

The *bdr* Gene Families of the Lyme Disease and Relapsing Fever Spirochetes: Potential Influence on Biology, Pathogenesis, and Evolution

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Appendix

Bacterial Cultivation, DNA Isolation, and Southern Hybridization Analyses

Isolates belonging to the *Borrelia burgdorferi* sensu lato complex were cultivated in complete BSK-H media (Sigma) at 33°C. To cultivate the relapsing fever borreliae and other *Borrelia* species, the complete BSK-H media were supplemented with additional rabbit sera (Sigma) to a final concentration of 12% (vol/vol). Bacteria were harvested by centrifugation and washed with phosphate buffered saline (pH 7.0), and DNA was extracted (25). For Southern hybridization analyses, 5µg of DNA from each isolate was digested under standard conditions with *Xba*I and fractionated by electrophoresis in 0.8% GTG agarose gels. The DNA was transferred onto membranes for hybridization by vacuum blotting using the VacuGene system as described by the manufacturer (Pharmacia). All other Southern hybridization methods were as previously described (39).

Immunoblot Analyses

Bacterial cultures were grown and harvested as described above. One OD600 equivalent of cells was pelleted and resuspended in 100µl of standard SDS-sample buffer with reducing agents. The cell lysates (7µl) were fractionated by electrophoresis in 15% SDS-PAGE gels and electroblotted onto Immobilon P membranes (38). The immunoblots were blocked overnight in

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blocking buffer (1X PBS, 0.2% Tween, 0.002% NaCl, and 5% nonfat dry milk) and then incubated with a 1:1,000 antisera dilutions. ImmunoPure Goat anti-mouse IgG (H+L) peroxidase conjugate served as the secondary antibody. The secondary antibody was incubated with the blots for 1 hour at room temperature at a 1:40,000-fold dilution and then the blots were washed three times with wash buffer. For chemiluminescent detection, the Supersignal West Pico Stable Peroxide solution and the Supersignal West Pico Luminol/Enhancer solution were used. Both reagents were from Pierce and were used as described by the manufacturer. The immunoblots were exposed to film for time frames of 5 to 30 seconds.

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