Transcriptional profiling of *Borrelia burgdorferi* containing a unique *bosR* allele identifies a putative oxidative stress regulon

Jenny A. Hyde, J. Seshu† and Jonathan T. Skare

Correspondence

Jonathan T. Skare

jskare@medicine.tamhsc.edu

Department of Microbial and Molecular Pathogenesis, Texas A&M University Health Science Center, College Station, TX 77843-1114, USA

Borrelia burgdorferi regulates gene expression in response to environmental conditions, including temperature, pH, redox potential and host factors. *B. burgdorferi* encodes a PerR homologue designated BosR, which presumably serves as a global regulator of genes involved in the oxidative stress response. Infectious *B. burgdorferi* strain B31 is resistant to oxidative stressors *in vitro*, whereas the non-infectious isolate was sensitive due, in part, to a point mutation that converts an arginine to a lysine at residue 39 of BosR. Subsequent insertional inactivation of this *bosRR39K* allele (*bosRR39K::kan^R*) restored resistance to oxidative stressors. These observations suggest that the *B. burgdorferi* non-infectious *bosRR39K::kan^R* strain may transcribe genes that are also expressed in infectious *B. burgdorferi* cells, but are repressed in the *bosRR39K* background, thus explaining the different oxidative stress phenotypes observed between these isolates. To test this hypothesis, macroarray technology and quantitative RT-PCR were utilized to compare the transcriptional profiles from the isogenic *bosRR39K* and *bosRR39K::kan^R* isolates. Array data indicated that 88 ORFs were significantly expressed in the absence of BosRR39K. Since most affected genes mapped to the chromosome, it is likely that these genes define an important physiologic response for *B. burgdorferi*. Included within the genes identified was the detoxification gene *sodA*, as well as other loci not overtly linked to oxidative stress. These results suggest that a putative BosR regulon, as defined by the *bosRR39K* allele, is required to combat toxic oxidative intermediates, but may also be involved in adaptive strategies that are independent of reactive oxygen species.

Received 14 March 2006

Revised 31 April 2006

Accepted 18 May 2006

INTRODUCTION

Lyme disease, caused by the pathogenic spirochaete *Borrelia burgdorferi*, is the most common arthropod-borne disease in the USA, causing a multi-systemic, inflammatory disorder that, if untreated, can result in significant morbidity (Steere *et al.*, 2004). In 2002, the Center for Disease Control and Prevention reported 23 763 cases of Lyme disease in the USA, a 40 % increase from the previous year, indicating that it is a re-emerging infectious disease. The distinctive host environments that *B. burgdorferi* occupies (i.e. the arthropod vector and mammalian host) suggest that it must be able to quickly adapt to disparate conditions. The response

to temperature and pH accounts for some of the environmental cues that modulate gene expression in *B. burgdorferi* (Brooks *et al.*, 2003; Carroll *et al.*, 1999, 2000; Ojaimi *et al.*, 2003; Revel *et al.*, 2002; Schwan *et al.*, 1995; Yang *et al.*, 2000). Additional studies, using host-adapted spirochaetes, indicate that other unknown host factors also modulate gene expression in *B. burgdorferi* (Akins *et al.*, 1998; Brooks *et al.*, 2003). As an extension of these observations, previous studies have demonstrated that dissolved oxygen levels modulate the expression of *B. burgdorferi* genes and, as such, may be a possible cue for pathogenic and/or regulatory networks (Seshu *et al.*, 2004a). It is likely that the redox environment of an unfed versus a fed tick would be vastly different and this, coupled with the known differences in dissolved oxygen levels within mammalian host tissues, suggests that *B. burgdorferi* must cope with various oxidative stressors throughout its complex lifecycle.

A member of the Fur family of regulators has been identified in *B. burgdorferi*, which was subsequently designated BosR, for *Borrelia* oxidative stress regulator (Boylan *et al.*, 2003). Our recent observations, along with others, have

†Present address: South Texas Center for Emerging Infectious Diseases and the Department of Biology, University of Texas at San Antonio, San Antonio, TX 78249, USA.

Abbreviation: ROS, reactive oxygen species.

The array data discussed in this publication have been deposited in the NCBI Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo/>) and are accessible through GEO series accession number GSE4856.

demonstrated that BosR is involved in the regulation of the oxidative stress response in *B. burgdorferi* (Boylan *et al.*, 2003; Seshu *et al.*, 2004b). In other bacteria, BosR homologues, referred to as PerR, are metalloregulatory proteins that sense oxidative stress, and regulate the expression of genes involved in the detoxification of reactive oxygen species (ROS) (Bsar *et al.*, 1998; Fuangthong *et al.*, 2002; Helmann *et al.*, 2003; Herbig & Helmann, 2001; Horsburgh *et al.*, 2001a, b; Mongkolsuk & Helmann, 2002). Several independent studies have demonstrated that BosR (also designated Fur) binds to regulatory elements in *napA* (Boylan *et al.*, 2003), the borrelial *dps/dpr* homologue *sodA* (Seshu *et al.*, 2004b), *bosR/fur*, as well as a gene adjacent to *bosR/fur*, *bb0646* (Katona *et al.*, 2004), and presumably regulates the expression of these genes in *B. burgdorferi*. A previous study identified a unique allele, *bosRR39K*, in the non-infectious strain B31 derivative CHP100, which manifests a unique regulatory activity relative to the wild-type *bosR* allele (Seshu *et al.*, 2004b). Specifically, characterization of the *bosRR39K*-containing non-infectious strain (CHP100), relative to infectious MSK5 (containing the *bosR* allele), has indicated that CHP100 is more sensitive to ROS than MSK5 (Seshu *et al.*, 2004b). Genetic inactivation of the *bosRR39K* allele (*bosRR39K::kan^R*) results in strain JS167, which is as resistant to ROS as the low-passage infectious *B. burgdorferi* clonal isolate MSK5 (Seshu *et al.*, 2004b). The model that has emerged suggests that the redox-resistant phenotype observed for JS167 is due to the de-repression of genes involved in the oxidative stress response that are repressed in the redox-sensitive parent strain CHP100 by the binding of BosRR39K to transcriptional regulatory elements. Hence, the objective of this study was to identify the borrelial genes that are transcribed in the ROS-resistant isolate versus an ROS-sensitive isolate, as a first step in delineating the oxidative stress response in this pathogen, with the long-term goal of relating this to infectious isolates to define an ROS-responsive regulon. Based on the hypothesis indicated above, the transcriptional profile analysis of strain JS167 relative to its parent CHP100 might identify genes that account for the differential sensitivities to ROS observed between these two derivatives. Results presented herein demonstrate that 88 genes are significantly affected in the JS167 strain relative to its isogenic parent CHP100. Inasmuch as strain JS167 and infectious *B. burgdorferi* are resistant to ROS, some of the genes identified in this study may be involved in host adaptation, in addition to those required for the oxidative stress response. If so, some of the genes identified herein may be involved in pathogenic processes operative in *B. burgdorferi* infection.

