Published in final edited form as:
FEMS Microbiol Lett. 2011 April ; 317(2): 172–180. doi:10.1111/j.1574-6968.2011.02226.x.

Functional analysis of *Borrelia burgdorferi uvrA* in DNA damage protection

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Abstract

Bacterial pathogens face constant challenges from DNA-damaging agents generated by host phagocytes. Although *Borrelia burgdorferi* appears to have many fewer DNA repair enzymes than pathogens with larger genomes, it does contain homologues of *uvrA* and *uvrB* (subunits A and B of excinuclease ABC). As a first step to exploring the physiologic function of $uvrA_{Bbu}$ and its possible role in survival in the host in the face of DNA damaging agents, a partially deleted *uvrA* mutant was isolated by targeted inactivation. While growth of this mutant was markedly inhibited by UV irradiation, mitomycin C (MMC) and hydrogen peroxide at doses which lacked effect on wild-type *B. burgdorferi*, its response to pH 6.0 – 6.8 and reactive nitrogen intermediates was similar to that of the wild-type parental strain. The sensitivity of the inactivation mutant to UV irradiation, MMC and peroxide was complemented by an extrachromosomal copy of $uvrA_{Bbu}$. We conclude that $uvrA_{Bbu}$ is functional in *B. burgdorferi*.

Keywords

Borrelia burgdorferi; Lyme disease; *uvrA*; DNA damage; nucleotide excision repair; UV radiation

Introduction

All organisms face constant challenges to the chemical and physical integrity of their genomes from exogenous and endogenous DNA-damaging agents (Nathan & Shiloh, 2000; Pereira *et al.*, 2001; Fang, 2004), and all possess an array of DNA repair systems to counteract these challenges (Sancar, 1996; Rivera *et al.*, 1997; Reardon & Sancar, 2005). Activation of these repair systems is triggered by recognition of a signal implying DNA damage (Black *et al.*, 1998; Smith *et al.*, 2002; Aertsen *et al.*, 2004; Liveris *et al.*, 2004). In *Escherichia coli* and many other bacteria, DNA damage is associated with the presence of significant quantities of ssDNA (Black *et al.*, 1998; Smith *et al.*, 2002; Aertsen *et al.*, 2004; Liveris *et al.*, 2004) that when bound to RecA, induces its co-proteinase activity which

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enhances autocatalysis of the LexA repressor and activates the SOS response. This results in a choreographed transcription of multiple genes (UvrA, UvrB, UvrC, UvrD, DNA polymerase I, DNA ligase) which repair intrachain DNA damage by nucleotide excision (Black *et al.*, 1998; Aertsen *et al.*, 2004; Maul & Sutton, 2005; Fry *et al.*, 2005). Not all bacteria have an SOS response or induction of *uvrA* transcription in response to DNA damage. In *Pseudomonas aeruginosa* (Rivera *et al.*, 1997) and *Neisseria gonorrhoeae* (Davidsen *et al.*, 2007), DNA damage does not trigger an SOS response and does not induce *uvrA* (Black *et al.*, 1998), suggesting that *E. coli* and *B. subtilis* paradigms regarding the regulation of *uvrA* are not universal.

Since many host defenses involve production of DNA-damaging reactive oxygen species (ROS) and reactive nitrogen species (RNS), the ability of pathogenic bacteria to repair damaged DNA is important to their survival in hosts. In *Mycobacterium tuberculosis*, *uvrA* mutants show decreased ability to survive within macrophages (Graham & Clark-Curtiss, 1999) and *uvrB* mutants are attenuated in mice (Darwin & Nathan, 2005). Similarly, in *Helicobacter pylori* and *Yersinia* sp., defects in *uvrA* are accompanied by attenuation in mice (Bijlsma *et al.*, 2000; Garbom *et al.*, 2004). These experimental results strongly suggest that lack of DNA repair mediated by the *uvrA* gene product attenuates bacterial pathogens because they cannot overcome the DNA damaging systems of the host (Janssen *et al.*, 2003).

The genome of *Borrelia burgdorferi*, the cause of Lyme disease, contains a minimal set of genes devoted to DNA repair and appears to lack an SOS response despite the presence of orthologues of *uvrA*, *uvrB*, *uvrD*, DNA polymerase I and DNA ligase (Fraser *et al.*, 1997). It also lacks an orthologue for the repressor of the SOS response, *lexA*, and none of the genes potentially involved in DNA repair display consensus LexA binding boxes similar to those found in *E. coli* (Fraser *et al.*, 1997). *recA* also does not appear to be involved in repair of UV-induced DNA damage in *B. burgdorferi* (Liveris *et al.*, 2004; Putteet-Driver *et al.*, 2004). *B. burgdorferi* is exposed to antibacterial levels of ROS and RNS in infected ticks (Pereira *et al.*, 2001) and mammals (Benach *et al.*, 1984; Cinco *et al.*, 1997; Hellwage *et al.*, 2001), intracellularly following phagocytosis, and extracellularly, by diffusion from intracellular sources or by production at the phagocyte plasma membrane (Putteet-Driver *et al.*, 2004). *B. burgdorferi* can also be exposed to solar UVB radiation in the erythema migrans skin lesion (Born & Born, 1987). *B. burgdorferi* must therefore have functional DNA repair systems to overcome these exposures if it is to survive and proliferate in its hosts.

