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Identification of *Vibrio natriegens uvrA* and *uvrB* Genes and Analysis of Gene Regulation Using Transcriptional Reporter Plasmids

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Nucleotide excision repair (NER) rectifies a variety of chemically and structurally distinct DNA lesions. The current model of NER is based upon the enteric bacterium *Escherichia coli* and there is scarce information about how other bacterial species respond to, and correct, DNA damage. Here we report the isolation and functional analysis of the *uvrA* and *uvrB* genes from *Vibrio natriegens*, a naturally occurring marine bacterium. Genetic studies were completed to assess the repair capabilities of *V. natriegens uvrA* and *uvrB* in *E. coli uvrA* and *uvrB* mutants. In addition to the genetic studies, transcriptional fusions between the luciferase gene and the 5′ regulatory regions of *uvrA* and *uvrB* gene of *V. natriegens* and *E. coli* were constructed. Luminescent measurements from *E. coli* transformed with these constructs showed that whilst the response to UV irradiation of either *E. coli* or *V. natriegens uvrA* regulatory sequences was similar, both the rate and induction of luminescence detected from the *uvrB* regulatory regions differed.

***Keywords*:** NER, *uvrA*, *uvrB*, *V. natriegens*, *luxAB*

Nucleotide excision repair (NER) is a major prokaryotic DNA repair pathway that repairs a diverse range of DNA damage from bulky DNA adducts [cyclo-butane pyrimidine dimers (CPD's)] to non-bulky lesions such as methlyated bases (Sancar, 1996; Moller and Wallin, 1998). NER in *E. coli* has been examined in detail and serves as the paradigm for this pathway in bacterial species (Van Houten, 1990; Van Houten *et al.*, 2005; Truglio *et al.*, 2006).

The pathway involves the co-ordinated activity of multiple key proteins: UvrA, UvrB, and UvrC. UvrA is principally involved in DNA recognition (Seeberg and Stenium, 1982) and forms a damage recognition complex that contains either UvrA2-UvrB heterotrimer or UvrA2-UvrB2 heterotramer (Thesis *et al.*, 2000). After damage detection, UvrA dissociates and UvrB remains bound to the DNA forming a pre-incision complex and acting as a binding site for UvrC (Orren and Sancar, 1989; Delagoutte *et al.*, 2002). UvrC incises 4 nucleotides (nt) towards the 3′ end and then incises 7nt toward the 5′ end of the lesion (Lin and Sancar, 1992; Verhoeven *et al*., 2000). The damaged nucleotide, along with surrounding nucleotides, is removed as the incision complex is released. The resultant gap, caused by the excision, is filled by DNA polymerase (Pol I) and DNA ligase seals the newly repaired DNA strand (Van Houten *et al*., 2005).

A universal bacterial repair pathway, known as the SOS response, becomes activated when DNA is damaged or replication is blocked. De-repression of the genes involved in the SOS response is under direct or indirect negative control of the LexA repressor. During the SOS response, a RecA nucleoprotein filament cleaves LexA, thus releasing repressed SOS genes. The LexA regulon includes the NER genes, *uvrA* and *uvrB* and both genes are up regulated as a part of this global repair pathway in *E. coli* (Kenyon and Walker 1980; Fogliano and Schendel, 1981; Crowley and Hanawalt, 1998).

Vibrio natriegens is a marine bacterium that inhabits surface waters and is therefore exposed to a diversity of exogenous mutagenic substances. These include UVR and environmental pollutants, such as waste water from industrial, agricultural and domestic sources (Ohe *et al*., 2004). Initial studies exploring the role of RecA in DNA repair in *V. natriegens* have been undertaken (Booth *et al*., 2001; Mead, 2003), yet little is known about the physiological state and transcriptional response of these bacteria during DNA repair processes. Given that marine bacteria represent a unique microbial population exposed to a high mutagenic load, we aimed to identify components of the NER pathway in *V. natriegens* with the ultimate goal of designing biosensors for application in an aquatic environment. Here we report on the first steps towards this goal, using a two step approach. Firstly, we identified the *uvrA* and *uvrB* genes in *V. natriegens* and studied their complementation and transcriptional activities in *E. coli*. *V. natriegens uvrA* was shown to fully complement an *E. coli uvrA* mutant strain under native regulation, whereas *V. natriegens uvrB* was found to partially complement an *E. coli uvrB* mutant strain, but only when regulated by *E. coli uvrB* promoters. Secondly, *V. natriegens* NER::*luxAB* reporter plasmids was constructed to determine rates of gene induction. *V. natriegens uvrA::luxAB* showed a similar gene induction profile to that of the *E. coli uvrA* counterpart, as opposed to the *V. natriegens uvrB:: luxAB* reporter plasmid did not induce to the same level.

Material and Methods

General cloning methods

All PCR's were performed using high fidelity Phusion Polymerase

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(FINNzymes, Espoo, Finland) using the primers listed in Table 1. Primers were purchased from Geneworks Pty Ltd. (Thebarton, Australia). PCR primers were designed and optimised using Netprimer (www.premierbiosoft.com/primerdesign/netprimer) (Table 1). Plasmid DNA was purified from overnight cultures with Wizard Plus SV Mini prep DNA purification system (Promega Corporation, Australia). All restriction endonucleases and T4 DNA ligase was purchased from Promega. Transformations of AB1157 cells with purified plasmid DNA was performed by $CaCl_2$/heatshock method (Sambrook, 1989). After restriction digestion, DNA was purified by a Qiagen Nucleotide Clean-up kit (QIAGEN Pty Ltd, Australia). All sequence analysis was performed by the Australian Genome Research Facility (AGRF Ltd., Australia).

Bacterial strains, plasmids, and media

The bacterial strains and plasmids used in this study are presented in Table 2. *E. coli* strains were grown on either nutrient agar (NA) or nutrient agar supplemented with the required antibiotic, where appropriate, to maintain plasmid DNA. Brain heart infusion (BHI) agar/broth supplemented with 1× Marine Salts (MS) was used for *V. natriegens* culture. Marine salts were prepared as a 10× stock and consisted of NaCl (20 g/L), $MgCl_2{\cdot}6H_2O$ (10.2 g/L), and KCl (3.73 g/L). All cultures were grown at 37°C with aeration. Spectrophotometric readings at A_{600} were taken to monitor growth.

Amplifcation of *V. natriegens uvrA* and *uvrB* internal gene regions from *V. natriegens* genomic DNA

Gene sequences of *uvrA* and *uvrB* were sourced from the National Centre for Biotechnology Information (NCBI) for *Vibrio cholera* (AAF93567.1), *V. vulnificus* (BAC60975.1), and *V. parahaemolyticus* (AAO09867.1). Alignment of gene sequences revealed highly-conserved regions from which specific PCR primers were designed. Primers VuvrAF1 and VuvrAR1 for *V. natriegens uvrA*, along with primers VuvrB 1.1 and VuvrB 3.1 for *V. natriegens uvrB* were created (Table 1). Chromosomal DNA extracted from *V. natriegens* (Ausubel *et al.*, 1989) was used as template to amplify *V. natriegens uvrA* (2 kb) and *uvrB* (1.5 kb) internal gene fragments. PCR products were A-tailed before ligating into pGEM T-easy (Promega Corporation). Plasmid DNA from resulting ampicillin resistant colonies was purified and the sequence was found to be highly similar to *uvrA* and *uvrB* genes from other *Vibrio* species in the NCBI database. These internal gene sequences of *V. natriegens uvrA* and *uvrB* were used to design primers for inverse PCR (Ochman *et al*., 1988). IPCR products were cloned into pGEM T-easy and plasmid DNA from transformants was extracted and sequenced to determine identity.

Construction of *V. natriegens uvrA* and *uvrB* plasmids

Following the identification of 5′ and 3′ sequences of the *V. natriegens uvrA* and *uvrB* genes, primers were designed that flanked the genes (Table 1: Inverse PCR). Using chromosomal DNA as a template, the complete *V. natriegens uvrA* (3.2 kb) and *uvrB* genes (2.5 kb), along with flanking regulatory regions were amplified and cloned into pBR322. The resultant PCR products were cloned into a *Pst*I site of pBR322 (*V. natriegens uvrA*) or a *Bam*HI site (*V. natriegens uvrB*) to obtain pKLS$uvrA_{VN}$ and pKLS$uvrB_{VN}$, respectively. Ligation reactions were transformed into competent AB1885 (*uvrB*$^-$, *V. natriegens uvrB*) or AB1886 (*uvrA*$^-$, *V. natriegens uvrA*) cells (Sambrook *et al.*, 1989). From selected transformant colonies, plasmid DNA was purified and the inserts sequenced to confirm error-free cloning of the genes.

