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Characterization of the *Burkholderia thailandensis* SOS Response by Using Whole-Transcriptome Shotgun Sequencing

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The bacterial SOS response is a well-characterized regulatory network encoded by most prokaryotic bacterial species and is involved in DNA repair. In addition to nucleic acid repair, the SOS response is involved in pathogenicity, stress-induced mutagenesis, and the emergence and dissemination of antibiotic resistance. Using high-throughput sequencing technology (SOLiD RNA-Seq), we analyzed the *Burkholderia thailandensis* global SOS response to the fluoroquinolone antibiotic, ciprofloxacin (CIP), and the DNA-damaging chemical, mitomycin C (MMC). We demonstrate that a *B. thailandensis* recA mutant (RU0643) is ~4-fold more sensitive to CIP in contrast to the parental strain *B. thailandensis* DW503. Our RNA-Seq results show that CIP and MMC treatment (*P < 0.01*) resulted in the differential expression of 344 genes in *B. thailandensis* and 210 genes in RU0643. Several genes associated with the SOS response were induced and include *lexA*, *uvrA*, *dnaE*, *dinB*, *recX*, and *recA*. At the genome-wide level, we found an overall decrease in gene expression, especially for genes involved in amino acid and carbohydrate transport and metabolism, following both CIP and MMC exposure. Interestingly, we observed the upregulation of several genes involved in bacterial motility and enhanced transcription of a *B. thailandensis* genomic island encoding a *Siphoviridae* bacteriophage designated phiE264. Using *B. thailandensis* plaque assays and PCR with *B. mallei* ATCC 23344 as the host, we demonstrate that CIP and MMC exposure in *B. thailandensis* DW503 induces the transcription and translation of viable bacteriophage in a RecA-dependent manner. This is the first report of the SOS response in *Burkholderia* spp. to DNA-damaging agents. We have identified both common and unique adaptive responses of *B. thailandensis* to chemical stress and DNA damage.

*B. thailandensis* is a Gram-negative motile rod commonly found in stagnant waters, soils, and rice paddies in the central and northeastern areas of Thailand (1). *B. thailandensis* is genetically and physiologically similar to *Burkholderia pseudomallei*, the etiologic agent of melioidosis (1–3). In fact, extensive chromosomal similarities exist between *B. thailandensis* and *B. pseudomallei*, with the exception of various virulence genes that are encoded by *B. pseudomallei* and absent in *B. thailandensis* (4). The most notable difference between *B. thailandensis* and *B. pseudomallei* is the ability to cause disease in animals and humans. Brett et al. reported a 50% lethal dose (LD₅₀) of <10 CFU for *B. pseudomallei* in a hamster model in contrast to an LD₅₀ of >10⁶ CFU for *B. thailandensis* (1). Likewise, the LD₅₀ in BALB/c mice for *B. thailandensis* is >10⁹ CFU compared in contrast to an ~LD₅₀ of 182 CFU for *B. pseudomallei* (5, 6).

Both Gram-negative and Gram-positive bacteria respond to environmental stresses that lead to DNA damage by the induction of the bacterial SOS response (7–9). The SOS response involves upregulation of a number of genes that are important for DNA repair and the regulation of cell division (10). The majority of the genes involved in the SOS response are negatively regulated by the LexA repressor protein, which binds to an approximately 16- to 20-mer consensus sequence upstream of the target gene (11, 12) and is known as the LexA binding site or SOS box. The SOS response is induced when DNA damage uncouples the replicative DNA polymerase from helicase, resulting in the formation of single-stranded DNA (ssDNA). RecA binds to ssDNA and becomes activated by the formation of a multimeric, nucleoprotein filament. Upon ATP binding, this nucleoprotein functions as a protease that cleaves LexA, the SOS repressor protein, which leads to depression of the genes involved in the bacterial SOS response (13).