The *B. burgdorferi uvrA* homologue (BB0837) encodes a protein of 950 amino acids ($UvrA_{Bbu}$) whose deduced amino acid sequence has 23–54% homology to UvrA of *Treponema pallidum*, *Leptospira interrogans*, *Bacillus subtilus*, and *E. coli*, and like these others, contains two zinc finger motifs and two ATP binding sites (Savery, 2007). The function of BB0837 has not been experimentally verified, and study of its function, expression and regulation in *B. burgdorferi* is therefore likely to shed light on its role in DNA repair and bacterial survival. To this end, we inactivated $uvrA_{Bbu}$ and found that the resulting *B. burgdorferi* disruption mutant was more sensitive to UV radiation, MMC and

ROS than the parental strain. This increased sensitivity was reversed by extrachromosomal complementation with a wild-type copy of *uvrA*$_{Bbu}$.

Materials and methods

Strains and culture conditions

Low-passage infectious *Borrelia burgdorferi* 297, clone BbAH130, was obtained from Dr. M. V. Norgard, University of Texas Southwestern Medical Center. PCR analysis using appropriate primers (Iyer *et al.*, 2003) indicated that this clone contained lp25 but lacked lp28-1. Cultures were routinely grown at 34°C in Barbour-Stoenner-Kelly medium supplemented with 6% rabbit serum (BSK-H) (Sigma Chemical Co., St. Louis, MO). *E. coli* DH5α (GIBCO/Life Technologies, Grand Island, NY) was routinely used for cloning, and was grown and maintained in Luria-Bertani medium.

DNA and RNA manipulations

Genomic DNA was isolated from pelletted *B. burgdorferi* grown at 34°C to 3×10^8 cells mL^{-1} with High Pure PCR Template Preparation Kit (Roche Diagnostics Corporation, Indianapolis, IN), total RNA was isolated using TRizol Reagent (Invitrogen Life Technology, Carlsbad, CA), both according to the manufacturer's instructions. Traces of genomic DNA were removed from isolated RNA by treatment with RNase-free DNase. RNA was dissolved in RNase free water (Ambion, Austin, TX) and stored in aliquots at −80°C. cDNA was generated by AMV reverse transcriptase with random primers using the Access RT-PCR system (Promega Corporation, Madison, WI). Controls with the omission of reverse transcriptase were always included in each experiment. PCR reactions were performed using Taq polymerase (Denville Scientific Inc., Metuchen, NJ) or Expend Long Template DNA polymerase mix (Roche Applied Science) using parameters according to Tm of primers. All constructs were confirmed by restriction enzyme analysis, PCR and DNA sequencing using standard procedures (Sambrook & Russell, 2001). The primers used in this study are listed in Table 1.

Generation of *uvrA*$_{Bbu}$ inactivation construct

The *uvrA*$_{Bbu}$ inactivation construct (Fig. 1A) was generated using overlap extension PCR fusion (Shevchuk *et al.*, 2004). Flanking fragments of *uvrA* were amplified from *B. burgdorferi* 297genomic DNA (Fraser *et al.*, 1997) using target-specific primers. Briefly, the 544 bp upstream region of *uvrA*$_{Bbu}$ was amplified from *B. burgdorferi* genomic DNA using primers 12.4 and 12.3 (nt 889980-890523 in the *B. burgdorferi* chromosome) (Table 1). The 700 bp downstream region of *uvrA*$_{Bbu}$ was amplified using primers 12.2 and 12.1 (nt 891827-892526). The kanamycin resistance gene *aph(3′)-IIIa* from *Enterococcus faecalis* was amplified with its own promoter and stop codon from pBLS500 using primers III and IV (Shevchuk *et al.*, 2004). Parameters for PCR reactions were denaturation at 94°C for 2 min, 32 cycles of 94°C for 15 sec-56°C for 20 sec-68°C for 2 min, and final extension at 68°C for 5 min. PCR-fragments were fused by long PCR (Shevchuk *et al.*, 2004), and the final 2,279 kb PCR product containing the *uvrA*$_{Bbu}$ gene with a kanamycin resistance gene insertion was cloned into pGEM-T (Promega), a vector that cannot replicate in *B. burgdorferi*, to yield pBL12. Selection and maintenance of *E. coli* DH5α transformants with

pBL12 was done using solid and liquid Luria-Bertani medium containing 100 μg mL^{-1} of ampicillin.

Construction of pAB63 and pMS9 for complementation of *uvrA*$_{Bbu}$ inactivation mutant

To obtain pAB63 (Fig. 1B), a 3.4 kb PCR fragment containing *uvrA*$_{Bbu}$ and 504 bp 5′ to its translational start site (possible promoter region) was amplified from *B. burgdorferi* 297 genomic DNA using primers AVB3 (containing a *Sac*I restriction site) (Table 1) and AVB4 (containing a *Pst*I restriction site) (Table 1), and ligated into the multiple cloning site of pKFSS1 (Frank *et al.*, 2003) digested with *Sac*I and *Pst*I. To obtain pMS9 (Fig. 1B), the *flaB*$_{Bbu}$ promoter and *uvrA*$_{Bbu}$ were amplified from *B. burgdorferi* 297 genomic DNA using primers FflaB/RflaB (containing *Sac*I and *Kpn*I restriction sites) (Table 1) and FuvrA/RurvA (containing *Kpn*I and *Pst*I restriction sites) (Table 1), respectively, and cloned into pKFSS1, first the *flaB*$_{Bbu}$ promoter, then the ORF for *uvrA*$_{Bbu}$, using the appropriate restriction enzymes.