Table 1. Primers used in this study

Species	Name	Sequence
Internal gene region		
V. natriegens uvrA	VuvrAF1	5′-GAAGGCAGCAAAATGATG-3′
	VuvrAR1	5′-CCTTTACACACATCACAA-3′
V. natriegens uvrB	VuvrB1.1	5′-TCTTACTACGATTATTACCA-3′
	VuvrB3.1	5′-GTTTCATCCATCGCTTT-3′
Inverse PCR		
V. natriegens uvrA	INVPAF1	5′-GTCAGGTTCAGGCAAGTCAA-3′
	INVPAR1	5′-GCAATACCGCCAGACAACTC-3′
V. natriegens uvrB	INVPBF1	5′-CTGAATCCGATCAAGATGCT-3′
	INVPBR1	5′-CCTCGCTCAAAGGCTACATC-3′
Complete gene		
V. natriegens uvrA	VNuvrApstFOR	5′-AACTGCAGAACCAATGCACGATTTGATTGTGGTAGCGTAG-3′
	VNuvrApstREV	5′-AACTGCAGAACCAATGCAAAGGAGAAACTTGCGATTGAGA-3′
V. natriegens uvrB	VNuvrBFORBam	5′-CGGGATCCCGATATGCAACACGGTTT-3′
	VNuvrBREVBam	5′-CGGGATCCTCCATGCTCATTTAACTCTGACCC-3′
E. coli uvrA	ECuvrAPstFOR	5′-AACTGCAGAACCATTGCAGCGCTTTTACTATGTTGTGA-3′
	ECuvrAPstREV	5′-AACTGCAGAACCATTGCACAAGCCATCTGAATAAA-3′
E. coli uvrB	ECuvrB 3/2	5′-GAAAATCCAGAAAATGCGGC-3′
	ECuvrB 2/2	5′-CGGTGAAAGAGCGGATTGTG-3′
E. coli uvrB promoter primers		
E. coli uvrB promoter	ECuvrBPF1	5′-CGGGATCCGGCGAATGTGGGAATTGCCAG-3′
E. coli uvrB promoter	ECuvrBPR1	5′-CGGATCCCATATGTCGCTACCTGAAGGAGTTG-3′

Restriction cut sites are underlined.

Table 2. Bacterial strains and plasmids used for this study

Strain or plasmid	Genotype or construction	Source/Reference
Strains		
E. coli		
AB1157	*thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 rac hisG4 rbfD1 mgl-51 rspL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	G. Walker
AB1885	As for AB1157 *uvrB5*	*E. coli* Stock Centre
AB1886	As for AB1157 *uvrA6*	*E. coli* Stock Centre
JM109	F'[*traD36 lacI^q (lacz*Δ*M15 proAB$^+$] RecA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ *(lac-proAB)*	Promega
TK501	*thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 glnV44(AS) galK2 Δ(bioA-moa)301 LAM- umuc36 hisG451 rspL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	*E. coli* stock Centre
Vibrio		
V. natriegens PJ164	*thr-2 ser-1 Str^R*	D.G. MacPhee
Plasmids		
pGEM® T-easy	PCR cloning vector: Amp^R	Promega
pGEM®-7Zf(+)	Ap^R	Promega
pBR322	Tc^R, Amp^R	New England Biolabs
pKLS001	2 kb *V. natriegens uvrA* gene partial sequence in pGEM® T-easy, Amp^R	This study
pKLS002	1.5 kb *V. natriegens uvrB gene* partial sequence in pGEM® T-easy, Amp^R	This study
pKLS003	9 kb Inverse PCR-derived partial sequence *V. natriegens uvrA* in pGEM® T-easy, Amp^R	This study
pKLS004	6 kb Inverse PCR-derived partial sequence *V. natriegens uvrA* gene in pGEM® T-easy, Amp^R	This study
pKLS005	4 kb Inverse PCR-derived partial sequence *V. natriegens uvrB* gene into pGEM® T-easy, Amp^R	This study
pKLS*uvrA*$_{EC}$	3 kb *E. coli uvrA* gene with flanking sequence ligated into the *Pst1* site of pBR322, Amp^R	This study
pKLS*uvrB*$_{EC}$	3 kb *E. coli uvrB* gene with flanking sequence ligated into the *Not1* site of pBR322, Amp^R	This study
pKLS*uvrA*$_{VN}$	3.2 kb *V. natriegens uvrA* gene with flanking sequence ligated into the *Pst1* site of pBR322, Amp^R	This study
pKLS*uvrB*$_{VN}$	2.8 kb *V. natriegens uvrB* gene with native flanking sequence ligated into the *BamHI* site of pBR322, Amp^R	This study
pGEM *uvrB*$_{VN1}$	2.8 kb *V. natriegens uvrB* gene fused to *E.coli uvrB* promoter sequence in pGEM-7Zf(+), Amp^R	This study
pKLSEC$_{uvrB}$VN$_{uvrB}$	2.8 kb *V. natriegens uvrB* gene fused to *E.coli uvrB* promoter sequence in pBR322, Amp^R	This study
pKLS*lux*	Tc^R, Ap^R pBR322 containing 2,100 bp *V. harveyi luxAB* fragment	This study
pKLS*lux*::E*uvrA*	Derivative of pKLS*lux* containing 400 bp *E. coli uvrA* promoter region fused to *luxAB*	This study
pKLS*lux*::E*uvrB*P3$^+$	Derivative of pKLS*lux* containing 509 bp *E. coli uvrB* (P3 present) promoter region fused to *luxAB*	This study
pKLS*lux*::E*uvrB*P3$^-$	Derivative of pKLS*lux* containing 352 bp *E. coli uvrB* (P3 absent) promoter region fused to *luxAB*	This study
pKLS*lux*::V*uvrA*	Derivative of pKLS*lux* containing 541 bp *V. natriegens uvrA* promoter region fused to *luxAB*	This study
pKLS*lux*::V*uvrB*P3$^+$	Derivative of pKLS*lux* containing 565 bp *V. natriegens uvrB* (P3 present) promoter region fused to *luxAB*	This study
pKLS*lux*::V*uvrB*P3$^-$	Derivative of pKLS*lux* containing 390 bp *V. natriegens uvrB* (P3 absent) promoter region fused to *luxAB*	This study

Construction of *E. coli uvrB* promoter::*V. natriegens uvrB* fusion plasmid

To verify *V. natriegens uvrB* function in *E. coli*, a fusion between the *E. coli uvrB* promoter and the *V. natriegens uvrB* coding sequence was made transformed into *E. coli*. A 490 bp *uvrB* promoter fragment was amplified from *E. coli* chromosomal DNA and digested with *Bam*HI and inserted into pGEM®-7Zf(+) to obtain pGEM/ECuvrBpromo.

The *V. natriegens uvrB* gene coding region was PCR amplified from *V. natriegens* chromosomal DNA using primers VNuvrBFN and VNuvrBRN. The ~2 kb *V. natriegens uvrB* gene product was digested with *Nde*I and inserted into pGEM/ECuvrBpromo. Ligation reactions were transformed into competent AB1885 cells (Sambrook *et al*., 1989) and positive clones screened by plasmid preparation and restriction enzyme analysis. The *E. coli uvrB* promoter::*V. natriegens uvrB* coding sequence was sub-cloned from pGEM®-7Zf(+) into the pBR322 vector as a *Bam*HI fragment to obtain pKLSECuvrBVNuvrB. Ligation reactions were transformed into competent AB1885 cells (Sambrook *et al.*, 1989) and colonies screened by a colony PCR procedure (Casali and Preston, 2003). Plasmid DNA was purified from positive PCR colonies, analysed by restriction enzyme digestion and sequenced. Sequence information indicated that *E. coli uvrB* promoter region has been fused in-frame to the *V. natriegens uvrB* gene coding sequence.

As positive controls for UV sensitivity and mutation reversion studies, *E. coli uvrA* and *uvrB* genes along with their respective promoter regions were cloned into pBR322. The fidelity of these clones was determined by sequence analysis and comparison with the GenBank accessions, M13495 (*uvrA*) and X03678 (*uvrB*).

UV sensitivity and mutation reversion assays

UV sensitivity and mutation reversion assays were performed using logarithmic phase cultures. Bacteria were grown to stationary phase overnight in nutrient broth (NB) with antibiotic selection. The culture was diluted 10-fold, re-inoculated into NB and grown at 37°C, 250 rpm until OD 600 nm=0.5. Cells were centrifuged, washed twice in 0.85% (w/v) NaCl and 2.5 ml aliquots were irradiated in 60 mm petri dishes. The UVC light was a 254 nm bulb positioned to give a dose of 0.4 J/m^2/sec. The cultures were exposed to UV light for the time required to deliver between 0 and 2.8 J/m^2/sec. Irradiated bacteria (100 µl) were spread plated on Minimal medium O solid media plates containing trace levels of histidine. In histidine revertant bacterial colonies, the histidine defect is corrected by mutation and thus supports growth on minimal medium O solid media plates. A mutation frequency was reported as the number of mutant revertant colonies per 10^8 surviving cells. Irradiated bacteria were also diluted and spread plated on nutrient agar to obtain a viable cell count. Percentage survival was derived from the number of colonies obtained for irradiated and untreated bacteria.