METHODS

Strains and growth conditions. Strain CHP100, a high-passage, non-infectious, clonal isolate of *B. burgdorferi* B31 that contains the *bosRR39K* allele, and JS167, the *bosRR39K::kan^R* derivative of CHP100, were used in this study (Seshu *et al.*, 2004b). For RNA isolation, *B. burgdorferi* was grown statically at 32 °C under

conventional microaerophilic conditions (in a 1% CO₂ atmosphere) in complete Barbour–Stoenner–Kelly (BSK)-II medium to a density of 5×10^7 cells ml⁻¹.

PCR. PCR was performed to evaluate the plasmid content of strains, using oligonucleotides specific for all linear plasmids, cp9 and cp26, as previously described by Labandeira-Rey & Skare (2001), and for the cp32 plasmids as previously indicated by Purser & Norris (2000).

Macroarray used in this study. The *B. burgdorferi* nylon macroarrays were generated as described previously by Ojaimi *et al.* (2003). Briefly, 1628 of the 1697 known ORFs of *B. burgdorferi* strain B31 were spotted in duplicate onto nylon membranes (Sigma–Genosys).

RNA isolation and probe synthesis. Three independent cultures of either *B. burgdorferi* strain CHP100 or JS167 were grown to the exponential growth phase, and total RNA was isolated from 1×10^9 cells using a Versagene kit (Gentra) for each extraction. RNA samples were treated with DNase I (Roche) and Suprase-In (Ambion) to eliminate contaminating DNA and inhibit RNase activity. The three independent RNA samples for both strains tested were separately pooled and examined for DNA contamination and crude RNA yield by PCR and RT-PCR, respectively. The purified RNA was converted to [α -³²P]-labelled cDNA using an Ambion Strip-EZ RT kit together with 5 µg pooled total RNA and [α -³²P]dATP (Perkin–Elmer). The probe was then purified on a gel filtration spin column, and the specific activity was determined by scintillation counting. Probes with specific activities $>1 \times 10^6$ c.p.m. µg⁻¹ were used for hybridization.

Hybridization. The nylon macroarray membranes were prehybridized with 10 ml ExpressHyb (Clontech) at 50 °C for 1 h. The ³²P-labelled probes were hybridized to the macroarray membranes for 18 h at 50 °C, washed with a low-stringency solution [2 × saline sodium citrate (SSC), 0.05% SDS] at 37 °C for 10 min, and then incubated in a high-stringency wash (0.1 × SSC, 0.1% SDS) at 50 °C for 10 min. The membranes were exposed to a phosphor screen (Amersham Pharmacia Biotech) for 48 h at room temperature. Signals were detected using the Storm 860 phosphorimager (Amersham Biosciences). Macroarray membranes were stripped of probe as per the Strip-EZ RT kit (Ambion), exposed to a phosphor screen, and analysed using the Storm 860 phosphorimager to confirm that the signal was removed, and then reprobed as described above.

Array data analysis. Following scanning with the Storm 860 phosphorimager, the detected signals were analysed with Array Vision software version 6.0 (Imaging Research) to quantify background-adjusted spot densities. Values were imported into a Microsoft Excel spreadsheet for analysis, as previously described (Brooks *et al.*, 2003; Conway *et al.*, 2002). Raw density values for an individual spot were converted into percentage density values relative to the total density, and then duplicate spots were combined and averaged. Data from three independent arrays were analysed by two-tailed Student's *t* test to ascertain genes expressed at a significant level ($P < 0.01$) between the different *B. burgdorferi* isolates. Density values ≤ 2 SD above background densities, and induction values <1.5 -fold were excluded from the final datasets. ORFs whose transcripts represented $\leq 0.002\%$ of the total transcripts, or that were located on plasmids absent from the non-infectious B31 strains, were removed from the final datasets. The complete macroarray dataset is posted at the NCBI Gene Expression Omnibus (GEO) at <http://www.ncbi.nlm.nih.gov/geo/>, and is accessible through GEO Series accession number GSE4856.

Quantitative RT-PCR. A defined set of genes was subjected to quantitative RT-PCR to corroborate the macroarray data. Oligonucleotide primers were designed using Primer Express software (PE Biosystems) (Table 1). Selected primer pairs were tested to confirm

Table 1. Oligonucleotides used for quantitative RT-PCR

ORF	Forward/reverse primer sequence (5'–3')
BB0010	GGTAGCATTATAGAAAAGTTAGCTGGAAA/TTATATTGCAACAGTGGACTTAATGCTT
BB0028	GCAAAACAGGCCTTAATGATATTATTC/AAACCCCTGCCCTGAGTTAATATG
BB0043	GCTCTAGCAGGGACAATAACATTG/TTAGAAGTTGCCCCGCTAATACTC
BB0047	TTGCTTAAGGCAATAGCGGTTAT/GTCTAATTTAGATCTCTCCCATATTAC
BB0137	GAACAACAGCCAACCCAAAG/CACATGCGTATCAACCATTAATACTAA
BB0219	TGCCTTTAAGAAGAGGTAATGTTGCT/AAGCCCCCCCACAATTC
BB0243	AATGCTCTGTTCTATATTACGATGATTCA/AGGGCAATGCCTCCTTTTTTC
BB0269	CAGAGAAGTAATAACAAAAGACCGAGTACA/ACATCAGAGAGATCTAAAAGCTCCATT
BB0603	ACCACAACCCCTAATCTGACATT/TGCTATTGGAATTGCATAATCAAGA
BB0690	GAATGCTTGGATATGATTCTGAATTTAG/TCTCATTCCAAAAATATTCTTCAAAATCT
BB0735	CTGCTAATGGCGAAAAATTTGA/CGGTAGTATTAAAGGGCAATTCCTTG
BB0749	GGCACGGCGAGCTGATTA/CAAGCATTTCTTTTTCAACTCTCTCA
BBA15	GGCCTAAAAGCTGACAAAAGTAAAGTA/TAGTGTTTTGCCATCTTCTTTGAAAA
BBA59	GGTCGTGGGATTTTAATAGATTCTATTC/GGGTTGTTGTCAGCATTTTCTTC
BBB19	CGGATTCTAATGCGCTTTTACTTG/CAATAGCTTTAGCAGCAATTTTCATCT

that they specifically amplified a single product of known size, using genomic *B. burgdorferi* DNA as template. Reverse transcription reactions were performed by combining TaqMan reverse transcription reagents (Applied Biosystems) with purified *B. burgdorferi* total RNA. The total RNA used for quantitative RT-PCR was independently isolated and distinct from that used in the macroarray analysis. A control lacking reverse transcriptase was performed for each primer set using total RNA from each *B. burgdorferi* strain, to confirm the absence of contaminating DNA. Real-time PCR reactions were performed using an Applied Biosystems 7500 Real-time PCR system. SYBR Green PCRs were performed in triplicate, and each experiment was repeated in triplicate, resulting in nine data points for each gene of interest for each *B. burgdorferi* strain tested. A constitutively expressed gene, *flaB*, was used to normalize all transcripts tested for each *B. burgdorferi* strain. Fold levels of ORFs were determined by the $\Delta\Delta C_t$ method, as previously described by Brooks *et al.* (2003), Conway *et al.* (2002) and Seshu *et al.* (2004a).