In this investigation, we analyzed the global SOS response in *B. thailandensis* DW503 and an isogenic recA mutant (RU0643) to the fluoroquinolone antibiotic, ciprofloxacin (CIP), and the DNA-damaging chemical, mitomycin C (MMC). Using optical density readings at 600 nm (OD₆₀₀) and bacterial viability counts (plate enumerations), we demonstrate that a *B. thailandensis* recA mutant (RU0643) is substantially more sensitive to CIP and MMC. At the transcriptional level, *B. thailandensis* DW503 and RU0643 respond similarly to CIP and MMC. Numerous genes shown to be involved in the bacterial SOS response were induced after CIP and MMC exposure. Most notable was the downregulation of protein-encoding genes involved in amino acid and carbohydrate transport and metabolism, in addition to the induction of a *B. thailandensis* *Siphoviridae* prophage. These results identified both common and unique adaptive responses of *B. thailandensis* to chemical stress and DNA damage.

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TABLE 1 Bacterial strains, plasmids, and primers used in this study

<table>
<thead>
<tr>
<th>Strain, plasmid, or primer</th>
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</table>

a Km, kanamycin; Gm, gentamicin; Sm, streptomycin; Tp, trimethoprim; Ap, ampicillin. The EcoRI and BamHI sites incorporated into the oligonucleotides for directional cloning are indicated in boldface.

b In the I0643 primers, “i” indicates internal gene primers and “c” indicates conformation primers. The BTH PCR primers were used for the directional cloning of the B. thailandensis lexA CDS. The II2217 PCR primers were used for RT-PCR for RNA sequencing validation. The GI PCR primers were used for B. thailandensis prophage detection and differentiation.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains and plasmids used in this investigation are described in Table 1. B. thailandensis DW503, which was derived from B. thailandensis E264, is aminoglycoside sensitive due to a mutation in the amrA gene. B. thailandensis and Escherichia coli were cultured in Luria-Bertani (LB) broth or on LB agar at 37°C. When needed, antibiotics were added at the following concentrations: 25 μg of kanamycin (Sigma, St. Louis, MO)/ml, 50 μg of trimethoprim (Sigma)/ml. For recombinant clone screening, E. coli was grown on LB plates containing the appropriate antibiotic concentrations described above with or without 50 μg of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (Sigma)/ml using standard procedures (14).

Construction of a B. thailandensis DW503 recA mutant. For PCR primer design, an in silico search of the B. thailandensis E264 genome was performed using the JCVI Comprehensive Microbial Resource site (http://cmr.jcvi.org/tigr-scripts/CMR/CMRHomePage.cgi) to identify the recA ortholog BTH_I0643. A 419-bp internal gene fragment within BTH_I0643 was amplified with PCR using the primer pairs shown in Table 1 with B. thailandensis genomic DNA purified using a Wizard DNA purification kit (Promega, Madison, WI). PCR was performed using the following conditions: one cycle at 94°C for 5 min; 30 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s; followed by a final 7-min extension at 72°C. PCR products were analyzed using a 3% agarose gel and a shift in amplicon size of ∼4 kb indicates site specific plasmid integration and gene disruption.

CIP MIC determination for B. thailandensis DW503 and RU0643. B. thailandensis DW503 and RU0643 were grown for 18 to 24 h on an LB agar plate at 37°C. A loopful of bacteria was resuspended in 10 ml of LB broth,
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and the OD_{600} was determined using a SmartSpec 3000 (Bio-Rad, Hercules, CA). Bacterial suspensions were diluted to a concentration of 10^{6} CFU/ml (conversion factor of 5 x 10^{8} CFU/ml per OD_{600} unit). In each well of a 96-well plate, 50 µl of this bacterial suspension was added to plate wells containing LB medium with the appropriate concentrations of CIP to obtain a final bacterial concentration of ~5 x 10^{4} CFU/well. Plates were incubated for 18 to 24 h at 37°C and MICs determined both visually and turbidimetrically at OD_{600} using a Tecan Infinite M1000 plate reader (Tecan, San Jose, CA). All MICs were determined in triplicate, and the MIC was defined as the lowest concentration of CIP that prevented any detectable growth. *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *E. coli* ATCC 25922 were used as quality control strains to ensure antibiotic efficacy.

**Bacterial viability and growth rate determination after CIP and MMC exposure.** A single colony of *B. thailandensis* DW503 or RU0643 was used to inoculate 20-ml cultures of LB broth that were cultured at 37°C for 18 to 20 h with aeration. To 20 ml of prewarmed LB broth, 1 ml of the overnight culture was added, followed by incubation until the cultures reached the mid-log phase (OD_{600} ~ 0.5). Either CIP (6.5 µg/ml) or MMC (4 µg/ml) was added to the bacterial cultures at 10 X the MIC, and the OD_{600} and cell viability were monitored at 30, 60, and 120 min. To determine cell viability, serial dilutions of each culture were prepared, and aliquots were plated onto LB agar plates, followed by incubation at 37°C for 18 to 20 h.