Construction of luminescent reporter plasmids

The construction of the reporter plasmids was done by PCR in a step-wise manner. All other reporter constructs were made using this strategy with the exception of different primers used to amplify the promoter regions. The *luxAB* genes were amplified from purified plasmid DNA of pTJ10 (Justus and Thomas, 1998) using primers luxABF1/R1 (Table 1). A *Bgl*II site was engineered 3′ the *Hin*dIII site in the luxABF1a primer to facilitate the following cloning step. Both the purified PCR product and the pBR322 plasmid DNA were digested with *Hin*dIII and ligated to produce plasmid pKLS*lux*. The orientation of the *luxAB* genes within the vector were confirmed by restriction digest mapping (data not shown) and verified by sequencing. To insert the promoter region 5′ of the *luxAB* genes in pKLS*lux*, primers specific to the *uvrA* and *uvrB* promoter regions of *E. coli* and *V. natriegens* were designed to amplify 5′ regions of approximately 400-500 bp adjacent to the *luxAB* start codon.

The sequence of the primers, the position of the restriction sites, and the transcriptional fusion that were generated between promoter regions and the *luxAB* coding sequence are shown for the construction of the pKLSlux::VuvrBP3$^+$ plasmid in Fig. 1. The following PCR products containing the 5′ regulatory sequences were amplified: *E. coli 5′ uvrA* (primers $P_{EuvrA}F^b/P_{EuvrA}R$) (400 bp), *E. coli 5′ uvrB* P3$^+$ (primers $P_{EuvrBP3}F/P_{EuvrB}R$) (509 bp), *E. coli 5′* P3$^-$ (primers $P_{EuvrB}F/P_{EuvrB}R$) (352 bp), *V. natriegens 5′ uvrA* (primers $P_{VuvrA}F/P_{VuvrA}R$) (541 bp), *V. natriegens 5′ uvrB* P3$^+$ (primers $P_{VuvrBP3}F/P_{VuvrB}R$) (565 bp) and *V. natriegens 5′ uvrB* P3$^-$ (primers $P_{VuvrB}F/P_{VuvrB}R$) (390 bp). All promoters were found to be 100% homologous to their derived sequence. The *E. coli* K12 strain AB1157 was used as a host for luminescence assays.

Luminescence assays

Luminescence assays were performed as previously described (Justus and Thomas, 1998). Luminescence was detected by adding 2 µl N-

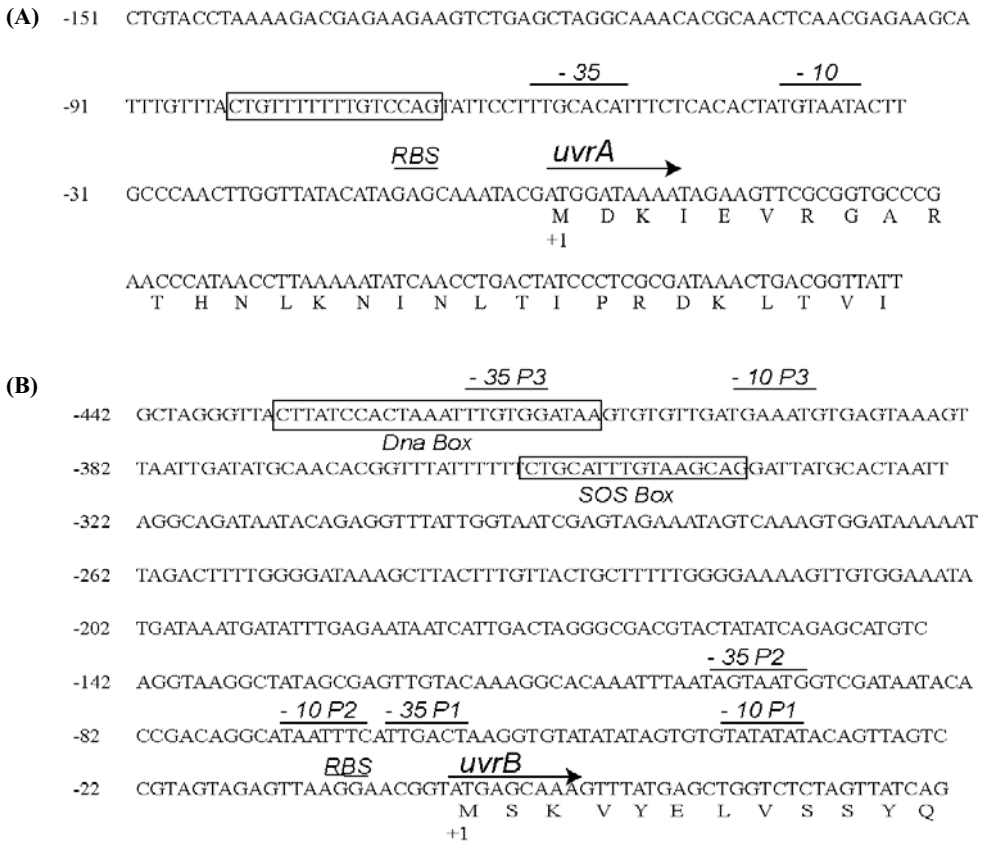

Fig. 1. Nucleotide and amino acid sequences of *V. natriegens uvrA* (A) and *V. natriegens uvrB* (B) upstream regions. Translational starting points are indicated by an arrowhead. The predicted -35 and sequences for all three promoters, as well as putative Shine Dalgarno sequence (RBS) are indicated above the nucleotide sequence. The *V. natriegens uvrA* and *uvrB* SOS-like boxes are found upstream of the start codon and is boxed. The deduced amino acid sequence of UvrA and UvrB are shown below the corresponding nucleotide sequences.

decyl aldehyde (33% v/v in 100% ethanol) (Sigma D 7384) to 1 ml of culture, using a Turner 20e luminometer. The UV light was a 254 nm bulb positioned at a height that resulted in a dose of 0.4 J/m^2/sec. Variations to Justus and Thomas, 1998 were as follows: 1) upon reaching log phase, cultures were diluted 1 in 100 before irradiation 2) At each time point after luminescence reading, 10 µl of treated cells were diluted and plated to obtain viable counts and 3) luminescence values are presented as relative light units (RLU). The specific luminescence unit (SLU) for each sample was calculated by dividing the relative luminescence units by cell viability at each time point (RLU/viable cells). Induction factors (F*i*) were calculated as previously described (Norman *et al*., 2005) as SLUx/SLUO, where SLUX is the treated sample and SLUo is the untreated sample at the same time point.

Plasmid stability assay

Adapted from Szpirer and Milinkovitch (2005), treated cells were plated onto both NA and NA with 50 µg/ml ampicillin. Cells present on NA plates represented the total number of viable cells. Cells present on NA/ampicillin plates represented the number of viable cells containing reporter constructs. Plasmid stability was determined by dividing the total number of cells by those carrying the plasmid. An average over time for each construct was determined.

Statistical analysis

All values presented are mean±SE for n=3. Data for UV sensitivity and mutation reversion assays along with luminescence data were analysed using the SPSS for Windows (Version 17) program (SPSS Inc., USA). AUC (area under the curve) data was determined via a one way ANOVA followed by the Tukey test. Statistical significance was accepted at $P<0.05$.

Results

Isolation of *V. natriegens uvrA* and *uvrB* genes

Nucleotide sequences of *uvrA* and *uvrB* genes from *V. cholera (*AAF93567.1), *V. vulnificus* (BAC60975.1), and *V. parahaemolyticu*s (AAO09867.1) were aligned and highly conserved sequence regions were selected for primer design (Table 1). Subsequent PCR products derived from *V. natriegens* genomic DNA yielded internal fragments of the *uvrA* and *uvrB* genes. The sequence identity of these fragments to *uvrA* and *uvrB* sequence from *V. cholera*, *V. vulnificus*, and *V. parahaemolyticus* was 91-96%. From this internal sequence information, primers were designed to amplify the 3′ and 5′ ends and flanking regions of the *V. natriegens uvrA* and *uvrB* genes by inverse PCR (Ochman *et al*., 1988). Primers were designed at least 200 bp inside the existing sequence so that overlapping sequence information permitted the verification of the identity of the inverse PCR product. Inverse PCR using *V. natriegens* genomic DNA digested with *Pst*I and *Hin*dIII yielded products of 9 and 6 kb for *V. natriegens uvrA* respectively (data not shown). A two step approach was necessary to amplify the 3′ and 5′ flanking sequences of the *V. natriegens uvrB* gene. The ~3.5 kb 3′ region was amplified from *Sal*I digested and ligated genomic DNA (data not shown), whereas the 5′ region was isolated by inverse PCR using *Afl*III genomic digested DNA that generated a ~4 kb product (data not shown). These fragments were cloned and sequenced, and upon examination of the 5′ regions of the *V. natriegens uvrA* and *uvrB* genes, -10 and -35 promoter regions were identified. Primers were designed that enabled the complete *V. natriegens uvrA* and *uvrB* genes and putative regulatory regions to be amplified. The resultant PCR products, 3.2 kb for *V. natriegens uvrA* and 2.8 kb for *V. natriegens uvrB*, were cloned and sequenced for subsequent complementation analysis.

Sequence analysis of *V. natriegens uvrA* and *uvrB* genes

Primers designed from the 3′ and 5′ flanking region of the *V. natriegens uvrA* gene were used to amplify a 3,238 bp PCR fragment. Within this sequence, a 2,823 bp ORF was identified capable of encoding a 940 aa protein with a molecular mass of 104 kDa. When compared with known bacterial UvrA proteins, the *V. natriegens* UvrA protein shared 56-96% sequence identity and 71-97% sequence similarity.