Transformation of *B. burgdorferi*

Spirochetes grown to mid-logarithmic phase were electroporated with 5–20 μg of plasmid DNA (Samuels, 1995). Individual clones were obtained by serial dilution of aliquots taken from antibiotic-resistant cultures in complete BSK-H containing antibiotics.

Sensitivity to MMC

1×10^5 *B. burgdorferi* cells (midlog phase) were inoculated into 0.5 mL of complete BSK-H containing 0.01, 0.1, 1, 5 or 10 μg of MMC (Sigma), were cultured at 34°C for 12–13 days, and spirochetes counted in duplicate every 1–4 days by dark field microscopy (Sicklinger *et al.*, 2003). Bacteria were always kept in the dark during these experiments. Two independent experiments with each complementing plasmid were performed.

Sensitivity to UV radiation

Cells grown to a density of 3×10^7 cells mL^{-1} in complete BSK-H were harvested by centrifugation, resuspended in phosphate buffered saline, pH 7.4 (PBS) to 1×10^5 cells mL^{-1} and exposed to 800 or 1000 μJ cm^{-2} 280 nm UV radiation (Spectrolinker XL-1000 UV crosslinker, Spectronics Corporation, Westbury, NY). Survival of cells after culture at 34°C on semisolid BSK-H was determined at 14–18 days (Liveris *et al.*, 2004). *B. burgdorferi* not exposed to UV irradiation served as a control. Bacteria were always kept in the dark during these experiments. Results from two independent experiments with each complementing plasmid have been combined.

Sensitivity to ROS

B. burgdorferi, 3×10^7 cells mL^{-1}, were harvested by centrifugation, and diluted in triplicate to a density of 5×10^5 cells mL^{-1} in PBS containing 0, 0.1, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 mM H_2O_2 (Sigma). After incubation for 1 h at 34°C, cells were washed with PBS, resuspended in complete BSK with appropriate antibiotics and cultured in capped 0.5 mL tubes or in 96-well plates in 3% CO_2 at 34°C for 12 days. End points were determined by the change of color of the medium denoting bacterial growth (Terekhova *et al.*, 2002).

Results from 2–4 independent experiments have been combined and are reported as minimal inhibitory concentrations (MIC).

Sensitivity to NOS

$NaNO_2$ (10, 25, 50, 100, 150 mM), (Z)-1-[N-(3-ammoniopropyl)-N-[4-(3-aminopropylammonio) butyl]-amino]-diazen-1-ium-1,2-diolate (0.01, 0.1, 1 mM) (SPER/NO, Sigma) and S-nitroso-N-acetylpenicillamine (0.05, 0.1, 0.5, 1 mM) (SNAP, Sigma) were used as sources of NOS. For treatment with $NaNO_2$, 5×10^5 borrelia were inoculated into capped tubes containing 1 mL complete BSK-H and various concentrations of $NaNO_2$ and cultured at 34°C. For treatment with SPER/NO and SNAP, 5×10^5 cells were incubated in PBS with various concentrations of these reagents for 1 hour at 37°C, harvested by centrifugation, and resuspended and cultured at 34°C in 1 mL complete BSK-H with appropriate antibiotics. Growth of *B. burgdorferi* was determined by counting under dark field microscopy every 2–3 days for 8 days. Results from two independent experiments have been combined.

Sensitivity to acid

Acidity of complete BSK-H (pH 7.5) was adjusted to pH 5.5, 6.0, 6.5 and 6.8 by addition of HCl. *B. burgdorferi*, 5×10^5 cells, were inoculated into 1 mL of pH unadjusted and adjusted medium, and cultured at 34°C for 9 days. Bacterial growth was assessed by counting under dark field microscopy. Results from two independent experiments have been combined.

Statistical analysis

Data were analyzed by one-way analysis of variance with a post-hoc Bonferroni multiple comparisons test. The level of significance was set at $P < 0.05$.