RESULTS

Transcriptional profiling of the *bosRR39K::kan^R* *B. burgdorferi* isolate relative to the *bosRR39K* genetic background

Previous studies demonstrated that the phenotypes of the infectious *B. burgdorferi* strain B31 clonal isolate MSK5 and the non-infectious B31 isolate CHP100, which encode the wild-type *bosR* and *bosRR39K* alleles, respectively, are vastly disparate, with the infectious isolate demonstrating a near 5 log difference in viability when exposed to 1 mM t-butyl hydroperoxide (Seshu *et al.*, 2004b). Subsequently, all assays conducted with other ROS indicated that the infectious MSK5, was significantly more resistant relative to the non-infectious strain, CHP100 (Seshu *et al.*, 2004b). The insertional inactivation of *bosRR39K* allele in CHP100 (*bosRR39K::kan^R*) resulted in a strain, designated JS167, that was as resistant to ROS as the infectious MSK5 strain (Seshu *et al.*, 2004b), suggesting that these resistant strains derepressed/induced genes required for resistance to oxidative stressors relative to the ROS-sensitive CHP100 strain.

Unfortunately, it is difficult to compare strain MSK5 with either CHP100 or JS167, since the plasmid content of the infectious and non-infectious isolates are so disparate. In addition, attempts to inactivate *bosR* in MSK5 (and other low passage isolates) have been unsuccessful. Thus, a focused comparison of transcriptional profiles between the redox-sensitive CHP100 relative to the redox resistant JS167 isolate was conducted to identify genes that are responsible for the phenotype observed between these genotypic strains. It is important to note that the growth rate (i.e. doubling times) are indistinguishable between strains CHP100 and JS167 when these strains are grown at 32 °C (data not shown).

To test the hypothesis that differential gene expression accounts for the aforementioned sensitivity/resistant phenotypes observed, macroarrays containing 1628 *B. burgdorferi* ORFs were probed with cDNA derived from total RNA extracted from ROS-sensitive parent strain CHP100 and the ROS-resistant JS167 isolates. Macroarrays were probed with cDNA derived from pooled total RNA obtained from three independent cultures for each strain tested. In addition, for each strain analysed, hybridizations were performed in triplicate on alternating membranes to reduce the bias of the individual macroarray and possible spotting inconsistencies. A strong correlation was observed between the different macroarrays probed, indicating equivalent transcript populations, cDNA labelling and membrane hybridizations, resulting in high *r* values for JS167 (mean *r* value of 0.95) and CHP100 (mean *r* value of 0.87). It is important to note that the clonal non-infectious B31 isolate, CHP100, is missing the following plasmids relative to infectious strain B31 *B. burgdorferi*: cp9, cp32-6, cp32-8, lp25, lp28-4, lp36 and lp38 (Fig. 1). PCR analysis, using primers that flank *bosR*, amplified 0.5 and 2.5 kb fragments in the parent CHP100 and JS167, respectively, confirming that the transposon insertion in the *bosRR39K* allele was stably maintained within JS167 (Fig. 1). The

Fig. 1. Plasmid profiles of *B. burgdorferi* isolates, CHP100 (*bosRR39K*) and JS167 (*bosRR39K::kan^R*), used for macroarray analysis and quantitative RT-PCR. The second lane shows the amplified product for the *bosR* allele present in either CHP100 or JS167. Circular and linear plasmids are indicated by cp and lp, respectively, with the approximate size (in kb) of the plasmids indicated numerically.

bosRR39K::kan^R isolate is apparently missing lp5 and lp28-2, but was otherwise identical to its non-infectious parent (Fig. 1). Previous studies indicated that the sensitivity to oxidative stressors in the redox-resistant JS167 strain (missing lp5 and lp28-2) could be converted back to a redox-sensitive strain via complementation with intact *bosRR39K* (Seshu *et al.*, 2004b), indicating that the phenotypic difference observed is due to the status of the *bosR* allele and is independent of lp5 and lp28-2.

Eighty-eight ORFs were differentially expressed in JS167 after statistical analysis (up- or down-regulated more than 1.5-fold; $P < 0.01$) (Table 2). Of these 88 ORFs, 66 (75 %) were located on the chromosome and 22 on plasmids (25 %) (Fig. 2). Among the 88 significantly expressed ORFs, 51 had no known function, including 18 ORFs categorized as hypothetically conserved, 31 as hypothetical and two as 'other' by The Institute for Genomic Research (TIGR; Fig. 3) (Fraser *et al.*, 1997). Several genes of known function were also affected by inactivation of the *bosRR39K* allele, including superoxide dismutase (*sodA*), as well as all of the genes that define a putative *glp* operon involved in glycerol uptake and utilization (*bb0240–bb0243*). The observed induction of *sodA* is consistent with the increased resistance to oxidative stressors seen for JS167 (Seshu *et al.*, 2004b), whereas the increased expression of the *glp* operon implies that *BosRR39K* represses genes important for physiological responses, specifically, the utilization of glycerol as a carbon source. Additional genes of interest include: (i) *bb0116*, a *malX* homologue involved in the transport of maltose and glucose (induced 5.25-fold); (ii) *bb0042*, a *phoU* homologue involved in the regulation of phosphate transport (induced 3.11-fold); and (iii) *p66* (induced 1.83-fold), a putative

porin (Skare *et al.*, 1997) and integrin adhesin (Coburn & Cugini, 2003). In the case of the *malX* and *phoU* borrelial homologues, the variable expression of these distinct transporters suggests that the compounds they import may affect cellular homeostasis and directly or indirectly alter the observed response to oxidative stressors. The induction of *p66* suggests that metabolite transport may also be involved in the resistance to ROS. Two independent array-based studies have indicated that *p66* is induced under host-adapted conditions independent of temperature (Brooks *et al.*, 2003; Revel *et al.*, 2002), and suggest that *BosRR39K* may directly or indirectly affect *p66* transcription. Finally, the gene encoding a two-component regulatory protein (*bb0763*) exhibited decreased transcription in JS167 (Table 2). The *bb0763* locus encodes a response regulator designated Rrp-2 that modulates the expression of *rpoS* in a RpoN-dependent manner (Yang *et al.*, 2003). *rrp-2* exhibited a 1.8-fold decrease in transcription in JS167. The reduction in *rrp-2* transcripts in a borrelial cell that demonstrates significant resistance to ROS suggests that the two-component system does not require additional Rrp-2 to mediate this response. It is interesting to note that the transcriptional profiles for *sodA*, *p66* and *rrp-2* observed in the JS167 isolate are identical in infectious *B. burgdorferi* isolate MSK5 (J. A. Hyde and J. T. Skare, unpublished observations), suggesting that these genes play a key role in the ROS-resistant phenotype observed in these strains.