The sequence 5′ to the predicted start codon of *uvrA* revealed several distinguishable regulatory elements. A putative Shine Dalgarno sequence (GAG) could be found -8 bp 5′ of the start codon (Fig. 1A). Sequences of the -10 and -35 promoter elements (TGTAAT and TTGCCG respectively) were identified that differed from the *E. coli* consensus by 3 and 1 bp's, respectively (Fig. 1A) (Table 3) (Harley and Reynolds, 1987). An additional element common to prokaryotic damage induced genes was found in the *uvrA* 5′ sequence. This 'SOS box' is common to genes that are induced by the release of the transcriptional repressor LexA. The SOS box defines the binding site of this repressor and has the

Table 3. Comparison of -35, -10 promoter regions and SOS boxes for *uvrA* and *uvrB* genes between *E. coli* and *V. natriegens* to consensus sequences (Harley and Reynolds, 1987). Highlighted bases are the same as consensus sequence.

Promoter	-35	-10	SOS Box
E. coli			
E. coli uvrA	TGTATA	CATATT	CTGTATATTCATTCAG
E. coli uvrB P3	TTCCTG	TAGAGT	CTGCATTTGTAAGCAG
E. coli uvrB P2	TGGTGA	TATAAT	
E. coli uvrB P1	TTGGGA	TAAAAT	
V. natriegens			
V. natriegens uvrA	TTGCAC	TGTAAT	CTGTTTTTTTGTCCAG
V. natriegens uvrB P3	TTGTGG	TGAAAT	CTGCATTTGTAAGCAG
V. natriegens uvrB P2	TAGTAA	TAATTT	
V. natriegens uvrB P1	TTGACT	TATATA	
CONSENSUS	TTGACA	TATAAT	CTG (10 nt) CAG

consensus sequence of CTG-10nt-CAG. A putative SOS box was found between -83 to -68 bp 5′ of the start codon in the *V. natriegens uvrA* gene.

A 2,557 bp *uvrB* fragment was amplified from *V. natriegens* genomic DNA using primers designed 5′ and 3′ to the sequence of the gene. As cloned copies of this fragment contained the P1 and P2 promoters only, the PCR product was stable in an *uvrB*$^-$ *E. coli* background. A 2,032 bp ORF was identified that would encode a 676 aa protein with a molecular mass of 77 kDa. Depending upon the species, the deduced *V. natriegens* UvrB amino acid sequence showed 50-97% identity and 74-98% similarity.

The nucleotide sequence of the 5′ end, and the regulatory elements, of the *V. natriegens uvrB* gene are shown in Fig. 1B. 5′ UTR *V. natriegens uvrB* sequence information was obtained from purified plasmid DNA containing the *V. natriegens Afl*III inverse PCR product propagated in an *uvrB*$^-$ background. A putative Shine Dalgarno sequence (GGA) could be found -6 bp 5′ of the start codon (Fig. 1B). At first glance the *V. natriegens uvrB* gene promoter region is more complex than that observed for *uvrA*. Three putative promoters (P1, P2, and P3) were identified which is consistent with that reported for *E. coli uvrB* (Sancar *et al.*, 1982). The sequences of the -35 elements for the three promoters were (P1) TGAAAT, (P2) TAGTAA, and (P3) TTGACT (Table 3). The most divergent element was that of P3 located approximately 400 bp 5′ of the start codon (Fig. 1B). The -35 promoter sequence that differed by only one base from the consensus sequence is that of P1 located 58 bp 5′ of the start codon. The sequences of the -10 elements for the three promoters were (P1) TGAAAT, (P2) TAGATT and (P3) TATATA (Fig. 3B). All three of the -10 elements differed by two base pairs when compared to the consensus sequence generated from *E. coli* promoter regions (Table 3) (Harley and Reynolds, 1987). A putative SOS box can be found 352 bp 5′ to the start codon of the *V. natriegens uvrB* gene. P3 in *V. natriegens* also overlapped with a DnaA binding box located 433 bp 5′ to the start codon. The sequence of the DnaA binding region in *V. natriegens* differed by 5 bp to the corresponding 20 bp sequence from *E. coli*.

Effect of third promoter (P3) region upon cloning of *V. natriegens uvrB* gene

During the *V. natriegens uvrB* inverse PCR cloning process, plasmid instability and sequence editing was noted. PCR was used to screen plasmid inserts in colonies obtained from transformation of the inverse PCR/ pGEM®T-easy ligation mix into JM109 cells. The expected PCR product size of 3 kb was not observed in any of the 15 random colonies that were analyzed. Plasmid DNA was extracted from two colonies that yielded smaller than expected PCR. Sequence analysis revealed these plasmids contained a truncated version of the inverse PCR product where the points of truncation were 665 bp and 395 bp 5′ of the ATG. The points of truncation varied between all 15 colonies analysed. This indicated that the *V. natriegens uvrB* gene inverse PCR products were unstable during the cloning process and that regions of the *uvrB* 5′ region were being edited in the *E. coli* host cell. To ascertain whether the *uvrB*$^+$ background of JM109 was responsible for the sequence modification, the *V. natriegens uvrB* inverse PCR/ pGEM®T-easy ligation mix was transformed into both AB1885 (*uvrB* mutant strain) and TK501 (*uvrB* deletion strain) (data not shown). Plasmid DNA from two colonies from the AB1885 transformation experiment were purified and sequenced. Sequence data was compared with *E. coli uvrB* 5′ UTR and confirmed that the *V. natriegens uvrB* inverse PCR product contained the complete 5′ flanking regions of *V. natriegens uvrB*. No evidence of sequence trimming/modification was detected when either AB1885 or TK501 background was used for cloning. Consequently, AB1885 (*uvrB* mutant strain) was used for all transformation experiments.

The collective results of cloning of the 5′ IPCR products and the complete *uvrB* gene indicate instability associated with plasmid containing the 5′ regulatory region of the gene. This result is consistent with that of plasmids containing the *E. coli uvrB* gene reported by Van den Berg *et al.* (1985). These authors suggest that the instability is associated with plasmids containing the P3 element of *uvrB* gene. This is also consistent with our results, where the truncation removed the putative P3 element, and for this reason, complementation experiments involving *V. natriegens uvrB*, contained the gene only under P2 and P1 regulation. These two promoters had been previously been identified as the genuine promoters of *E. coli uvrB* (Arikan *et al.*, 1986). This permitted the assessment of gene function in an *E. coli uvrB* mutant without the interference of plasmid instability or sequence modification due to the presence of P3.

Evaluation of the *V. natriegens uvrA* and *uvrB* genes in NER

E. coli uvrA and *uvrB* mutants are hyper sensitive to DNA damaging agents (Sancar and Tang, 1993). To ascertain whether the *V. natriegens uvrA* and *uvrB* genes can complement an *E. coli uvrA* or *uvrB* mutant, UVC irradiation was chosen as a treatment known to generate DNA damage, and thus provide substrates for the Uvr proteins involved in NER. The assessment of *V. natriegens* gene complementation was conducted by mutation reversion and UV sensitivity assays.

V. natriegens uvrA

Reversion of a histidine requirement (his$^-$→his$^+$) was used as an assay for determining the mutation frequency induced by exposure to UV at doses ranging from 0-4.2 J/m^2. AB1157 (DNA repair wild type strain) maintained a low mutation rate for all UV doses in contrast to the *uvrA*$^-$ strain (AB1886) (Table 4). The absence of any colonies found for the AB1886 strain at UV doses greater than 1.8 J/m^2 can be attributed to lethality. Of greatest significance to this study was that AB1886/pKLS*uvrA*$_{VN}$, AB1157, AB1886/pKLS*uvrA*$_{EC}$, and AB1157/pKLS*uvrA*$_{EC}$ all had similar mutation rates (no significant difference). There was no significant difference between AB1886/pKLS*uvrA*$_{VN}$ and AB1886/pKLS*uvrA*$_{EC}$ and that of the wild type strain (AB1157) indicating the complete restoration of NER by pKLS*uvrA*$_{VN}$ and pKLS*uvrA*$_{EC}$. That is, a functional *uvrA* gene from either *E. coli* or *V. natriegens* was capable of fully restoring DNA repair to an *E. coli uvrA*$^-$ strain. This shows that the *E. coli* and *V. natriegens uvrA* genes are functionally interchangeable.