Results and discussion

To inactivate $uvrA_{Bbu}$, a 2.3 kb DNA segment was constructed by long PCR (Shevchuk *et al.*, 2004). This segment contained a small portion of the original $uvrA_{Bbu}$ gene lacking a domain necessary for function and an inserted kanamycin resistance gene (Fig. 1A). It was cloned into pGEM-T (a plasmid that cannot replicate in *B. burgdorferi*) to yield the suicide plasmid pBL12. After electroporation of pBL12 into low passage, infectious *B. burgdorferi* 297, multiple kanamycin-resistant clones were obtained; two were selected for genotyping. Genetic inactivation of $uvrA_{Bbu}$ in these clones was confirmed by PCR of genomic DNA using primers 12.1 and 12.4 (Supplementary Figure, panel A, compare lanes 1 and 2). Sequencing a 5.8 kb PCR fragment obtained with primers 12.5 (upstream gene BB0835) and 12.6 (downstream gene BB0838) confirmed homologous DNA exchange between the wild-type chromosomal $uvrA_{Bbu}$ gene in the chromosome and the disrupted $uvrA_{Bbu}$ allele in pBL12. Two plasmids were constructed to complement this mutant. Because promoters of *B. burgdorferi* often overlap the preceding ORF (Cabello *et al.*, 2006), one of these, pAB63 (Fig. 1B), contained both the $uvrA_{Bbu}$ ORF and 504 bp upstream of the translational start of *uvrA*. The other, pMS9 (Fig. 1B), contained the $uvrA_{Bbu}$ ORF under the control of the borrelial *flaB* promoter. Electroporation of these plasmids and the pKFSS1 vector control into *B. burgdorferi* $\Delta uvrA_{Bbu}$ followed by selection and passaging yielded clones containing

both full-length and disrupted *uvrA*$_{Bbu}$ (Supplementary Figure, panel A) which expressed *uvrA* mRNA transcripts (Supplementary Figure, panel B). Reactions performed without reverse transcriptase showed no amplicons and confirmed the lack of DNA contamination in total RNA samples (data not shown).

UV irradiation damages DNA by generating intrachain thymine dimers (Black *et al.*, 1998; Aertsen *et al.*, 2004; Fry *et al.*, 2005; Maul & Sutton, 2005). Exposure of the parental strain to 800 and 1000 μJ cm^{-2} of UV radiation had little effect on its survival, while exposure of Δ*uvrA*$_{Bbu}$ or its derivative containing only the pKFSS1 cloning vector to these doses resulted in complete loss of viability (Figs. 2A, 2B). Significant complementation of the phenotypic defect of the inactivation mutant was obtained with both pAB63 and pMS9 ($P < 0.001$). The inability of the inactivation mutant to survive UV radiation was partially corrected by pAB63 (*uvrA*$_{Bbu}$ and 504 bp 5′ up to the *uvrA*$_{Bbu}$ start codon, Fig. 2A) and fully corrected by pMS9 (Fig. 2B). This indicates that the *uvrA*$_{Bbu}$ gene product is involved in the ability of *B. burgdorferi* to repair intrachain DNA damage.

MMC, a nucleotide akylating agent, cross-links DNA (Iyer & Szybalski, 1963). Bacterial mutants with various defects in DNA repair have been found to be more susceptible to growth inhibition by this agent than are wild-type (Bijlsma *et al.*, 2000; Liveris *et al.*, 2004). In the absence of MMC, wild type, the Δ*uvrA*$_{Bbu}$ inactivation mutant and its pAB63 (not shown), pMS9 or pKFSS1 derivatives (Fig. 3A) grew equally well in complete BSK-H. All strains reached log-phase density (about 10^8 cells mL^{-1}) by day 4 of culture. In the presence of MMC, the growth of Δ*uvrA*$_{Bbu}$ was significantly ($P < 0.001$) inhibited [concentrations examined: 0.1 μg mL^{-1} (data not shown), 1 μg mL^{-1} (data not shown), 5 μg mL^{-1} (Fig. 3B), 10 μg mL^{-1} (Fig. 3C)]. This growth inhibition was reversed by extrachromosomal complementation of Δ*uvrA*$_{Bbu}$ with pMS9 (*uvrA*$_{Bbu}$ under the control of *flaB*p) but not with the cloning vector pKFSS1 (Figs. 3B, 3C). Similar results were obtained using pAB63 (*uvrA*$_{Bbu}$ under the control of 504 upstream nt) to complement Δ*uvrA*$_{Bbu}$ (data not shown). This indicates that the *uvrA*$_{Bbu}$ gene product is involved in repair of interchain repair of DNA damage in *B. burgdorferi*, in striking difference to the situation in *E. coli* (Sancar, 1996; Savery, 2007). This functional difference between the *uvrA*$_{Bbu}$ and *uvrA*$_{Eco}$ gene products is not surprising given the evolutionary distance between *E. coli* and *B. burgdorferi* (Wu *et al.*, 2009).

B. burgdorferi Δ*uvrA*$_{Bbu}$ was significantly more susceptible to H_2O_2 than the wild-type parental strain (Table 2), with the MIC of H_2O_2 for the wild type *B. burgdorferi* being as much as 5-fold higher than that of the Δ*uvrA*$_{Bbu}$ mutant. This increased sensitivity to ROS was partially reversed by complementation with either pAB63 or pMS9 (Table 2). Complementation was not affected by the presence of 3% CO_2 (studies using pAB63), or its absence (studies using pMS9) during culture. Because the inserted kanamycin resistance gene contained its own stop codon, it seems unlikely that polarity effects on the downstream BB0838 gene contributed to the phenotype of the Δ*uvrA*$_{Bbu}$ mutant. However, homologues of BB0838 are present in other *Borrelia* as well as in *Treponema*, and because the function of this a hypothetical protein is unknown, it is not possible to give a definitive answer.