Quantitative RT-PCR validates the trend of gene expression observed in the macroarray analysis

Quantitative RT-PCR was utilized to independently confirm the transcriptional profile data generated by the macroarray

Table 2. Fold expression of genes from JS167 (*bosRR39K::kan^R*) relative to CHP100 (*bosRR39K*)

Gene categories: ARS, amino acid biosynthesis; B, biosynthesis; CE, cell envelope; CH, chemotaxis proteins; D, division; F, flagellar biosynthesis; FM, fatty acid metabolism; GM, growth and metabolism; HE, haemolytic proteins; HS, heat-shock proteins; HX, hypothetical conserved domains; IM, intermediary metabolism; NM, nucleotide metabolism; PD, protein degradation; PE, protein export; PM, protein metabolism; R, replication; RP, ribosomal proteins; TF, translation factors; TP, transporter proteins; TR, transcriptional regulation proteins; U, hypothetical proteins; X, other.

Description	Paralogous family	Gene category	Replicon	Fold expression	P value
BB0813 hypothetical protein		U	Chromosome	11.75	0.00000274
BB0242 hypothetical protein		U	Chromosome	11.68	0.00001402
BB0410 hypothetical protein		U	Chromosome	7.82	0.00745573
BB0012 pseudouridylate synthase I (<i>hisT</i>) (<i>B. burgdorferi</i>)		ARS	Chromosome	7.50	0.00017301
BB0115 ribosomal protein S6 (<i>rpsF</i>) (<i>Odontella sinensis</i>)		RP	Chromosome	6.83	0.00056509
BB0802 ribosome-binding factor A (<i>rbfA</i>) (<i>Bacillus subtilis</i>)		ARS	Chromosome	6.20	0.00000756
BB0116 PTS system, maltose and glucose-specific IIABC component (<i>malX</i>) (<i>Escherichia coli</i>)	16 family	TP	Chromosome	5.25	0.00180917
BB0243 glycerol-3-phosphate dehydrogenase, anaerobic (<i>glpA</i>) (<i>Haemophilus influenzae</i>)		IM	Chromosome	4.77	0.00000409
BB0199 hypothetical protein		U	Chromosome	4.53	0.00973596
BB0247 conserved hypothetical protein (<i>Methanococcus jannaschii</i>)		HX	Chromosome	4.49	0.00053278
BB0117 haemolysin III (<i>yplQ</i>) (<i>Bac. subtilis</i>)		HE	Chromosome	4.29	0.00002855
BB0824 hypothetical protein		U	Chromosome	3.74	0.00000411
BB0312 purine-binding chemotaxis protein (<i>cheW-1</i>) (<i>B. burgdorferi</i>)	33 family	CH	Chromosome	3.68	0.00410814
BB0823 hypothetical protein		U	Chromosome	3.59	0.00003011
BB0241 glycerol kinase (<i>glpK</i>) (<i>E. coli</i>)		IM	Chromosome	3.50	0.00010727
BB0042 phosphate transport system regulatory protein (<i>phoU</i>) (<i>Pseudomonas aeruginosa</i>)		GM	Chromosome	3.11	0.00519846
BB0153 superoxide dismutase (<i>sodA</i>) (<i>H. influenzae</i>)		X	Chromosome	2.97	0.00959025
BBB01 conserved hypothetical protein (<i>E. coli</i>)		HX	cp26	2.96	0.00028073
BB0047 conserved hypothetical protein (<i>Synechocystis</i> PCC6803)		HX	Chromosome	2.94	0.00082564
BB0294 flagellar basal-body rod protein (<i>flgB</i>) (<i>B. burgdorferi</i>)		F	Chromosome	2.92	0.00039481
BB0711 hypothetical protein		U	Chromosome	2.84	0.00069426
BB0424 hypothetical protein		U	Chromosome	2.84	0.00040330
BBD09 hypothetical protein (<i>B. burgdorferi</i>)		U	lp17	2.76	0.00073821
BB0010 holo-acyl-carrier protein synthase, putative (<i>E. coli</i>)		CE	Chromosome	2.74	0.00095449
BB0245 hypothetical protein	125 family	U	Chromosome	2.62	0.00806398
BB0425 hypothetical protein		U	Chromosome	2.58	0.00716470
BB0263 signal peptidase I (<i>lepB-3</i>) (<i>Salmonella typhimurium</i>)		PE	Chromosome	2.37	0.00513844
BB0083 hypothetical protein		U	Chromosome	2.36	0.00095698
BB0819 conserved hypothetical protein (<i>M. jannaschii</i>)		NM	Chromosome	2.31	0.00313473
BB0366 aminopeptidase I (<i>yscI</i>) (<i>B. burgdorferi</i>)	131 family	PD	Chromosome	2.29	0.00002167
BB0799 conserved hypothetical protein (<i>E. coli</i>)		HX	Chromosome	2.25	0.00000203
BB0076 signal recognition particle-docking protein FtsY (<i>ftsY</i>) (<i>E. coli</i>)	10 family	D	Chromosome	2.22	0.00941598
BB0280 flagellar motor rotation protein B (<i>motB</i>) (<i>B. burgdorferi</i>)		F	Chromosome	2.20	0.00186128
BB0244 conserved hypothetical protein (<i>Bac. subtilis</i>)		HX	Chromosome	2.18	0.00019725
BB0123 ribosomal protein S2 (<i>rpsB</i>) (<i>Pediococcus acidilactici</i>)		RP	Chromosome	2.15	0.00598027
BB0814 pantothenate permease (<i>panF</i>) (<i>E. coli</i>)		TP	Chromosome	2.13	0.00000069
BB0051 conserved hypothetical integral membrane protein (<i>Bac. subtilis</i>)	47 family	HX	Chromosome	2.10	0.00350700
BB0082 hypothetical protein		U	Chromosome	1.94	0.00503889
BB0213 hypothetical protein		U	Chromosome	1.87	0.00104922
BB0028 hypothetical protein		U	Chromosome	1.86	0.00111039
BB0839 hypothetical protein		U	Chromosome	1.84	0.00118860
BBB29 PTS system, maltose and glucose-specific IIABC component (<i>malX</i>) (<i>E. coli</i>)	16 family	TP	cp26	1.83	0.00040202
BB0603 membrane-associated protein p66 (<i>B. burgdorferi</i>)		CE	Chromosome	1.83	0.00000057
BB0050 conserved hypothetical integral membrane protein (<i>Bac. subtilis</i>)	47 family	HX	Chromosome	1.80	0.00831225

Table 2. cont.