The same strains for the mutation reversion assay were also used in UV sensitivity assays. AB1886 was shown to be extremely UV sensitive (Fig. 2A) as the number of surviving

Table 4. uvrA mutation reversion assay showing mutants per 10^8 surviving cells after exposure to UV irradiation

Exposure (J/m^2)	Mutants per 10^8 surviving cells					
	AB1157	AB1886	AB1157/pKLSuvrA$_{EC}$	AB1886/pKLSuvrA$_{EC}$	AB1886/pKLSuvrA$_{VN}$	AB1886/pBR322
0	5.0±0.6	8.0±3.8 (p<0.000)	7.0±1.9 (p=0.995)	8.0±5.8 (p=0.997)	7.0±2.8 (p=1.000)	8.0±2.7 (p<0.000)
0.6	9.0±4.6	686.0±196.0 (p<0.000)	5.0±1.2 (p=0.995)	9.0±4.3 (p=0.997)	5.0±1.9 (p=1.000)	900.0±300.0 (p<0.000)
1.8	10.0±4.1	18, 708.0±5, 976.0 (p<0.000)	11.0±2.8 (p=0.995)	9.0±1.9 (p=0.997)	12.0±2.4 (p=1.000)	21, 208.0±8, 083.0 (p<0.000)
3.0	11.0±1.3	No data	10.0±2.7 (p=0.995)	10.0±3.0 (p=0.997)	12.0±2.1 (p=1.000)	No data
4.2	12.0±1.4	No data	11.0±2.2 (p=0.995)	8.0±1.9 (p=0.997)	14.0±1.3 (p=1.000)	No data

Results shown are Mean±SE (n=3).

colonies decreased rapidly over time, even at a low UV exposure. In contrast, AB1157 grew at all UV doses. The dose response of the AB1886/pKLS*uvrA*$_{VN}$ cells showed no signify-cance difference to that of the AB1157 cells (Fig. 2A). No significant difference was also detected in the dose response curve of AB1157 and AB1886/pKLS*uvrA*$_{VN}$ cells. No significant difference was detected when AB1886/pKLS*uvrA*$_{VN}$ was compared to AB1886/pKLS*uvrA*$_{EC}$ and AB1157/pKLS*uvrA*$_{EC}$. These results support the mutation reversion data, that indicate that the *V. natriegens uvrA* can completely complement the *E. coli uvrA* defect and significantly reduce UV sensitivity.

V. natriegens uvrB

The same assays for measuring mutation frequencies and UV sensitivity were applied to the *uvrB* complementation study. In gene complementation assays, both AB1157/pKLS*uvrB*$_{EC}$ and AB1885/pKLS*uvrB*$_{EC}$ showed a mutation frequency similar to AB1157, but significantly different to that observed for AB1885 ($p<0.000$). When however, *uvrB* from *V. natriegens* was introduced in AB1885 (AB1885/pKLS*uvrB*$_{VN}$), the mutation frequency was significantly elevated compared to both AB1157 and AB1157/pKLS*uvrB*$_{EC}$. This indicates that *uvrB* from *V. natriegens* is incapable of restoring the mutation frequency to wild type levels. Despite this, a significant difference was found between AB1885 (*uvrB*$^-$) and AB1885/pKLS*uvrB*$_{VN}$. This indicates that *V. natriegens uvrB*, under the control of its native promoter, partially restores the phenotype of the highly mutable *E. coli uvrB*$^-$ strain.

The results of the UV sensitivity study concur with the mutation study for *uvrB* complementation. AB1885 and AB1885/pBR322 both showed extreme UV sensitivity with cell death or very limited survival at a dose greater than 0.6 J/m^2 (Fig. 2B). In contrast, AB1157 grew at all UV doses with a dose response curve that was not significantly different to

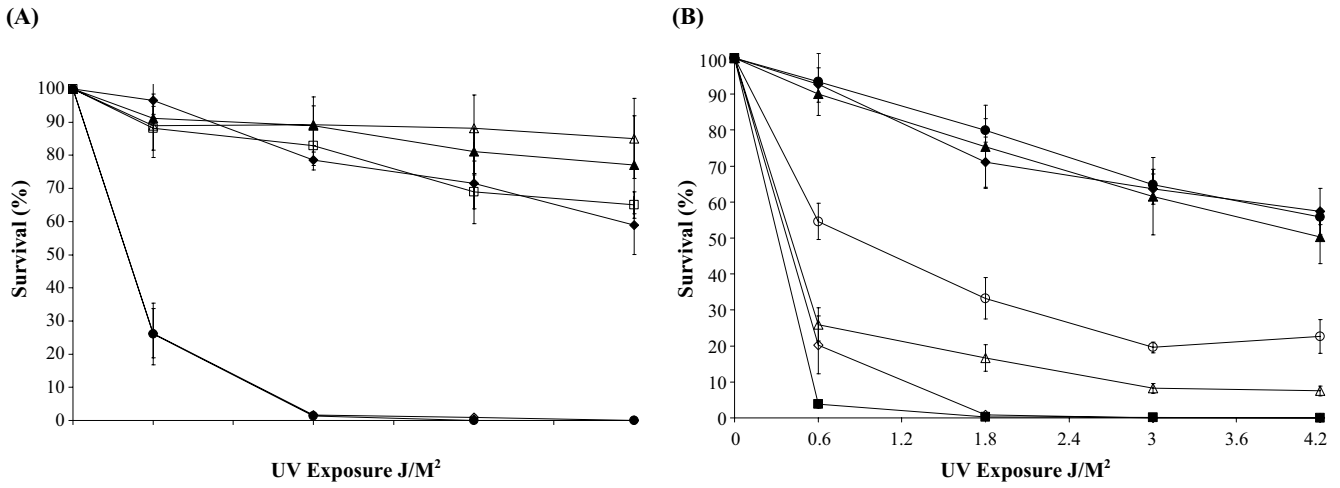

Fig. 2. (A) Percentage survival of *E. coli* AB1157 (◆), AB1886 (*uvrA*$^-$ ◇), AB1886 carrying plasmid pKLS*uvrA*EC containing *E. coli uvrA* gene (△), AB1886 carrying plasmid pKLS*uvrA*VN containing *V. natriegens uvrA* gene (□), AB1157 carrying plasmid pKLS*uvrA*EC containing *E. coli uvrA* gene (▲). AB1886 (*uvrA*$^-$) strain carrying the vector pBR322 was included as a control (●). Data presented are means for three independent experiments±SE. (B) Survival of *E. coli* AB1157 (◆), AB1885 (*uvrB*$^-$ ◇), AB1885 carrying plasmid pKLS*uvrB*EC containing *E. coli uvrB* gene (▲), AB1885 carrying plasmid pKLS*uvrB*VN containing *V. natriegens uvrB* gene (△), AB1157 carrying plasmid pKLS*uvrB*EC containing *E. coli uvrB* gene (●), AB1885 carrying plasmid pKLSECuvrBVNuvrB containing *E. coli uvrB* promoter::*V. natriegens uvrB* (○). AB1885 (*uvrB*$^-$) strain carrying the vector pBR322 was included as a control (■). Data presented are means for three independent experiments±SE.

Table 5. uvrB mutation reversion assay showing mutants per 10^8 surviving cells after exposure to UV irradiation

Exposure (J/m^2)	Mutants per 10^8 surviving cells						
	AB1157	AB1885	AB1157/ pKLSuvrB$_{EC}$	AB1885/ pKLSuvrB$_{EC}$	AB1885/ pKLSuvrB$_{VN}$	AB1885/ pKLSEC$_{uvrB}$VN$_{uvrB}$	AB1885/pBR322
0	8.0±1.8	7.0±6.1 (p<0.000)	7.0±0.3 (p=1.000)	12.0±3.2 (p=1.000)	31.0±14.6 (p=0.221)	7.0±2.4 (p=0.009)	26.0±7.1 (p<0.000)
0.6	12.0±2.1	2, 157.0±791.2 (p<0.000)	10.0±0.8 (p=1.000)	19.0±4.4 (p=1.000)	649.0±35.4 (p=0.221)	23.0±3.3 (p=0.009)	2, 128.0±387.0 (p<0.000)
1.8	18.0±1.6	17, 778.0±1, 222.2 (p<0.000)	13.0±1.2 (p=1.000)	21.0±3.9 (p=1.000)	1, 257±537.0 (p=0.221)	71.4±41.2 (p=0.009)	18, 556.0±3, 245.1 (p<0.000)
3.0	18.0±0.5	No data	21.0±1.5 (p=1.000)	30.0±5.8 (p=1.000)	1, 342±808.0 (p=0.221)	160.0±92.5 (p=0.009)	No data
4.2	18.0±0.3	No data	26.0±1.7 (p=1.000)	40.0±4.3 (p=1.000)	3,798.0±1, 017.0 (p=0.221)	1, 120±54.6 (p=0.009)	No data

Results shown are Mean±SE (n=3).

that of AB1885/pKLS*uvrB*$_{EC}$. That is, as anticipated, an *E. coli uvrB* gene could fully restore NER in an *uvrB*$^-$ *E. coli* strain. This was, however, not the case for the *V. natriegens uvrB* gene. UV sensitivity of AB1885/pKLS*uvrB*$_{VN}$ was not significantly different to AB1885 and AB1885/pBR322 and significantly less than AB1157 and AB1885/pKLS*uvrB*$_{EC}$. These data therefore supports the conclusion that the *V. natriegens uvrB* gene, under its native promoter, failed to fully complement the *E. coli uvrB* defect and did not significantly reduce UV sensitivity.