In contrast to the sensitivity of the Δ*uvrA*$_{Bbu}$ mutant to ROS, its growth and that of its derivatives was not inhibited by exposure to $NaNO_2$, SNAP or SPER/NO (Table 2). The lack of effect of exposure to any of these RNS generators on *B. burgdorferi* growth even though exposure to SNAP and SPER/NO was in PBS while exposure to $NaNO_2$ was in BSK-H suggests that this lack of effect was not likely caused by the serum component of BSK-H (Sohaskey & Barbour, 1999). There were also no significant differences in growth of *B. burgdorferi* and its derivatives in complete BSK-H at pH 6.0, 6.5 or 6.8 (Table 2). None of the strains used in the study (wild-type, *uvrA* inactivation mutant, complemented mutants) were able to grow at pH 5.5 in complete BSK-H (data not shown).

The ability of pathogenic bacteria to repair challenges to their genomes from various DNA-damaging agents produced by host phagocytes is critical to their survival in their hosts (Fang, 2004; Steere *et al.*, 2004). In the absence of DNA-damaging agents, the *uvrA*$_{Bbu}$ inactivation mutant grew as well as the wild-type strain but was markedly inhibited by exposure to UV radiation (Fig. 2), MMC (Fig. 3A) and ROS (Table 2). Extrachromosomal complementation with wild-type *uvrA*$_{Bbu}$ restored growth. In contrast, growth of the inactivation mutant was identical to that of the wild-type after exposure to RNS or decreased pH, conditions under which *uvrA* has been shown to be protective in other bacteria (Aertsen *et al.*, 2004; Fang, 2004). The *uvrA*$_{Bbu}$ gene product is thus involved in the repair of DNA damage caused by UV-radiation, ROS and MMC in *B. burgdorferi*, but not involved in damage due to RNS or decreased pH.

Repair of DNA damage caused by UV irradiation involves UvrA recognition of this damage and nucleotide excision (Sancar, 1996, Savery, 2007). Although both pAB63 and pMS9 restored UV resistance to the Δ*uvrA*$_{Bbu}$ mutant, they differed in the extent of this complementation (Fig. 2). Interestingly, the UV survival curve of the infectious *B. burgdorferi* 297 (clone BbAH130) wild-type strain used in the present study was likely similar to that of the infectious *B. burgdorferi* B31 clone (5A18NP1) used by Lin *et al*. (Lin *et al.*, 2009), but was distinctly different from that reported for the infectious *B. burgdorferi* B31M1 strain studied by Liveris and co-workers (Liveris *et al.*, 2004; Liveris *et al.*, 2008). The reason for this difference is at present unclear, but may be strain-related, since the design of our experiments and those of Liveris et al. was otherwise identical.

In vitro growth of *B. burgdorferi uvrA* inactivation mutants was inhibited by ROS but not by RNS. Dissociation of *in vitro* susceptibility to ROS and RNS has been reported to occur in a *Mycobacterium tuberculosis uvrB* mutant (Darwin & Nathan, 2005). In this case, the mutant was more susceptible to RNS than the wild-type parent but showed similar susceptibility to ROS. It was not possible to examine the *in vivo* function of *B. burgdorferi uvrA*$_{Bbu}$ because Δ*uvrA*$_{Bbu}$ and its derivatives, in contrast to the parental strain, lacked lp25 (Purser & Norris, 2000; Iyer *et al.*, 2003) (data not shown) and were non-infectious. Studies are currently underway to develop an infectious *uvrA* inactivation mutant in order to examine its *in vivo* virulence.

Several lines of evidence suggest that the ability of *B. burgdorferi* to overcome DNA damage following phagocytosis is critical to its ability to survive and produce disease in the host. Mutants of *mutS* and *mutS-II*, genes whose products are involved in DNA mismatch

repair, display decreased infectivity in immunocompetent mice (Lin *et al.*, 2009). Furthermore, resistance of *B. burgdorferi* to rapid killing in vitro by phagocytes has been correlated with in vivo infectivity (Georgilis *et al.*, 1991). Although the majority of phagocytosed borrelia are rapidly killed after ingestion, some remain viable and cultivable (Montgomery *et al.*, 1993), and can stimulate a phagocytic oxidative burst (Georgilis *et al.*, 1991). Plausibly, these few viable organisms could be sufficient to initiate infection of the mammalian host.

In summary, homologous recombination and extrachromosomal complementation have been used to show that $uvrA_{Bbu}$ is needed to repair DNA damage in *B. burgdorferi* exposed in vitro to UV, ROS and MMC but not in *B. burgdorferi* exposed to RNS or low pH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

M.S. and L.B.I. contributed equally to this work which was supported by grant R01 AI 048856 to F. C. C. We would like to thank Dr. M. Norgard, University of Texas Southwestern Medical Center, Dallas, TX, for providing *B. burgdorferi* 297, clone BbAH130, and Dr. Julia Bugrysheva for advice.