Description	Paralogous family	Gene category	Replicon	Fold expression	P value
BB0209 hypothetical protein		U	Chromosome	1.78	0.00821345
BB0043 hypothetical protein		U	Chromosome	1.76	0.00133781
BB0157 hypothetical protein		U	Chromosome	1.73	0.00150062
BB0535 hypothetical protein		U	Chromosome	1.73	0.00969767
BBQ09 conserved hypothetical protein (<i>B. burgdorferi</i>)	50 family	HX	lp56	1.71	0.00961474
BB0313 cell division protein (<i>ftsJ</i>) (<i>M. jannaschii</i>)		D	Chromosome	1.70	0.00675262
BB0625 N-acetylmuramoyl-L-alanine amidase, putative (<i>Enterococcus faecalis</i>)		CE	Chromosome	1.69	0.00184625
BB0269 <i>minD</i> -related ATP-binding protein (<i>ylxH-1</i>) (<i>Bac. subtilis</i>)	32 family	TP	Chromosome	1.64	0.00441824
BB0344 DNA helicase (<i>uvrD</i>) (<i>E. coli</i>)	18 family	R	Chromosome	1.57	0.00909871
BB0240 glycerol uptake facilitator (<i>glpF</i>) (<i>Bac. subtilis</i>)		TP	Chromosome	1.57	0.00079502
BB0367 hypothetical protein		U	Chromosome	1.55	0.00403575
BB0477 ribosomal protein S10 (<i>rpsJ</i>) (<i>B. burgdorferi</i>)		RP	Chromosome	1.55	0.00422148
BB0339 ribosomal protein L13 (<i>rplM</i>) (<i>H. influenzae</i>)		RP	Chromosome	1.52	0.00801772
BB0301 cell division protein (<i>divIB</i>) (<i>B. burgdorferi</i>)		D	Chromosome	1.51	0.00398555
BB0559 PTS system, glucose-specific IIA component (<i>crr</i>) (<i>B. burgdorferi</i>)		TP	Chromosome	1.51	0.00378774
BBH15 hypothetical protein		U	lp28-3	-1.51	0.00000005
BB0751 hypothetical protein		U	Chromosome	-1.53	0.00098450
BB0228 conserved hypothetical protein (<i>Saccharomyces cerevisiae</i>)		HX	Chromosome	-1.53	0.00134828
BBR07 conserved hypothetical protein (<i>B. burgdorferi</i>)	150 family	HX	cp32-4	-1.53	0.00452178
BB0335 oligopeptide ABC transporter, ATP-binding protein (<i>oppF</i>) (<i>Bac. subtilis</i>)	4 family	TP	Chromosome	-1.54	0.00086446
BB0330 oligopeptide ABC transporter, periplasmic oligopeptide-binding protein (<i>oppA-3</i>) (<i>B. burgdorferi</i>)	37 family	TP	Chromosome	-1.56	0.00110352
BBS07 conserved hypothetical protein (<i>B. burgdorferi</i>)	150 family	HX	cp32-3	-1.58	0.00803546
BB0649 heat-shock protein (<i>groEL</i>) (<i>B. burgdorferi</i>)		HS	Chromosome	-1.58	0.00639412
BB0014 primosomal protein N' (<i>priA</i>) (<i>B. burgdorferi</i>)		R	Chromosome	-1.67	0.00220464
BBN13 hypothetical protein, paralogous family 154, authentic frameshift	154 family	U	cp32-9	-1.67	0.00036413
BBS04 hypothetical protein	148 family	U	cp32-3	-1.70	0.00391660
BBH17 hypothetical protein		U	lp28-3	-1.72	0.00835877
BB0071 hypothetical protein		U	Chromosome	-1.75	0.00035815
BBN07 conserved hypothetical protein (<i>B. burgdorferi</i>)	150 family	HX	cp32-9	-1.76	0.00496727
BBA76 thymidylate synthase-complementing protein (<i>thy1</i>) (<i>Dictyostelium discoideum</i>)	65 family	X	lp54	-1.78	0.00455087
BB0763 response regulatory protein (<i>rrp-2</i>) (<i>E. coli</i>)	14 family	GM	Chromosome	-1.80	0.00716378
BB0596 methyl-accepting chemotaxis protein (<i>mcp-2</i>) (<i>Treponema denticola</i>)	13 family	CH	Chromosome	-1.80	0.00054325
BBD18 hypothetical protein (<i>B. burgdorferi</i>)		U	lp17	-1.84	0.00000899
BBR22 conserved hypothetical protein (<i>B. burgdorferi</i>)	142 family	HX	cp32-4	-1.84	0.00892219
BBP11 hypothetical protein	152 family	U	cp32-1	-1.92	0.00080733
BBP29 conserved hypothetical protein (<i>B. burgdorferi</i>)	161 family	HX	cp32-1	-1.99	0.00071963
BBO07 conserved hypothetical protein (<i>B. burgdorferi</i>)	150 family	HX	cp32-7	-2.12	0.00929785
BBB28 hypothetical protein		U	cp26	-2.22	0.00743511
BBA03 outer-membrane protein (<i>B. burgdorferi</i>)		CE	lp54	-2.25	0.00120672
BBA66 antigen, P35, putative (<i>B. burgdorferi</i>)	54 family	CE	lp54	-2.32	0.00011441
BB0525 conserved hypothetical protein (<i>M. jannaschii</i>)		HX	Chromosome	-2.38	0.00000084
BBQ42 conserved hypothetical protein (<i>B. burgdorferi</i>)	80 family	HX	lp56	-2.43	0.00693411
BB0646 hypothetical protein		U	Chromosome	-2.71	0.00000022
BBA01 conserved hypothetical protein (<i>B. burgdorferi</i>)	48 family	HX	lp54	-3.97	0.00343611

analysis. In contrast to previously published array-based studies of *B. burgdorferi* demonstrating high correlation coefficients (Anderton *et al.*, 2004; Brooks *et al.*, 2003; Ojaimi *et al.*, 2003; Tokarz *et al.*, 2004), the comparison presented herein indicated that the quantitative RT-PCR

and macroarray analyses yielded a correlation coefficient (*r*) of 0.70, indicative of a linear relationship between these two methodologies. It is important to note that the RNA used for quantitative RT-PCR analysis was isolated independently from that used for the macroarray. In most cases the same

Fig. 2. Genomic distributions of significantly expressed ORFs. The distribution of ORFs significantly expressed in the *bosRR39K::kan^R* background (JS167) relative to the non-infectious *bosRR39K* parental isolate (CHP100) is shown. The histogram bars above zero represent the number of ORFs that had greater transcript numbers for strain JS167 relative to CHP100, whereas those below zero indicate the number of ORFs with reduced transcript levels in the same comparison.

trend in the expression of a given gene between these different experimental modalities was observed (Fig. 4).

DISCUSSION

The unique lifecycle of *B. burgdorferi* involves disparate environments, specifically an arthropod vector and several mammalian hosts, which serve as either reservoirs or dead-end hosts for *B. burgdorferi* (Steere *et al.*, 2004). Each stage in the transmission of the pathogen and the subsequent dissemination within the host(s) represents a unique and complex environment for the spirochaete. Within the

midguts of unfed *Ixodes* ticks, the dissolved oxygen level would presumably be quite low; however, once a tick takes a blood meal, temperature and respiration would increase, resulting in a concomitant increase in the levels of dissolved oxygen. Thus, as *B. burgdorferi* transits from the infected arthropod vector to the mammalian host(s), it must adapt and respond accordingly to oxygen and its potentially toxic byproducts.