Evaluation of *V. natriegens uvrB* gene under *E. coli uvrB* promoter regulation

The *V. natriegens* UvrB protein is 75% identical to its *E. coli* counterpart. Despite this, the *V. natriegens uvrB* gene did not complement an *E. coli uvrB* mutation. There are several possible explanations for this. Regulatory elements in the *V. natriegens uvrB* gene may not be recognised in *E. coli* and thus limit gene expression. Alternatively, or in combination, there maybe sub-optimal function of the *V. natriegens* UvrB protein when expressed in *E. coli*. To test whether the failure of *V. natriegens uvrB* complementation in *E. coli* is due to transcriptional or post-transcriptional inadequacies, we fused the *E. coli uvrB* promoter to the coding sequence of the *V. natriegens uvrB* gene and tested this construct in the *uvrB*$^-$ *E. coli* strain AB1885.

This construct, AB1885/pKLSEC$_{uvrB}$VN$_{uvrB}$, partially complemented the *E. coli uvrB* deficient strain in both the UV sensitivity (Fig. 2B) and mutation reversion assays (Table 5). When the AB1885/pKLSEC$_{uvrB}$VN$_{uvrB}$ construct was introduced into the AB1885 strain (*uvrB*$^-$), the resulting cells had a significantly lower sensitivity to UV-induced cell death than untransformed AB1885 (p=0.009), yet significantly higher sensitivity than the wild type strain AB1157 (p<0.000) (Fig. 2B). Despite a trend suggesting increased mutability of AB1885 cells transformed with pKLSEC$_{uvrB}$VN$_{uvrB}$ compared to that of the wild type strain, there is no significant difference between these data sets. In contrast, AB1885 cells containing pKLSEC$_{uvrB}$VN$_{uvrB}$ had a significantly lower mutation frequency than that of AB1885 and AB1885 transformed with *V. natriegens uvrB* under control of its native promoter. Collectively, these data indicate that the *V. natriegens* UvrB protein expression under the control of *E. coli uvrB* promoter improved DNA repair capability compared with *V. natriegens uvrB* under the controls of its native promoter.

Analysis and characterisation of transcriptional promoter fusions

Six different luminescent reporter plasmids were constructed and tested in this study (Table 2). The six variants were designed so that promoter regions of *uvrA*' and *uvrB*' from *E. coli* or *V. natriegens* were driving *luxAB* reporter gene expression. Similarity of the 5′ sequences of *E. coli* and *V. natriegens uvrA* and *uvrB*, with the exception of the position of the SOS box would suggest similar regulation of gene induction. To test this, we treated cells transformed with these constructs with UV radiation and measured luminescence as an indication of gene induction. As a control we compared these data with luminescence obtained from transformed yet untreated cells.

Response of NER-lux reporter plasmids after UV irradiation

Luminescence induction over a 300 min period was observed in treated cells following a range of UV irradiation doses. A UV dose of 48 J/M^2 was shown to generate a significant difference in the rate of SLU between the untreated and treated samples for all six reporter plasmids (Fig. 3). This dose was used in further analysis. Each reporter plasmid generated a level of luminescence (>0) that continued to significantly increase for treated samples for the duration of the assay, with the exception of the *V. natriegens uvrB* promoter. The level of induction of each of the promoters was evident after 60 min, but light emission continued to escalate and began to plateau after 180 to 240 min. The rate of luminescence was found to diminish after 300 min (data not shown).

Disparity of *uvrA* and *uvrB* induction between *E. coli* and *V. natriegens*

To determine if the rate of *uvrA* and *uvrB* induction varied between the two species, the induction profiles of *E. coli* and *V. natriegens uvrA'* and *uvrB'* promoters were compared. No significant difference between the rates of induction from *E. coli* and *V. natriegens uvrA'* was observed (Fig. 4A). The lag in luminescence, particularly noticeable for the first 60 min of induction in cells containing the *V. natriegens uvrA* 5′ promoter::*luxAB* fusion construct could be attributed to cellular

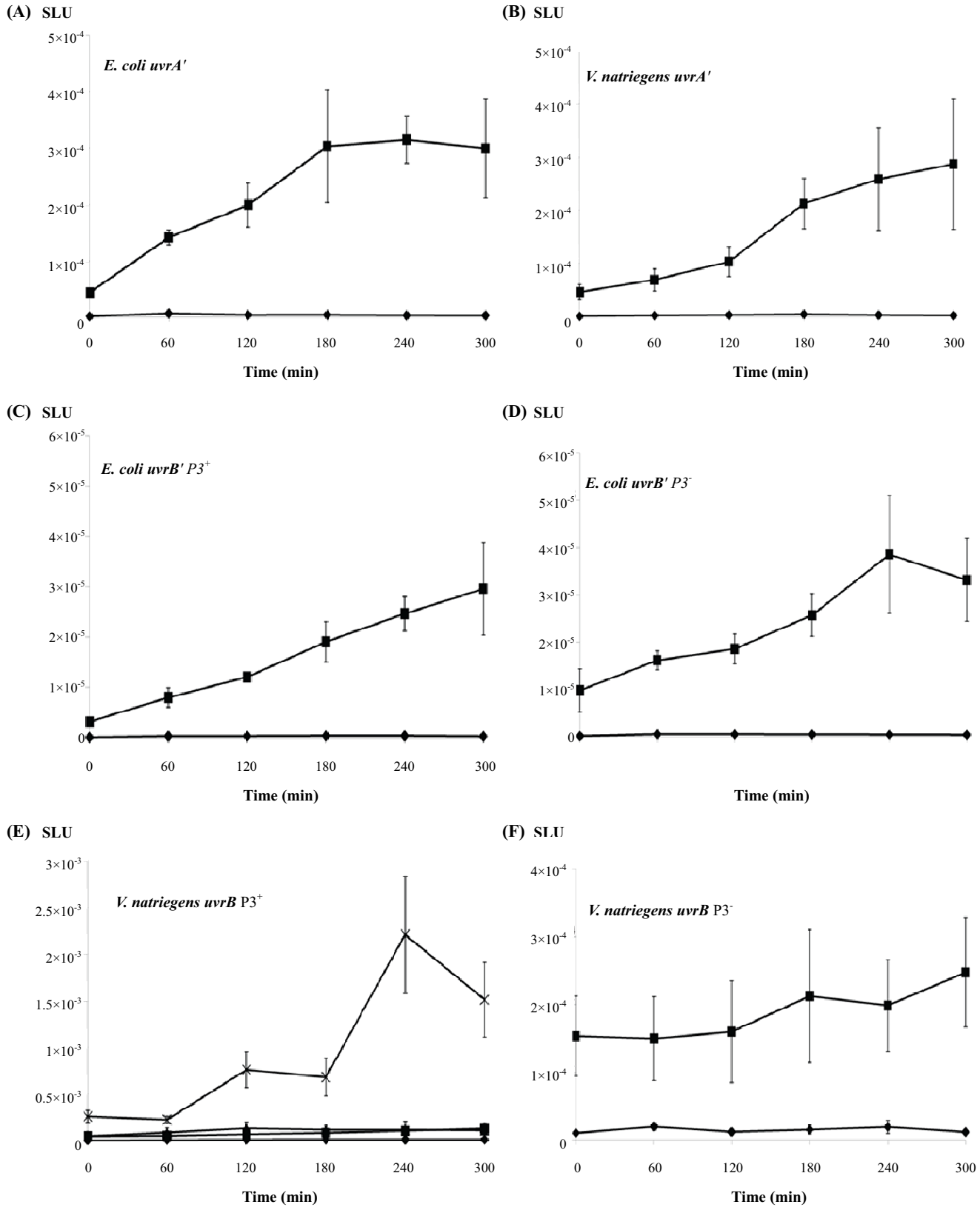

Fig. 3. Luminescence response of *E. coli* carrying various *luxAB* reporter plasmids following exposure of a high dose of UV irradiation. (A) pKSlux::EuvrA, (B) pKSlux::VuvrA, (C) pKSlux::EuvrBP3+, (D) pKSlux::EuvrBP3-, (E) pKSlux::VuvrBP3+ where (♦, 0 NA), (■, 48 J/M2 NA), (▲) NA + A), (× 48 J/M2 NA+ A), and (F) pKS::luxVuvrBP3- (◆) control (untreated cells). Values presented are means±SE, $n=3$.