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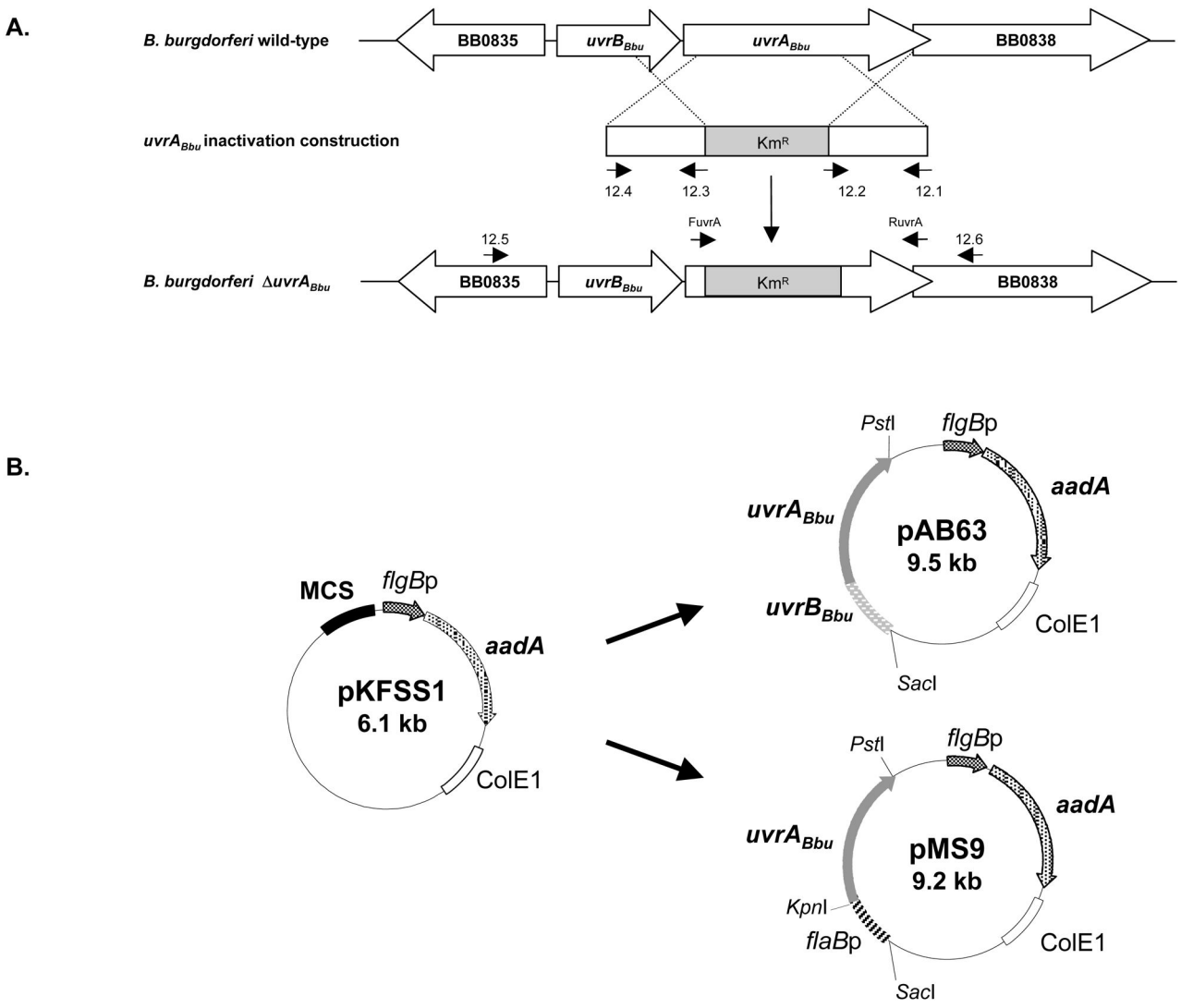

Fig. 1.

Construction of *B. burgdorferi uvrA*$_{Bbu}$ inactivation mutant and complementation plasmids. A. Generation of Δ*uvrA*$_{Bbu}$ inactivation mutant by substitution of part of *uvrA*$_{Bbu}$ with the kanamycin resistance gene *aph(3′)-IIIa* (Km^{R}) from *Enterococcus faecalis* under its own promoter (Shevchuk *et al.*, 2004). See Materials and Methods for details. B. Construction of plasmids for complementation of Δ*uvrA*$_{Bbu}$ inactivation mutant.