**Complementation of the recA mutation in RU0643.** To restore the SOS response in RU0643, the *B. thailandensis* DW503 recA coding DNA sequence (CDS) was directionally cloned into the broad-host-range plasmid pBHR1 (16). The recA gene was PCR amplified as described above using the primer pairs listed in Table 1. The forward primer contained an EcoRI restriction endonuclease site, while the reverse primer contained a BamHI site. PCRs were performed as described above using a 1-min extension time. PCR products were purified using a QIAquick nucleotide removal kit (Qiagen), digested with EcoRI and BamHI (New England BioLabs), and subcloned into similarly digested pBHR1. Ligations were electroporated into *E. coli* SM10 and mobilized into RU0643 according to previously described methods (15).

**CIP and MMC exposures.** *B. thailandensis* DW503 and RU0643 were cultured as described above for the bacterial viability and growth rate determination methods. At mid-log phase (OD_{600} ~ 0.5), CIP (6.5 µg/ml) or MMC (4 µg/ml) was added to the culture at concentrations equivalent to 10 X the MIC. After chemical treatment for 2 h, 5 ml of each bacterial culture was centrifuged, and cell pellets were resuspended in 1 ml of TRIzol reagent (Invitrogen), followed by total RNA purification as described by the manufacturer. Biological replicates were repeated three independent times on separate days, and RNA was pooled for sequencing as previously described (17, 18). Total RNA was processed and sequenced according to the methods described below by Cofactor Genomics (St. Louis, MO).

**Enrichment of RNA-free transcriptome RNA and RNA fragmentation.** Whole transcriptome RNA was enriched from 5 to 10 µg of total RNA by the removal of 23S and 16S rRNA using a RiboZero kit (Epicentre Biotechnologies) according to the manufacturer’s instructions. A SOLID Total RNA-Seq kit (Foster City, CA) was used to construct transcriptome RNA libraries according to the manufacturer’s instructions.

**cDNA synthesis.** 20 µl of reverse transcription master mix containing 13 µl of nuclease-free water, 4 µl of 10× RT buffer, 2 µl of 2.5 mM dNTP mix, and 1 µl of ArrayScript reverse transcriptase was added to the ligation reaction, followed by incubation at 42°C for 30 min. Synthesized cDNA was purified with a Qiagen MinElute PCR purification kit and eluted in 10 µl of EB buffer (10 mM Tris-HCl, pH 8.5). The purified cDNA was run on a Novex 6% Tris-borate-EDTA–urea gel (Invitrogen) for size selection. cDNA was excised from the gel at a size of 150 to 250 nucleotides and divided into four pieces, and two in-gel PCRs were performed to obtain enough material for subsequent emulsion PCR (ePCR). During this PCR, each library was barcoded using PCR primers containing different barcodes to allow for multiplex sequencing.

**ePCR and sequencing.** ePCR and emulsion break analyses were performed according to the Applied Biosystems SOLID 4 system templated bead preparation guide. The amplified beads were first run on a workflow analysis (WFA) slide to determine the quality and quantity of beads, which was followed by sequencing runs performed according to the Applied Biosystems SOLID 4 system instrument operation guide.

**Sequence alignments, clustering, and normalization.** Novoalign (version v1.01.13) was used for all sequence alignments with the parameters “-o SAM -r all -l 30 -t 140 -e 10” (detailed aligner algorithm specifics can be obtained from the Novocraft Technologies website). Alignment data were used to generate clusters of reads (or patches) which represent nonredundant genomic regions in *B. thailandensis* E264 (http://cmr.jcvi.org/tigr-scripts/CMR/CmrHomePage.cgi). Cluster boundaries were created by taking each experimental sample into account during the cluster generation process to define the left- and right-most end coordinates of each cluster. Multiple mapping reads are neither thrown out nor counted as 1× mapping at each of their respective regions; instead, they were given a fractional association if they map to multiple genomic regions (i.e., a read that maps equally well to four genomic regions would contribute 0.25× of coverage to each location). Linear normalization was performed by multiplying each experimental sample locus coverage by the total reads of the lowest read-count sample divided by the respective samples total reads. These normalized expression data were the basis for all subsequent comparisons of the experimental groups (i.e., CIP treated versus nontreated). The comparative expression approach steps through each cluster compares every possible pairing of samples and generates a log_{2}(A/B), in which A and B are the two normalized average coverages of each sample. Each read length was ~50 bp.