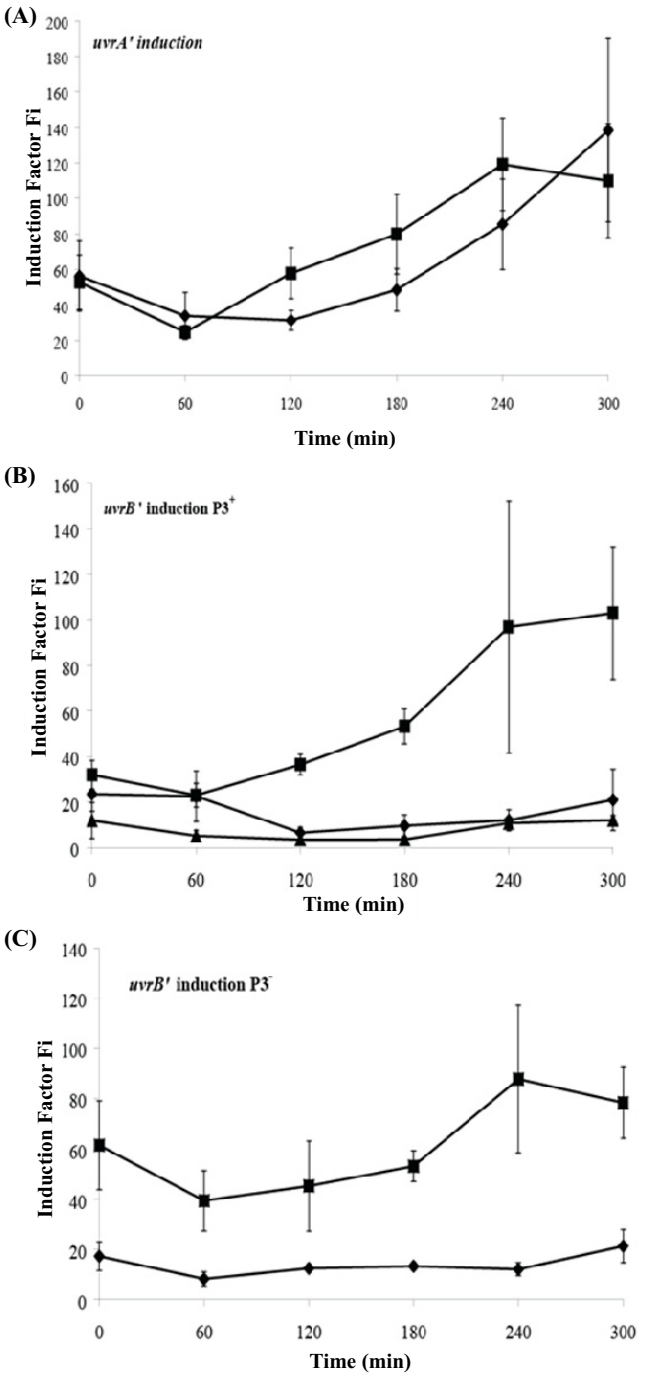

Fig. 4. Induction over time of *E. coli* carrying (A) pKSlux::EuvrA and pKSlux::VuvrA (B) pKSlux::EuvrBP3+ and pKSlux::VuvrBP3+ and (C) pKSlux::EuvrBP3- and pKS::luxVuvrBP3-, comparing the rate of induction between species following exposure to a high dose of UV irradiation 48 J/M2. *E. coli* (■) and *V. natriegens* (◆). Values presented are means±SE, $n=3$.

recovery from initial UV exposure. In contrast, the rate of *luxAB* induction derived from the *E. coli uvrB* promoter was significantly higher than that obtained for the *V. natriegens uvrB* promoter. This disparity was evident after approximately 60 minutes and continued for the duration of the assay.

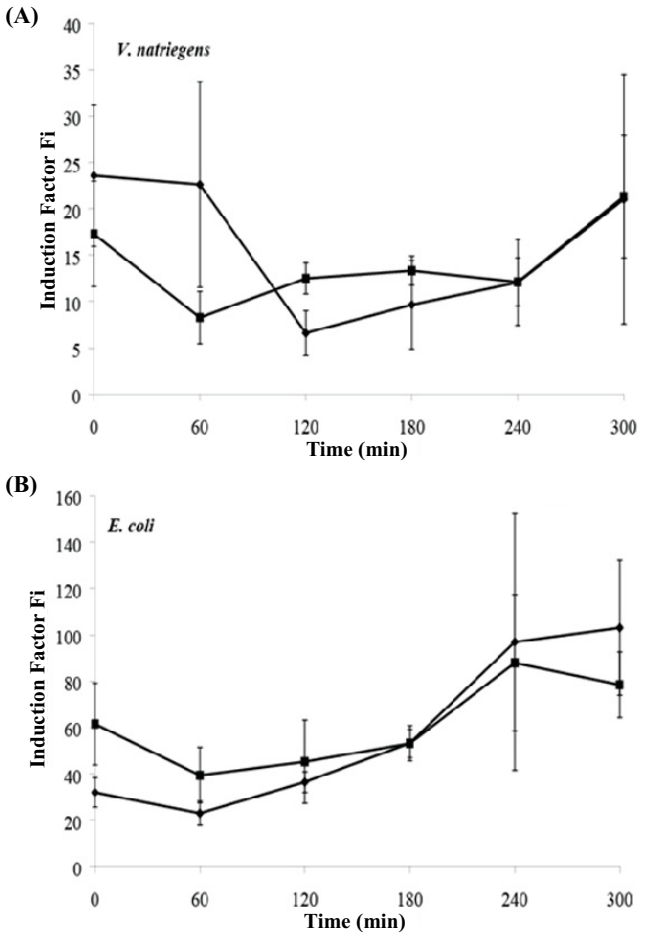

Fig. 5. Induction over time of *E. coli* carrying (A) pKSlux::VuvrBP3+ (◆) and pKS::luxVuvrBP3- (■), and (B) pKSlux::EuvrBP3+ (◆) and pKS::luxVuvrBP3- (■), comparing rate of uvrB induction in the presence and absence of P3.

Effect of P3 region of *uvrB* upon rate of induction

Constructs containing the *E. coli uvrB* 5′ regulatory region were found to be stable for the duration of the assay, irrelevant of the presence or absence of the P3 region (data not shown). In contrast, a plasmid containing the *V. natriegens uvrB* 5′ region was found to be unstable when the P3 element was present, and yet stable when P3 was absent (data not shown). As a consequence, SLU values derived from cells transformed with plasmids containing P3 were plotted relative to total viable cells [as determined by cells counts on NA plates (Fig. 3E)], and also relative to total viable cells containing the plasmid [as determined by cells counts on NA+Amp plates (Fig. 3E)]. Despite accounting for loss of plasmids containing the *V. natriegens* P3 element, a significant difference was still observed between luminescence obtained when the *luxAB* genes are driven by the *E. coli uvrB* promoter containing the P3 element, compared to the same region from *V. natriegens uvrB* (compare Fig. 4B with Fig. 4C). When comparing within species, the presence or absence of *uvrB* P3 element did not impact upon the rate of reporter gene induction (Figs. 5A and B).

Discussion

The ability of organisms to maintain genomic integrity is important for survival and is reflected in the diversity of DNA repair pathways. UvrA and UvrB are two of the central proteins involved in damage recognition and excision processes in NER, so it is not surprising that both *uvrA* and *uvrB* gene homologues were identified within the *V. natriegens* genome. *V. natriegens* is also known for its rapid replication rate and as such, its DNA repair capacities are of interest (Aiyar and Gourse, 2002). One would assume that an elevated rate of DNA replication would require an elevated rate of DNA repair to ensure the genomic integrity is maintained. Until now however, no attempt had been made to identify and study the regulation of *uvrA* and *uvrB* genes from this organism. The discovery therefore of these two genes in *V. natriegens* and how they are regulated has the potential to address these questions and thus enhance our understanding of prokaryotic DNA repair. In addition, the study of promoter regions of *uvrA* and *uvrB* from *V. natriegens* and also *E. coli* will contribute to the construction of a marine-based reporter plasmid for use as a bio-sensor.

Analysis of *V. natriegens uvrA* and *uvrB* gene sequences identified a transcription initiation site, Shine Dalgarno sequence (RBS), a putative SOS binding region (SOS Box) and putative promoter regions for each gene. A DnaA box overlapping the P3 region in *V. natriegens* was also identified. Each gene possessed a readily identifiable translational initiation site (Kozak, 1999). In *E. coli*, the ribosomal binding site or Shine-Dalgarno sequence is located within close proximity (10 bp) to the translational start site. The Shine-Dalgarno consensus sequence is a 2-6 bp element composed of the following nucleotides, GGAGG. Such an element (GAG) was located 7 bp 5′ from the start codon of the *V. natriegens uvrA* gene. The *V. natriegens uvrB* Shine-Dalgarno sequence (GGA) was located 5 bp 5′ to the start codon. Thus, *uvrA* and *uvrB* from *V. natriegens* have all the identifiable hallmarks of NER genes described from other species.

Comparison of the *V. natriegens* UvrA amino acid sequence to other prokaryotic UvrA proteins revealed similarities between 71-97% and identities between 56-96%. A phylogram based on the UvrA and UvrB protein sequences of *V. natriegens* and other *Vibrio* species and other bacterial species (Fig. 4) is consistent with that obtained for other protein and gene sequences (Thompson *et al*., 1998). In addition to the high homology between *V. natriegens* UvrA protein and other prokaryotic UvrA protein sequences, similarities were found between important functional domains defined by mutagenesis studies of *E. coli uvrA*. Complete conservation was found for all of the functional domains of the *V. natriegens* UvrA protein including; two ATP binding cassette (ABC ATPase domains) that include the Walker A, Walker B, and leading sequence motifs (LSGG). Two zinc finger domains (CXxCX20Cx2C), along with a Q-loop and His-loop were also found to be completely conserved (Myles and Sancar, 1991; Truglio *et al*., 2006; Linton, 2007). It is therefore not surprising that UV sensitivity and mutation reversion assay data showed that the *V. natriegens* UvrA protein can fully complement the repair defect in an *E. coli uvrA* mutant strain, AB1886. The *V. natriegens* UvrA protein restored a low mutation rate and UV resistant phenotype comparable to AB1157 (wild type in terms of DNA repair). The data indicates therefore that the *V. natriegens* UvrA protein can detect DNA damage within the *E. coli* genome and subsequently load *E. coli* UvrB onto the site of damage. Other studies assessing novel UvrA proteins in an enteric background (*E. coli*) showed *Deinococus radiodurains* UvrA was able to complement an *E. coli uvrA* mutant (Agostini *et al*., 1996).