ROS, such as superoxide anion, hydrogen peroxide and hydroxyl radicals, can damage DNA, proteins and lipids, and are an important parameter in a non-specific host defence toward pathogens. In addition, as a consequence

Fig. 3. Significantly expressed ORFs organized by gene categories. The percentage of significantly expressed ORFs in cells with the *bosRR39K::kan^R* allele (JS167) relative to its non-infectious parent strain carrying the *bosRR39K* allele (CHP100) is shown on the y axis, with the absolute numbers from each gene category listed above each histogram column.

Fig. 4. Quantitative RT-PCR analysis of *B. burgdorferi* ORFs verifies the trend of transcript production predicted by the macroarray data. Fifteen ORFs, specifically BB0010, BB0028, BB0043, BB0047, BB0137, BB0219, BB0243, BB0269, BB0603, BB0690, BB0735, BB0749, BBA15, BBA59 and BBB19, were selected for quantitative RT-PCR, and reactions were performed in triplicate (for array values for the genes listed above, refer to GEO accession no. GSE4856). The ratio of transcripts from JS167 relative to CHP100 was calculated for the quantitative RT-PCR data and compared to the macroarray data. The resulting comparison yielded a correlation coefficient (r) of 0.70. The table to the right indicates the numerical values for the macroarray analysis relative to the quantitative RT-PCR data for each gene tested (indicated by Array and Real time, respectively).

of respiration, ROS can be generated endogenously within bacterial cells, causing potentially lethal damage (Mongkolsuk & Helmann, 2002; Storz & Imlay, 1999). In response to these threats, micro-organisms have evolved several adaptable responses that sense oxidation and modulate the expression of appropriate genes. One such regulatory system is defined by PerR, a metalloregulatory protein of the Fur family of regulators (Bsai *et al.*, 1998; Fraser *et al.*, 1997). In *Bacillus* spp. and *Staphylococcus aureus*, PerR alters global gene expression in response to oxidative stress (Helmann *et al.*, 2003; Horsburgh *et al.*, 2001a). Under conditions of low redox in *Bacillus* spp., PerR binds to DNA and represses transcription of catalase (*katA*), peroxidases (*ahpC*, *ahpF*), a non-specific DNA binding protein (*dps*), and the regulatory locus *fur* (Fuangthong *et al.*, 2002; Herbig & Helmann, 2001). Furthermore, in *S. aureus*, PerR is autoregulatory (Horsburgh *et al.*, 2001a, b). The repressor activity of PerR in *Bacillus* is dependent on metal binding involving coordination of Mn^{2+} , Fe^{2+} or Zn^{2+} (Helmann *et al.*, 2003). Another redox-responsive transcriptional regulator is OxyR, which is activated by the oxidation of cysteine disulfide bonds that induce genes involved in the oxidative stress response (Aslund *et al.*, 1999; Zheng *et al.*, 1998, 2001). OxyR is able to bind DNA in the reduced or oxidized state; however, it is only able to activate transcription when oxidized (Aslund *et al.*, 1999). By analogy with other bacterial systems, some of the candidate genes that might constitute an oxidative stress regulon in *B. burgdorferi* include homologues to *sodA* (Nichols *et al.*, 2000; Whitehouse *et al.*, 1997), *dps* (*napA*), thioredoxin reductase (*trxB*), thioredoxin (*trxA*) and a CoA disulphide reductase

(*cdx*; previously annotated as *nox*) (Boylan *et al.*, 2006). To date no gene encoding a catalase or peroxidase has been identified in the *B. burgdorferi* genome.

In support of the hypothesis that BosR regulates the oxidative stress response in *B. burgdorferi*, *sodA* was significantly induced in JS167 relative to its genetic parent, CHP100 (Table 2). The transcriptional profile analysis also identified many genes of unknown function, suggesting that some of these loci may contribute to the resistance to ROS observed. Most genes identified generally had no predicted function, and no bias toward any particular paralogous family was observed (Fig. 3). Whether any of these genes are associated with the oxidative stress response will require additional characterization.

In addition to genes that could be overtly linked to redox resistance, analysis also indicated that the *glp* operon, composed of *glpF*, *glpK*, *bb0242* and *glpA*, was induced in borrelial strains that were resistant to ROS. It is worth noting that the *glp* operon is also induced at 23 °C relative to 35 °C (Ojaimi *et al.*, 2003), and, in that study, Ojaimi and others speculate that the *glp* operon may be required for borrelial cells to generate ATP as an adaptive response to low temperature. Data that indicate that JS167 induces the *glp* operon imply again that energy acquisition, via the metabolism of glycerol and its incorporation into the glycolytic pathway (Schwan *et al.*, 2003), is an important parameter in the oxidative stress response. Alternatively, it is tempting to speculate that the *glp* operon is used secondarily to eliminate or metabolize oxidized lipids. In this hypothetical scenario,

oxidized lipids would be deacylated by an as yet unknown lipase, with the resulting glycerol phosphate backbone shuttled into the glycolytic pathway in the form of either glyceraldehyde 3-phosphate or dihydroxyacetone phosphate (following an oxidation reaction mediated by a dehydrogenase in both cases).

Increased expression of the putative porin and integrin adhesin *p66* was also observed (Table 2). Previous transcriptional profile analysis of *B. burgdorferi* incubated within the peritoneal cavity of rats has indicated that *p66* is induced under these conditions (Brooks *et al.*, 2003). Earlier studies have estimated that interstitial fluid has significantly reduced dissolved oxygen levels (Siegemund *et al.*, 1999; Venkatesh *et al.*, 2000) and it is probable that the conditions within the peritoneal cavity have a comparable oxygen tension. If so, it is likely that *p66*, amongst other genes, is redox-regulated in a manner that may be directly or indirectly affected by BosR. DbpA, a decorin-binding protein, has been shown previously to have a higher level of synthesis in JS167 relative to the non-infectious CHP100 parent (Seshu *et al.*, 2004b). This corresponds with the observed transcriptional data for *dbpA*, with a 7.14-fold increase in expression and a *P* value 0.043, which fell slightly outside of the predetermined statistical parameter (data not shown). Thus, by analogy to *dbpA*, it is possible that BosR may regulate other genes that are also important in mammalian host adaptation and/or pathogenic mechanisms.

One of the two two-component regulatory systems found in *B. burgdorferi* was not transcribed to the same extent in the ROS-resistant JS167 strain relative to CHP100 (Table 2). This two-component system, defined by *bb0763* and *bb0764*, encodes the response regulator *rrp-2* and its cognate sensor kinase, respectively. Rrp-2 works together with borrelial RpoN to activate expression of RpoS, which in turn results in the transcription of several unlinked genes, including *ospC*, *dbpA* and *mlp8* (Hubner *et al.*, 2001; Yang *et al.*, 2003). Although unproven for this particular two-component system, several sensor kinases respond to the redox environment of the cells (Chou *et al.*, 1993). It is thus possible that BB0764 senses the redox status of *B. burgdorferi* and responds accordingly. If this hypothesis is true, then it is possible that an overlap or cooperative effort between the BosR and the RpoN-RpoS regulatory pathways may exist.