**Differential gene expression analysis.** The RNA expression was summarized at the gene level by mapping the sequencing reads to the *B. thailandensis* E264 chromosomes. The association between the log_{2} fold change value (M) for each relevant comparison (i.e., CIP-exposed cultures versus nontreated control cultures) and the average signal intensity (A) was removed by fitting a Loess curve (MA normalization, limma package for R system [Bioconductor]). The *B. thailandensis* “DNA stress signature” in response to both ciprofloxacin and MMC was determined by integrating the transcriptional response in both treatment groups. Statistical significance was obtained by a moderated analysis of variance test, implemented in the limma package (Bioconductor), which tests whether the response average is significantly different from zero. A moderated t test provides the same interpretation as an ordinary t-statistic except that the standard errors have been moderated across genes by using a simple Bayesian model. To determine functional analysis of genes significantly induced or repressed following drug exposure, searches were performed using clusters of orthologous groups (COG) classification (19).

**In silico searches of the B. thailandensis genome for LexA binding sites.** Promoter sequences enclosed within the *B. thailandensis* genome were identified by using the *B. thailandensis* E264 chromosomes (chromosome one [http://www.ncbi.nlm.nih.gov/nuccore/NC_007651.1] and chromosome two [http://www.ncbi.nlm.nih.gov/nuccore/CP000085.1]) with either the Gram-negative bacteria consensus LexA binding motif (CTGT-N8-ACAG) (20) or the *P. aeruginosa* CTGTAATAATACAG (100% conserved residues are represented in boldface) LexA box as the query sequence. Intergenic regions shorter than 50 bases were evaluated for operon structure using the following criteria: (i) flanking genes should be coded in the same orientation, (ii) flanking gene expression levels should be significantly correlated (P < 0.05 [Pearson correlation]), and (iii) 400 bases upstream of the start codon was considered the putative promoter region.

**RT-PCR RNA sequencing validation.** Template RNA used for reverse transcription-PCR (RT-PCR) was obtained using the methods described above for RNA sequencing. SOLID RNA sequencing results were validated using RT-PCR with the primer pairs listed in Table 1. RT-PCR was
performed using the Access RT-PCR System (Promega) as follows: RT of RNA was performed at 45°C for 45 min, followed by PCR amplification using 1 cycle at 95°C for 2 min; 30 cycles at 95°C for 30 s, 56°C for 30 s, and 72°C for 30 s; followed by a final extension at 72°C for 5 min. PCR products were resolved on a 0.8% agarose gel using standard methods. A representative gel to validate our RNA-Seq results is depicted in Fig. S2 in the supplemental material.

**Bacteriophage production and differentiation.** Bacteriophage biosynthesis by *B. thailandensis* DW503 and RU0643 were monitored using *B. mallei* ATCC 23344 as the host strain as previously described (19, 20). Briefly, 3 ml of *B. thailandensis* DW503 and RU0643 were grown overnight in LB broth at 37°C with shaking (250 rpm). Antibiotic selection was maintained in RU0643 during all bacterial propagation. Approximately 2 ml of each overnight culture was added to 60 ml of prewarmed LB broth, and cultures were grown to an OD<sub>600</sub> of ~0.5 as described above. CIP (6.5 μg/ml) or MMC (4 μg/ml) was added to the appropriate culture at concentrations equivalent to 10× the MIC. Cultures were divided into 20-ml aliquots and grown for an additional 2 h as described above. Each culture (2 ml) was centrifuged, and the supernatant was filtered using Millex HV 0.45-μm-pore-size filters (Millipore, Billerica, MA). Filtered supernatants were serially diluted in suspension medium (SM) (21), and 100 μl of the bacteriophage filtrate and 100 μl of a saturated *B. mallei* ATCC 23344 culture were mixed. The phage and bacterial suspension was incubated at 25°C for 20 min and then added to 4.8 ml of molten LB top agar (0.7%) containing 4% glycerol, and the PFU count was determined after 24 h at 37°C.