Comparison of *V. natriegens* UvrB protein sequence to other prokaryotic UvrB sequences revealed between 50-97% identity and 70-98% similarity. An ATP/GTP nucleotide binding site (Walker box A) at amino acids 39-46 (same location as *E. coli* UvrB) was identified (Sancar and Tang, 1993). Secondly, six motifs, with helicase activities, present in a super-family of proteins (including *E. coli* UvrB) were found in *V. natriegens* UvrB (Gorbalenya *et al*., 1989). Compared to *E. coli* UvrB, all of the helicase motifs were highly conserved. Helicase motifs I, Ia, and V shared 100% identify to *E. coli* UvrB. Helicase motifs II, III, IV, and VI shared an 80-97% identity to *E. coli* UvrB. Studies on *E. coli* UvrB proteins containing point mutations have identified specific amino acid residues that are essential for UvrB function. Ten such residues are E98, E256, D337, E338, F365, F496, G509, D510, E514, and R544 (Lin and Sancar, 1992; Moolenaar *et al*., 1994; Hsu *et al*., 1995). All of these residues are conserved in position and identity in the *V. natriegens* UvrB protein. The only deviation was E265 in *E. coli* which in *V. natriegens* UvrB is located one residue towards the c-termini (E266). In addition to these regions, the ß hair pin motif, essential for DNA binding and damage processing in *E. coli* UvrB was also identified for *V. natriegens* UvrB and shared an 88% identity (Skorvaga *et al.*, 2002). The location *V. natriegens* UvrB ß hair pin motif was identical *E. coli* UvrB ß hair pin motif, residues 96 to 111. The important functional domains of the *E. coli* UvrB were also found in the predicted *V. natriegens* UvrB protein. However, UV sensitivity and mutation reversion assay data showed that the *V. natriegens* UvrB protein, under the regulation of its native promoters (P2 and P1) failed to fully complement the repair defect in an *E. coli uvrB* mutant strain, AB1885. There are several possible causes for this, including the inefficiency of gene regulation by *V. natriegens* promoter elements when expressed in *E. coli*, and/or the inadequacy of the *V. natriegens* UvrB protein to function with the *E. coli* machinery of the NER pathway. In an attempt to ascertain whether the problem was promoter induction or protein function, the *V. natriegens uvrB* coding sequence was fused to the *E. coli uvrB* promoter region (P3, P2, and P1). All three promoters were used for the transcriptional fusion gene so as to best replicate *uvrB* gene induction in the wild type *E. coli* strain AB1157.

UV sensitivity and mutation reversion assay data, showed that the *V. natriegens* UvrB protein, under the regulation of *E. coli uvrB* promoters (P3, P2, and P1), was able to partially complement an *E. coli uvrB* mutant strain (AB1885). Data showed a significant difference between the *uvrB* mutant with and without the presence of $pKLSEC_{uvrB}VN_{uvrB}$. $pKLSEC_{uvrB}VN_{uvrB}$ provided some level of protection against UV sensitivity and also partially reduced the highly mutable phenotype of AB1885. This partial complementation suggests that the *V. natriegens* UvrB protein does not function fully in *E. coli*. This may involve its reduced catalytic activity on damaged

DNA or its sub-optimal interaction with either *E. coli* UvrA or UvrC or alternatively the variations detected with helicase motifs II, III, IV, and VI may be responsible for the partial restoration phenotype observed

In contrast to our study, *Pseudomonas aeruginosa* UvrB did complement an *E. coli uvrB* mutant (Rivera *et al*., 1996). There are however, key differences between *P. aeruginosa uvrB* and *V. natriegens uvrB*. No SOS box was observed in the 5′ untranslated region of the *P. aeruginosa uvrB* gene and it was not damage inducible. The *P. aeruginosa uvrB* gene was also under the regulation of only one promoter which is significantly different to the regulation of *E. coli uvrB* and the proposed promoter system for *V. natriegens*. *P. aeruginosa* UvrB also shares a higher identity to *E. coli* UvrB (84%) compared to *V. natriegens* (75%), potentially influencing the capacity of UvrB to bind with UvrA or be recognised by UvrC during NER. Thus, it could be a combination of regulatory and structural differences that lead to partial complementation.

The responses of cells containing the six reporter plasmids to UV irradiation over time revealed a similar induction pattern between species. This deviates from others studies that screen a range of mutagens at varying doses that lead to predominantly the induction of *E. coli* SOS genes (Vollmer *et al*., 1997; Norman *et al*., 2005). Here, interspecies promoter induction in an *E. coli* background is compared.

These six constructs varied according to the species from which the promoter were derived (*E. coli* or *V. natriegens*), and the gene from which the promoter sequences originated (*uvrA* or *uvrB*). Irrelevant of the promoter being tested, a significant difference between induced and un-induced cells was observed for all six reporter plasmids, indicating that DNA damage induces *uvr* genes in both species. This is consistent with the findings of Vollmer *et al.* (1997) for *E. coli uvrA* and represents the first such data for *E. coli uvrB*, *V. natriegens uvrA* and *V. natriegens uvrB*.

Further analysis of the reporter plasmids explored the rate of individual gene induction between the two species to determine the rate of gene expression. The induction factor derived from the two *uvrA*' UTRs was not significantly different in relation to rate and time of gene induction. This demonstrates that the regulation and induction of *uvrA* from *V. natriegens* is indistinguishable from its *E. coli* counterpart. This implies that the *E. coli* cellular machinery is capable of recognising the *V. natriegens uvrA'* promoter sequence elements and thus drives gene expression in a similar manner to that of the endogenous *uvrA'* gene. It can be postulated from this data, that *V. natriegens uvrA'* induction is similar to that reported for *E. coli*, in terms of levels of constitutive expression and also further up-regulation as a part of an SOS-like response.

In contrast to *uvrA*, the rate of *uvrB* induction differed between *E. coli* and *V. natriegens.* Both constructs built from the *E. coli uvrB* promoter where the P3 element was either present or absent were found to be stable for the duration of the luminescence assay. This is in contrast to the findings of van den Berg *et al.* (1985) who reported instability of plasmids containing the 5′ UTR of *E. coli uvrB* that included the P3 element. It should be noted however, that the 5′ UTR *uvrB* region used in our study did not contain any *uvrB* coding sequence, whereas the construct reported by van den Berg *et al*. (1985) included the first 15 codons of *uvrB* gene sequence. The presence of this gene coding sequence may influence plasmid stability.

Despite *E. coli uvrB* reporter constructs yielding a relatively low rate of luminescence, it's *V. natriegens uvrB* counterpart was significantly higher. It is possible, that repression of gene expression mediated by the *E. coli* LexA protein is inefficient due to poor recognition of the *V. natriegens* SOS box in the 5′ UTR. This would result in an increase in luminescence derived from the *V. natriegens uvrB* regulatory region relative to that observed for the same region of the *E. coli uvrB* gene. Whilst relative luminescence derived from the *V. natriegens uvrB'* promoter was higher than that of the same region of the *E. coli uvrB* gene, the *V. natriegens uvrB'* promoter showed no notable gene induction over time. This is compared with an approximate four fold increase in luminescence derived from the *E. coli uvrB* promoter and supported by the findings of Sancar (1987), Lin *et al.* (1997), and Crowley and Hanawalt (1998). Our data also shows no effect of the presence or absence of the P3 element in the promoter of the *V. natriegens uvrB* gene. The fact that the promoter of the *V. natriegens uvrA* gene could induce gene expression, whereas the *V. natriegens uvrB* promoter could not, further highlights the likely poor recognition of the *V. natriegens uvrB* promoter by the *E. coli* transcriptional machinery. This could only be tested by analysing the *V. natriegens uvrB* reporter plasmids in a *V. natriegens* background, and would thus require a transformation system. Transformation of *V. natriegens* is currently being investigated (Sbisa, unpublished data).

To conclude, we have isolated the *uvrA* and *uvrB* genes from *V. natriegens* and confirmed their function in DNA repair in *E. coli*. This provides strong evidence for the existence of a NER pathway in *V. natriegens* which occupies a unique environmental niche. Our results also indicate that the *uvrA* gene from *V. natriegens* is up-regulated in a DNA damage inducible manner. Whilst the rate of *uvrA* gene induction is similar between the two species, preliminary studies indicate that *V. natriegens uvrB* basal expression is higher than that of the *E. coli uvrB* gene, and yet does not appear to be DNA damage inducible. Our preliminary data on the *uvrB* regulon from *V. natriegens* reveals, therefore, some incompatibility between *Vibrio* and *E. coli* that requires further investigation. The *V. natriegens uvrA* regulon however presents a suitable candidate to develop an environmental biosensor for use in *V. natriegens.*

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