In order to determine if commonly expressed genes have a BosR binding consensus sequence that might define a regulon, the upstream sequences of several of the genes listed in Table 2 were compared and analysed. Phenogram analysis of the putative promoter regions of ORFs significantly expressed in JS167 did not identify any significant consensus sequence for a possible BosR regulatory binding site (data not shown), suggesting that BosRR39K alters the regulation of some of these genes indirectly.

A clonal isolate of non-infectious *B. burgdorferi*, CHP100, was used to isolate the strain (JS167) containing the

bosRR39K::kan^R allele (Seshu *et al.*, 2004b). CHP100 lacks cp9, lp25, lp28-4, lp36 and lp38, contributing to the non-infectious status of the isolate (Fig. 1). After isolating the *bosRR39K::kan^R* mutant, the levels of lp5 and lp28-2 were found to be reduced in this clonal isolate (Fig. 1). A role for lp5 and lp28-2 in the redox-resistant phenotype observed seems unlikely, based on the previous observation that the redox-resistant *bosRR39K::kan^R* isolate can be converted back to a redox-sensitive strain by adding intact *bosRR39K* alone *in trans* (Seshu *et al.*, 2004b). As such, if lp5 or lp28-2 were important in the redox-resistant phenotype, then one would expect that the aforementioned genetic complementation would not reverse the redox-resistant phenotype. This effect, coupled with the previous observation that the *bosRR39K::kan^R* strain JS167 is as resistant to ROS as the infectious clonal isolate MSK5 (Seshu *et al.*, 2004b), suggests that genes common to these isolates, either chromosomally or plasmid encoded, are responsible for the phenotype observed. Also, the effect of lp5 and lp28-2 in the resistance to ROS is further marginalized, in as much as all seven genes on lp5 and 20 of the 32 ORFs on lp28-2 have paralogues on one or several other plasmids, or within the chromosome, indicating that most of the activity lost from both of these plasmids could be provided by distal genes throughout the genome. Finally, none of the lp5 or lp28-2 paralogues was significantly expressed in the JS167 strain, suggesting that these genes do not contribute to the ROS resistance observed.

This study globally analysed the transcriptional profile of a *B. burgdorferi* mutant and its parental derivatives to begin defining genes involved in the oxidative stress response. The ideal study would directly investigate redox regulation via BosR, but the isolation of a *bosR* mutant within an infectious *B. burgdorferi* background has been unsuccessful. This is due presumably to the conditional lethality associated with the absence of *bosR* and the effect that this BosR deficiency has on borrelial viability. It is conceivable that under the proper experimental growth conditions, a *bosR* mutant can be isolated and characterized. In the interim, it is possible to address the potential role of a subset of genes identified in this analysis by genetically inactivating these loci and/or by conducting BosR-specific gel shift or footprint assays, to establish a link with their role in the borrelial oxidative stress response, and to ascertain whether BosR binds to regulatory domains within these genes, respectively. Experiments to characterize these genes, and thus refine the putative borrelial BosR regulon, are currently under way.

ACKNOWLEDGEMENTS

The authors wish to thank Ira Schwartz, Darrin Akins, Chad Brooks, Caroline Ojaimi and Jianmin Zhong for helpful advice regarding the array analyses. J. A. H. wishes to thank Clay Hancock for inspiration and encouragement. This work was supported by a fellowship from the Life Sciences Task Force at Texas A&M University (to J. A. H.) and Public Health Service grant AI-42345 (to J. T. S.) from the National Institute of Allergy and Infectious Diseases.