For bacteriophage differentiation, we used plaque PCR with primers designed to amplify an internal gene amplicon within BTH_I0115 (GI1), BTH_II1031 (GI12) (Table 1). A total of 10 plaques, all turbid and ca. 1 to 1.5 mm in size, from the *B. thailandensis* DW503 MMC-treated cultures were picked, and bacteriophages were resolved on a 0.8% agarose gel using standard methods. A representative gel to validate our RNA-Seq results is depicted in Fig. S2 in the supplemental material.

**RESULTS AND DISCUSSION**

**Bacterial treatment with MMC and CIP.** To determine whether the *B. thailandensis* SOS response is functional, we compared the sensitivity of the *B. thailandensis* DW503 and the SOS-defective *recA* mutant RU0643 to either CIP or MMC. The MIC values for CIP against *B. thailandensis* DW503 and RU0643 were 2.5 and 0.6 μg/ml, respectively, indicating the *B. thailandensis* SOS regulon is involved in the response to DNA damage produced by CIP (see Fig. S1 in the supplemental material). The growth and killing kinetics of cultures of *B. thailandensis* DW503 and RU0643 exposed to 10× the MIC of MMC and CIP were monitored by measuring the OD<sub>600</sub> and bacterial CFU at 30, 60, and 120 min (Fig. 1). Untreated-culture densities for both *B. thailandensis* DW503 and RU0643 showed a time-dependent increase in bacterial growth over the 2-h exposure period (Fig. 1A). No significant effect on growth in the absence of MMC or CIP was observed for RU0643 compared to *B. thailandensis* DW503 (Fig. 1A). In contrast, a notable reduction in cell density was observed for RU0643 after MMC or CIP treatment, in particular at the 120-min time point (Fig. 1A). Likewise, a decrease in the OD<sub>600</sub> for *B. thailandensis* DW503 in response to both MMC and CIP was observed but was not as pronounced as the response of RU0643, which demonstrates that disruption of the *B. thailandensis* SOS network enhances MMC and CIP sensitivity (Fig. 1A). As expected, analyses of the killing kinetics in the presence of MMC and CIP demonstrated that both compounds reduced the CFU/ml over the 2-h exposure period for both *B. thailandensis* strains characterized (Fig. 1B). Most notable, CIP and MMC treatment of RU0643 resulted in a ~10-fold decrease in CFU/ml compared to *B. thailandensis* DW503 (Fig. 1B). Similar findings in response to CIP exposure have been reported for *E. coli* in a ΔrecA background, while a *P. aeruginosa* PA01 ΔlexA mutant displayed no sensitivity to either CIP or MMC (22, 23). These results demonstrate that the *B. thailandensis* SOS response plays a role in DNA repair and responds to CIP and MMC treatment in a manner similar to that reported for *E. coli*.