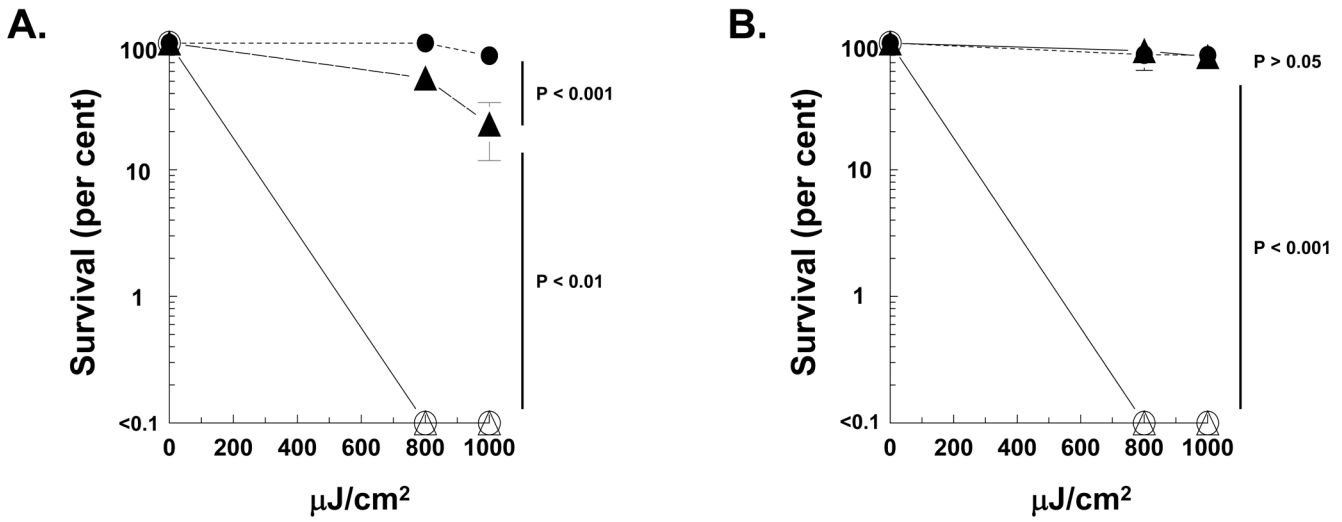

Fig. 2.

Mean (± SD) survival of *B. burgdorferi* 297 wild type, its *uvrA* inactivation mutant and transformed derivatives complemented with pAB63 (A) and pMS9 (B) after UV irradiation. *B. burgdorferi* 297 (●); *B. burgdorferi* Δ$uvrA_{Bbu}$ (○); *B. burgdorferi* Δ$uvrA_{Bbu}$ complemented with pAB63 or pMS9 (▲); *B. burgdorferi* Δ$uvrA_{Bbu}$ pKFSS1 (△). pAB63 provided partial complementation and pMS9 provided full complementation of *uvrA* inactivation mutant phenotype; complementation was significant in both cases compared to vector controls and uncomplemented mutants (one-way analysis of variance, Bonferroni post-test). Colonies grown in dark in semi-solid medium with appropriate antibiotics were counted on day 14 after UV exposure. Results from two (pAB63) or three (pMS9) independent experiments have been combined. In some cases, error bars are obscured by symbols. See Materials and Methods for details.

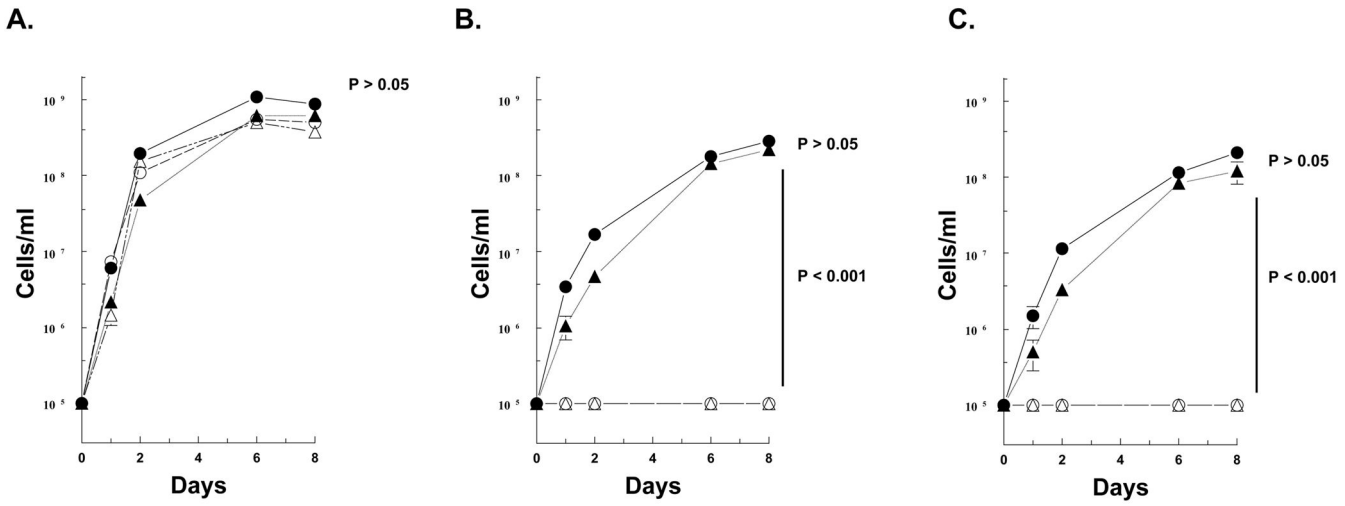

Fig. 3.

Mean (± SE) growth of *B. burgdorferi* 297 wild type, its *uvrA* inactivation mutant and transformed derivatives cultured at 34oC in complete BSK-H (A) in the absence of MMC, (B) in the presence of 5 μg mL^{-1} MMC, or (C) in the presence of 10 μg mL^{-1} MMC. *B. burgdorferi* 297 (●); *B. burgdorferi* Δ$uvrA_{Bbu}$ (○); *B. burgdorferi* Δ$uvrA_{Bbu}$ complemented with pMS9 (▲); *B. burgdorferi* Δ$uvrA_{Bbu}$ pKFSS1 (△). pMS9 provided full complementation of *uvrA* inactivation mutant phenotype; complementation was significant compared to vector controls and uncomplemented mutants (one-way analysis of variance, Bonferroni post-test). Data from one of three independent experiments are shown; results from the other experiments were similar. Error bars are obscured by symbols. See Materials and Methods for details.