REFERENCES

- Akins, D. R., Bourell, K. W., Caimano, M. J., Norgard, M. V. & Radolf, J. D. (1998). A new animal model for studying Lyme disease spirochetes in a mammalian host-adapted state. *J Clin Invest* **101**, 2240–2250.
- Anderton, J. M., Tokarz, R., Thill, C. D., Kuhlow, C. J., Brooks, C. S., Akins, D. R., Katona, L. I. & Benach, J. L. (2004). Whole-genome DNA array analysis of the response of *Borrelia burgdorferi* to a bactericidal monoclonal antibody. *Infect Immun* **72**, 2035–2044.
- Aslund, F., Zheng, M., Beckwith, J. & Storz, G. (1999). Regulation of the OxyR transcription factor by hydrogen peroxide and the cellular thiol-disulfide status. *Proc Natl Acad Sci U S A* **96**, 6161–6165.
- Boylan, J. A., Posey, J. E. & Gherardini, F. C. (2003). *Borrelia* oxidative stress response regulator, BosR: a distinctive Zn-dependent transcriptional activator. *Proc Natl Acad Sci U S A* **100**, 11684–11689.
- Boylan, J. A., Hummel, C. S., Benoit, S., Garcia-Lara, J., Treglown-Downey, J., Crane, E. J., 3rd & Gherardini, F. C. (2006). *Borrelia burgdorferi* bb0728 encodes a coenzyme A disulphide reductase whose function suggests a role in intracellular redox and the oxidative stress response. *Mol Microbiol* **59**, 475–486.
- Brooks, C. S., Hefty, P. S., Jolliff, S. E. & Akins, D. R. (2003). Global analysis of *Borrelia burgdorferi* genes regulated by mammalian host-specific signals. *Infect Immun* **71**, 3371–3383.
- Bsat, N., Herbig, A., Casillas-Martinez, L., Setlow, P. & Helmann, J. D. (1998). *Bacillus subtilis* contains multiple Fur homologues: identification of the iron uptake (Fur) and peroxide regulon (PerR) repressors. *Mol Microbiol* **29**, 189–198.
- Carroll, J. A., Garon, C. F. & Schwan, T. G. (1999). Effects of environmental pH on membrane proteins in *Borrelia burgdorferi*. *Infect Immun* **67**, 3181–3187.
- Carroll, J. A., Cordova, R. M. & Garon, C. F. (2000). Identification of 11 pH-regulated genes in *Borrelia burgdorferi* localizing to linear plasmids. *Infect Immun* **68**, 6677–6684.
- Chou, J. H., Greenberg, J. T. & Dimple, B. (1993). Posttranscriptional repression of *Escherichia coli* OmpF protein in response to redox stress: positive control of the *micF* antisense RNA by the *soxRS* locus. *J Bacteriol* **175**, 1026–1031.
- Coburn, J. & Cugini, C. (2003). Targeted mutation of the outer membrane protein P66 disrupts attachment of the Lyme disease agent, *Borrelia burgdorferi*, to integrin α v β 3. *Proc Natl Acad Sci U S A* **100**, 7301–7306.
- Conway, T., Kraus, B., Tucker, D. L., Smalley, D. J., Dorman, A. F. & McKibben, L. (2002). DNA array analysis in a Microsoft Windows environment. *Biotechniques* **32**, 110, 112–114, **116**, 118–119.
- Fraser, C. M., Casjens, S., Huang, W. M. & 35 other authors (1997). Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* **390**, 580–586.
- Fuangthong, M., Herbig, A. F., Bsat, N. & Helmann, J. D. (2002). Regulation of the *Bacillus subtilis* *fur* and *perR* genes by PerR: not all members of the PerR regulon are peroxide inducible. *J Bacteriol* **184**, 3276–3286.
- Helmann, J. D., Wu, M. F., Gaballa, A., Kobel, P. A., Morshedi, M. M., Fawcett, P. & Paddon, C. (2003). The global transcriptional response of *Bacillus subtilis* to peroxide stress is coordinated by three transcription factors. *J Bacteriol* **185**, 243–253.
- Herbig, A. F. & Helmann, J. D. (2001). Roles of metal ions and hydrogen peroxide in modulating the interaction of the *Bacillus subtilis* PerR peroxide regulon repressor with operator DNA. *Mol Microbiol* **41**, 849–859.
- Horsburgh, M. J., Clements, M. O., Crossley, H., Ingham, E. & Foster, S. J. (2001a). PerR controls oxidative stress resistance and iron storage proteins and is required for virulence in *Staphylococcus aureus*. *Infect Immun* **69**, 3744–3754.
- Horsburgh, M. J., Ingham, E. & Foster, S. J. (2001b). In *Staphylococcus aureus*, *fur* is an interactive regulator with PerR, contributes to virulence, and is necessary for oxidative stress resistance through positive regulation of catalase and iron homeostasis. *J Bacteriol* **183**, 468–475.
- Hubner, A., Yang, X., Nolen, D. M., Popova, T. G., Cabello, F. C. & Norgard, M. V. (2001). Expression of *Borrelia burgdorferi* OspC and DbpA is controlled by a RpoN-RpoS regulatory pathway. *Proc Natl Acad Sci U S A* **98**, 12724–12729.
- Katona, L. I., Tokarz, R., Kuhlow, C. J., Benach, J. & Benach, J. L. (2004). The *fur* homologue in *Borrelia burgdorferi*. *J Bacteriol* **186**, 6443–6456.
- Labandeira-Rey, M. & Skare, J. T. (2001). Decreased infectivity in *Borrelia burgdorferi* strain B31 is associated with loss of linear plasmid 25 or 28-1. *Infect Immun* **69**, 446–455.
- Mongkolsuk, S. & Helmann, J. D. (2002). Regulation of inducible peroxide stress responses. *Mol Microbiol* **45**, 9–15.
- Nichols, T. L., Whitehouse, C. A. & Austin, F. E. (2000). Transcriptional analysis of a superoxide dismutase gene of *Borrelia burgdorferi*. *FEMS Microbiol Lett* **183**, 37–42.
- Ojaimi, C., Brooks, C., Casjens, S. & 12 other authors (2003). Profiling of temperature-induced changes in *Borrelia burgdorferi* gene expression by using whole genome arrays. *Infect Immun* **71**, 1689–1705.
- Purser, J. E. & Norris, S. J. (2000). Correlation between plasmid content and infectivity in *Borrelia burgdorferi*. *Proc Natl Acad Sci U S A* **97**, 13865–13870.
- Revel, A. T., Talaat, A. M. & Norgard, M. V. (2002). DNA microarray analysis of differential gene expression in *Borrelia burgdorferi*, the Lyme disease spirochete. *Proc Natl Acad Sci U S A* **99**, 1562–1567.
- Schwan, T. G., Piesman, J., Golde, W. T., Dolan, M. C. & Rosa, P. A. (1995). Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. *Proc Natl Acad Sci U S A* **92**, 2909–2913.
- Schwan, T. G., Battisti, J. M., Porcella, S. F. & 7 other authors (2003). Glycerol-3-phosphate acquisition in spirochetes: distribution and biological activity of glycerophosphodiester phosphodiesterase (GlpQ) among *Borrelia* species. *J Bacteriol* **185**, 1346–1356.
- Seshu, J., Boylan, J. A., Gherardini, F. C. & Skare, J. T. (2004a). Dissolved oxygen levels alter gene expression and antigen profiles in *Borrelia burgdorferi*. *Infect Immun* **72**, 1580–1586.
- Seshu, J., Boylan, J. A., Hyde, J. A., Swingle, K. L., Gherardini, F. C. & Skare, J. T. (2004b). A conservative amino acid change alters the function of BosR, the redox regulator of *Borrelia burgdorferi*. *Mol Microbiol* **54**, 1352–1363.
- Siegemund, M., van Bommel, J. & Ince, C. (1999). Assessment of regional tissue oxygenation. *Intensive Care Med* **25**, 1044–1060.
- Skare, J. T., Mirzabekov, T. A., Shang, E. S. & 8 other authors (1997). The Oms66 (p66) protein is a *Borrelia burgdorferi* porin. *Infect Immun* **65**, 3654–3661.
- Steere, A. C., Coburn, J. & Glickstein, L. (2004). The emergence of Lyme disease. *J Clin Invest* **113**, 1093–1101.
- Storz, G. & Imlay, J. A. (1999). Oxidative stress. *Curr Opin Microbiol* **2**, 188–194.
- Tokarz, R., Anderton, J. M., Katona, L. I. & Benach, J. L. (2004). Combined effects of blood and temperature shift on *Borrelia burgdorferi* gene expression as determined by whole genome DNA array. *Infect Immun* **72**, 5419–5432.
- Venkatesh, B., Morgan, T. J. & Lipman, J. (2000). Subcutaneous oxygen tensions provide similar information to ileal luminal CO₂

tensions in an animal model of haemorrhagic shock. *Intensive Care Med* **26**, 592–600.

Whitehouse, C. A., Williams, L. R. & Austin, F. E. (1997). Identification of superoxide dismutase activity in *Borrelia burgdorferi*. *Infect Immun* **65**, 4865–4868.

Yang, X., Goldberg, M. S., Popova, T. G., Schoeler, G. B., Wikel, S. K., Hagman, K. E. & Norgard, M. V. (2000). Interdependence of environmental factors influencing reciprocal patterns of gene expression in virulent *Borrelia burgdorferi*. *Mol Microbiol* **37**, 1470–1479.

Yang, X. F., Alani, S. M. & Norgard, M. V. (2003). The response regulator Rrp2 is essential for the expression of major membrane lipoproteins in *Borrelia burgdorferi*. *Proc Natl Acad Sci U S A* **100**, 11001–11006.

Zheng, M., Aslund, F. & Storz, G. (1998). Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* **279**, 1718–1721.

Zheng, M., Wang, X., Doan, B., Lewis, K. A., Schneider, T. D. & Storz, G. (2001). Computation-directed identification of OxyR DNA binding sites in *Escherichia coli*. *J Bacteriol* **183**, 4571–4579.