**MMC and CIP induce the SOS response in *B. thailandensis* DW503 and RU0643.** To analyze the transcriptional response of *B. thailandensis* DW503 and RU0643 to DNA-damaging agents, we utilized SOLID RNA sequencing to profile the gene expression changes in mid-log-phase cultures of *B. thailandensis* DW503 and RU0643 with or without MMC and CIP treatment. It has been demonstrated with *P. aeruginosa* that over a 30- to 120-min CIP exposure, only the magnitude of gene induction or repression changes are different over the time course and, as a result, our transcriptional profiling analysis only focused on the 120-min time point (23). Using Gene Set Enrichment Analysis (GSEA) (24), we found that the *B. thailandensis* global transcriptional re-
spontaneous to MMC and CIP was very similar. To identify the RecA-
dependent DNA-stress signature, we identified the genes that are
differentially transcribed in response to DNA damage by combin-
ing the CIP and MMC transcriptional responses obtained from *B.
thailandensis*. A total of 344 genes were differentially transcribed
in *B. thailandensis* in response to MMC and CIP (Fig. 1A). To identi-
fy the RecA-regulated genes, we observed differential transcription in several genes
known to be involved in the bacterial stress response to DNA damage (Table 2). Both CIP and MMC induced similar SOS related genes in *B. thailandensis* in a RecA-dependent manner, with the most
upregulated alleles (ca. 2- to 4-fold) being involved in DNA repair
including *lexA* (SOS repressor protein), *uvrA* (excinuclease ABC
subunit A), *dnaE* (DNA polymerase III, alpha subunit), *dinB* (DNA polymerase IV which is an SOS error-prone polymerase), *recX* (regulator of RecA), and *recA* (DNA recombination and
repair protein) (Table 2). None of the SOS-associated genes de-
scribed above were significantly differentially transcribed in RU0643, which suggests a RecA-dependent induction following DNA damage (Table 2). As expected, these findings demonstrate that both CIP and MMC can induce an SOS-like response in *B.
thailandensis*. However, the number of SOS-regulated genes that are induced or repressed by CIP and MMC in *B. thailandensis* is not as high as what has been reported for *E. coli*, *Pseudomonas* spp., and *S. aureus* (7, 8, 23, 25–29). For example, in *S. aureus*, CIP induces the expression of numerous protein-encoding genes in-
deed in DNA metabolism, including *lexA* and *recA*, in addition to
*uvrA*, *uvrB*, *parE*, *parC*, *recF*, *gyrA*, and *gyrB* (29). Interestingly,
in *S. aureus*, the expression of numerous DNA repair genes in-
cluding *recX*, *recN*, *xerC*, *xerD*, and *rnuB* were either downregu-
lated or unchanged in response to CIP treatment (29). In contrast, CIP exposure of *P. aeruginosa* caused the repression of *dnaA*, *dnaN*, *recF*, and *gyrB*, and *rnuB* in addition to the downregulation of the *recG* and *rnuABC* genes, which have been shown to be involved in repairing DNA damage caused by CIP (23, 30, 31). However, and similar to our findings, the latter study did report a notable induc-
tion of *recA*, *recX*, *recN*, and a *dinB*-like *Y*-family polymerase (23).

**TABLE 2** *B. thailandensis* DW503 and RU0643 differentially transcribed SOS genes after a 120-min exposure to CIP or MMC

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<tr>
<td>BTH_110075</td>
<td>Excinuclease ABC subunit A</td>
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<sup>a</sup> That is, the fold change in *B. thailandensis* DW503 and RU0643 compared to untreated controls at 120 min after CIP and MMC treatment.

Similar findings have also been reported for *Pasteurella multocida*, in which microarray analysis showed that enrofloxacin, trim-
theprim, brodimoprim, and ceftuzine did not alter the gene expression profiles of their targets (32). However, our in vitro
findings using *B. thailandensis* DW503 and RU0643 CIP- and MMC-treated cultures did identify the upregulation of several *Betaproteobacteria* core SOS genes reported by Sanchez-Alberola et al. (33). The latter study utilized in silico methods to predict the SOS regulon in several *Betaproteobacteria* species encoding multiple chromosome genomes, including numerous *Burkholderia* spp. (33). Taken together, our results suggest that different signal types (i.e., UV versus CIP) may induce different gene sets in *B.
thailandensis* following DNA damage. Further, and although unclear, the *B. thailandensis* SOS response to DNA damage is unique compared to other reported SOS regulons and is likely a result of the niche in which this bacteria is adapted to survive.

**Common DT gene profiles of *B. thailandensis* DW503 and RU0643 following CIP and MMC treatment and comparison to other SOS regulons.** Through different mechanisms of induction, both CIP and MIC cause DNA damage using unique pathways. We observed both common and strain specific differential transcription patterns for *B. thailandensis* DW503 and RU0643 following chemical treatment. In *B. thailandensis* DW503, both CIP and MIC caused a repression of several open reading frames (ORFs; 17 common genes) encoding for proteins involved in amino acid and carbohydrate (maltose and ribose) transport and metabolism, primarily amino acid transporters and permeases (Table 3). A majority of these common differentially transcribed genes are located within a cluster of ORFs that encode prophage-like proteins (Table 3). Several hypothetical proteins were induced, along with genes involved in DNA replication, recombination, and repair (i.e., excinuclease ABC subunit A) (Table 3).