Table 1

Primers used in this study.

Primer	Sequence (5′- 3′)	Gene	Reference
12.1	CCTAGAGCTCACCCCTTGACTTTGCATCTGGAAG [a]	*uvrA*	This study
12.2	TTTACTGGATGAATTGTTTTAGTACCTCCGGATAGCGAGGCTCAGCGTATTAGGCTTGC [b]	Km^R/uvrA [c]	This study
12.3	TTGAAACCACAATTATGATAGAATTTTCCGGATATGCCTTGCATAAGCTGAAACAGATTCC [b]	Km^R/*uvrA* [c]	This study
12.4	CGTCTAGATTAGCTATGCGGGAGGCAATTGAG [d]	*uvrA*	This study
12.5	CCCCTTATTCCAGCAGTGCCAAATTCTAGA	BB0835	This study
12.6	GCCCATTTTTTTAAATCCTCATCGGTTGAA	BB0838	This study
AVB3	GTAGAGCTCTGCTTAGAGAGGGCTTAGATATTCCAGAAGT [a]	*uvrB*	This study
AVB4	TCATCCTGCAGCTGGGCAAAAATTGCATTAGAAAATGTT [e]	BB0838	This study
III	ATATCTAGAAAATTCTATCATAATTGTGGTTTCAA	Km^r	Shevchuk et al., 2004
IV	CTATCTAGAGGTACTAAAACAATTCATCCAGTAAA	Km^r	Shevchuk et al., 2004
387d	GATCCAAGGTTATACTTTTTGCACCAATTG	*uvrA*	This study
387r	CAAAGGACAACTGTTGTGCTCTGTAAATAG	*uvrA*	This study
RTF36	GCAACCGTATCTTCAATTTATGCTCTTGGATCTCCAG	*uvrB*	This study
RTR36	GACAATAAGGTCTTTCCATTGTACTTCCACTCAAATATTTAG	*uvrB*	This study
RTF37	GAATATCAGGTAGTCTATCTGGTGGCGAGGCTC	*uvrA*	This study
RTR37	CTCCCTTATCTGCCTTTCTTCTAGAGCTTGGAAC	*uvrA*	This study
RTF3637	GTTGGCTATGATTTTGAAAAAATTATTTCGGGTGAGAG	*uvrB*	This study
RTR3637	GCCTTGCATAAGCTGAAACAGATTCCATATACC	*uvrA*	This study
FflaB	TAAGAGCTCTGTCTGTCGCCTCTTGTGGCTTC [a]	*flaB*	Bono et al., 2000
RflaB	TGTGGTACCTCATTCCTCCATGATAAAATTTAAATTTCTGAC [f]	*flaB*	Bono et al., 2000
FuvrA	TCAGGTACCTTGGAAAAAAGTTTGAAAAAAAAAATTATTGTCAGAG [f]	*uvrA*	This study
RuvrA	TCTAGCTCAGTAAGGCCCATTTTTTTAAATCCTCATC[e]	BB0838	This study

[a] *Sac*I site (underlined).

[b] *Bsp*EI site (underlined).

[c] Km^R, *aph(3 ′)-IIIa*

[d] *Xba*I site (underlined).

[e] *Pst*I site (underlined).

[f] *Kpn*I site (underlined).

Table 2

Growth of *B. burgdorferi* and its Δ*uvrA* derivatives after exposure to DNA damaging agents

	H_2O_2 (MIC, mM)[a]	$NaNO_2$[b]	SPER/NO[c]	SNAP[d]	pH[e]
B. burgdorferi 297 wild-type	5	2.5	+	+	+	+
B. burgdorferi 297 Δ*uvrA*	1	0	+	+	+	+
B. burgdorferi 297 Δ*uvrA* pAB63	3	ND	+	+	+	+
B. burgdorferi 297 Δ*uvrA* pMS9	ND	1.5	ND	+	ND	ND
B. burgdorferi 297 Δ*uvrA* pKFSS1	1	0	+	+	+	+

[a] For determination of MIC using pAB63 for complementation of *B. burgdorferi* 297 Δ*uvrA*, cells were cultured on plates in 3% CO_2. For determination of MIC using pMS9, cells were cultured in capped tubes in the absence of CO_2. ND, not determined. See Materials and Methods for details.

[b] +, growth at 10, 25, 50, 100, 150 mM. Cells incubated and cultured in BSK-H with indicated concentration of $NaNO_2$. See Materials and Methods for details.

[c] +, growth at 0.01, 0.1, 1 mM. Cells incubated for 1 h with SPER/NO in PBS, washed, then cultured in BSK-H. See Materials and Methods for details.

[d] +, growth at 0.05, 0.1, 0.5, 1 mM. Cells incubated for 1 h with SNAP in PBS, washed, then cultured in BSK-H. See Materials and Methods for details.

[e] +, growth at pH 6.0, 6.5 and 6.8. Cells cultured in BSK-H at indicated pH. See Materials and Methods for details.

FEMS Microbiol Lett. Author manuscript; available in PMC 2013 January 30.