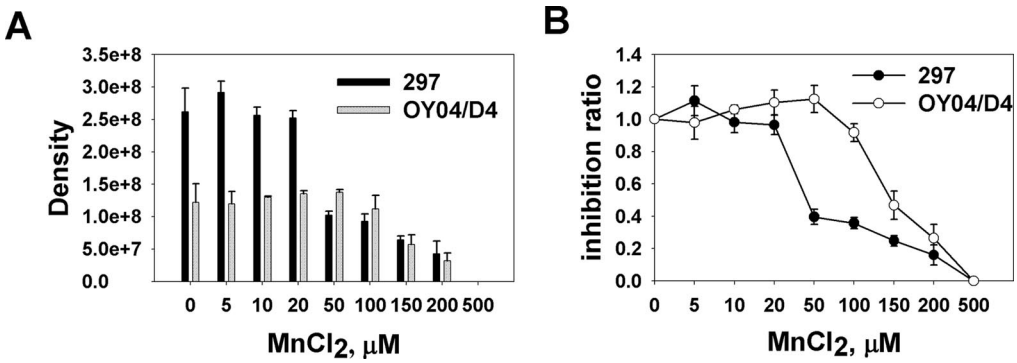

Fig. S3. Inactivation of *bmtA* renders spirochetes resistant to manganese toxicity. Growth yields (cell density) (*A*) or the inhibition ratio (*B*) of parental strain 297 (297) or the *bmtA* mutant (OY04/D4) were assessed after bacterial growth in BSK-II medium containing varying amounts of $MnCl_2$. The inhibition ratio was calculated by dividing the growth yield of each strain under each condition by the growth yield in BSK-II medium. Data are expressed as mean values with standard deviations from 3 independent experiments.

Table S1. Oligonucleotide primers used in this study*

Name	5′-3′
ZM010F	AGTTATGGTTCCAGCAGCTATTCCCG
ZM010R	GGCGCGCCTGATGTTTCTCGATGTTTCACTAGGT
ZM011F	GGCGCGCCGTCCCAATAATACAGGATGCAAAGT
ZM011R	TTAGCGCGCACTGATCATGTTAAAGCTGCTTGCT
ZM012F	GATGGACGAACCTACCTCTG
ZM012R	GGAGGGCTTGCCGATTATAC
ZM014F	GGGAACTTGATTAGCCTGCGCAAT
ZM014R	TCGAGCTTCTGATGATGCTGCT
ZM016F	CGGAATTCATGATAAAATCAATTTTAGATTATT
ZM016R	TATAAGCTTTTAACCTAGTGAAACATC
ZM21.2F	ATGGATCCTGTCTGTCGCCTCTTGTG
ZM21R	CGGAATTCTCATTCCTCCATGATAAA
297 ospC-444F	TGAAAATGTTACTGATGAGAATGCAA
297 ospC-556R	TTGCCAAGTTTTCTACTGCTTTAAATAG
Bb RpoS-542F	GGGTCATATTTTTCAGCAGCTCTTA
Bb RpoS-619R	GGGTAAAGGGTTAAAAATTGAAGACTT
Bb flaB-35F	TGATTAGCCTGCGCAATCATT
Bb flaB-115R	AATGACAGATGAGGTTGTAGCAGC
BBE22F	TTTAAAGGAACCGATCAATATTACGATAG
BBE22R	AAAGCTGAAGGCCCGTTTGT
a1	AAACCGCATAAGCACCCATAAGCC
a2	TTTGGAGCCTTGGCGTCTAATCCA
a3	AGAGAAATAGCTGCTCCTTCGGGA
a5	ATGATAAAATCAATTTTAGATTATT
a6	TTAACCTAGTGAAACATC
b1	TGAAGTTTGTGGAGGCCCTCATGT
b2	TCCAACCTCATCAATGTCGCCTGT
c1	ACCAAGGCAATTGAGCATGGAGAC
c2	CTCTCTTTGCGAATAAACCCTGGC
d1	TCTGTTGGTGAGGATGGCCACATA
d2	TGCTCTTTGAGAAGTCTGGCTGGA
e1	CTTTGGCAACCCAAAGCTTTATCC
e2	ACTGCTAATTGGCAAGCCTTGAT
f1	AGGCTAAGGGCATAAGCGAA
f2	TCGCTTATGCCCTTAGCCTT
g1	CGCACTTCTCCCAATCATGACAGA

*All primers were designed based on B31-MI sequence data, except for *ospC*, which was based on strain 297 sequence data. Restriction enzyme sites are underlined.