In RU0643, CIP and MIC treatment resulted in the downregu-
lation of 19 common genes that are involved in various cellular
processes, mainly genes involved in secondary metabolite biosyn-
thesis, transport, and catabolism (Fig. 2C). A majority of the common CIP and MMC repressed genes in RU0643 encode for hypo-
thetical proteins that have undetermined function(s) (Fig. 2C and Table 4). CIP and MIC exposure also induced 19 ORFs in RU0643 and, as observed for the downregulated genes, a majority of the

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**TABLE 3** Common genes DT by *B. thailandensis* in response to CIP and MMC

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Gene description</th>
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<th>COG&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>16.47 13.60 –</td>
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</table>

(Continued on following page)
induced genes encode for hypothetical proteins (Table 4). However, several upregulated genes involved in amino acid transport and metabolism, in addition to energy production and conversion, were identified as being induced (Table 4).

As observed for *P. aeruginosa* and *S. aureus*, the overall global response of *B. thailandensis* DW503 and RU0643 to CIP and MMC, was the downregulation of genes involved in essentially every aspect of cellular homeostasis (23, 29). Unlike the findings for *P. aeruginosa* and *S. aureus*, we observed no significant downregulation of *B. thailandensis* genes encoding proteins involved in general metabolism (i.e., ATP synthases and NADH dehydrogenases), DNA metabolism (*ruvA-C*), cell wall biosynthesis, cell division, efflux, and motility (Table 3; also see Table S1 in the supplemental material) (23). In *B. thailandensis* the most notable genes that are repressed in response to both CIP and MMC are amino acid transporters and permeases (Table 3 and Table S1 in the supplemental material). Oligopeptide transport systems (ABC) are essential for the import and export of various compounds, in particular peptide uptake and drug extrusion, and are linked to ATP hydrolysis (34–36). The exact roles of these *B. thailandensis* ABC transport systems have not been characterized, but our findings suggest that CIP and MMC exposure represses nutrient and/or substrate uptake in *B. thailandensis*, which is a plausible survival strategy to environmental stress.

Cirz et al. reported the downregulation of 41 genes required for *P. aeruginosa* motility in response to subinhibitory concentrations of CIP (23). In *B. thailandensis* DW503, our findings demonstrate that CIP specifically induces the upregulation of several genes involved in bacterial motility. The motility-associated protein-encoding genes that were induced are *fliA* (flagellar biosynthesis protein), *fliC* (major structural protein component of flagellin), *fliD* (flagellar hook-associated protein), *flhA* (flagellum-specific ATP synthase), *flG* (flagellar motor switch protein G), *fliF* (flagellar MS-ring protein), *fliD* (flagellar basal body rod modification protein), and *fliN* (flagellar motor switch protein) (see Table S1 in the supplemental material). Interestingly, bacterial motility, in particular swarming motility which requires flagella, has been directly linked to increased antibiotic resistance in both Gram-negative and -positive bacteria (37–39). Lai et al. reported that migrating swarming cells (planktonic cells) of *B. thailandensis* displayed enhanced resistance to the 10 antibiotics tested in their study, with CIP being one of the experimental compounds tested (39). Although the *B. thailandensis* strain used in this investigation is sensitive to CIP, it is conceivable that the upregulation of genes encoding flagellum components is a defensive mechanism that enables the bacteria to “escape” to an environment that contains lower concentrations of the inhibitory compound.

**FIG 2** Venn diagram of common and DT genes in *B. thailandensis* DW503 and RU0643 in response to CIP and MMC. (A) Genes repressed in *B. thailandensis* DW503; (B) genes upregulated in *B. thailandensis* DW503; (C) genes downregulated in RU0643; (D) induced genes in RU0643.

**TABLE 3 (Continued)**

<table>
<thead>
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<th>MMC</th>
<th>COG b</th>
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* Fold reduction in gene expression relative to untreated bacterial cultures; ↑, fold induction in gene expression relative to untreated bacterial cultures. CIP, ciprofloxacin; MMC, mitomycin C.

b COG groups: E, amino acid transport and metabolism; C, energy production and conversion; F, nucleotide transport and metabolism; G, carbohydrate transport and metabolism; I, lipid transport and metabolism; K, transcription; L, replication, recombination, and repair; M, cell wall/membrane biogenesis; N, cell motility; O, posttranslational modification, protein turnover, chaperones; P, inorganic ion transport and metabolism; Q, secondary metabolites biosynthesis, transport, and catabolism; R, general function prediction only; S, function unknown; T, signal transduction mechanisms. –, not in COGs.
CIP and MMC induce the transcription and biosynthesis of *Burkholderia thailandensis* prophage PI E264-3. It has been well documented in the literature that DNA damage and induction of the SOS response correlates with the lytic bacteriophage cycle (26, 40–43). *B. thailandensis* E264 encodes three prophage-like islands designated PI E264-1, PI E264-2, and PI E264-3 (44). Both CIP and MMC caused the induction of *B. thailandensis* DW503 putative *Siphoviridae* prophage PI E264-3 genes which are encoded on genomic island 12 (Table 5) (44). We refer to PI E264-3 as E264 throughout this study. E264 is a 52-Mb prophage that is located on *B. thailandensis* genome II and is encoded within ORFs BTH_I1011 to BTH_I1070 (44). A majority of the CIP- and MMC-induced phage genes are clustered in close proximity to each other on the chromosome, which suggests that their transcription could possibly result in the assembly of a mature phage.

To confirm our RNA-Seq results, we designed PCR primers for PI E264-1, PI E264-2, and PI E264 and performed plaque PCR for virus identification and differentiation. A total of 10 randomly

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*↓*, fold reduction in gene expression relative to untreated bacterial cultures; *↑*, fold induction in gene expression relative to untreated bacterial cultures. CIP, ciprofloxacin; MMC, mitomycin C.

*COG groups: C, energy production and conversion; E, amino acid transport and metabolism; G, carbohydrate transport and metabolism; H, coenzyme transport and metabolism; N, cell motility; P, inorganic ion transport and metabolism; Q, secondary metabolites biosynthesis, transport, and catabolism; R, general function prediction only; T, signal transduction mechanisms. –, not in COGs.*
### TABLE 5 DT *B. thailandensis* prophage E264-3 genes following CIP and MMC treatment

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<tr>
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<th>Description</th>
<th>Fold change&lt;sup&gt;a&lt;/sup&gt;</th>
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<sup>a</sup> That is, the fold change compared to untreated bacterial samples. CIP, ciprofloxacin; MMC, mitomycin C.
picked plaques were analyzed from CIP- and MMC-treated B. thailandensis DW503 cultures, and only /H9278 E264 was detected (Fig. 3B). /H9278 E264 produces turbid plaques ~1.5 mm in size on lawns of B. mallei ATCC 23344 (data not shown). These results are consistent with the finding of Woods et al., who demonstrated that B. thailandensis E264 produces two bacteriophages specific for B. mallei ATCC 23344: one produced turbid plaques, and the other produced clear plaques (19). Interestingly, the turbid-plaque-producing bacteriophage described by Woods et al. was induced 2-fold after brief UV light exposure, suggesting DNA damage is a signal for lytic cycle conversion (19). To further couple our RNA-Seq and PCR results, we analyzed B. thailandensis DW503 culture supernatants for viable bacteriophages in both untreated and chemically exposed cells. Figure 3A shows that /H9278 E264 is spontaneously produced by B. thailandensis DW503 and RU0643 at similar concentrations (~1.3 × 10^7 PFU/ml) by both strains. In contrast, exposure of B. thailandensis DW503 and RU0643 exposed to CIP (10× MIC) resulted in a notable decrease in viable /H9278 E264 production, in particular for RU0643 (1.6 × 10^7 PFU/ml versus 2.0 × 10^7 PFU/ml) (Fig. 3A). However, our SOLiD RNA sequencing results demonstrate a clear induction in the transcription of /H9278 E264 in B. thailandensis DW503 following CIP exposure (Table 5). It is plausible that the reduction in viable phage detected in B. thailandensis culture supernatants is the result of reduced bacterial viability after CIP treatment (Fig. 1B). The most drastic reduction in PFU/ml formation by /H9278 E264, despite /H9278 E264 gene induction, was observed in RU0643 after MMC treatment (Fig. 3A). Culture supernatants of B. thailandensis DW503 contained 4.4 × 10^7 PFU/ml, whereas supernatant filtrates of RU0643 contained 1.0 × 10^3 PFU/ml of /H9278 E264/ml, 44,000-fold reductions in viable bacteriophage detection, respectively (Fig. 3A). Taken together, these results demonstrate that B. thailandensis undergoes a SOS response and suggest that /H9278 E264 is under regulated by a RecA-dependent repressor. Similar results have been reported for CIP-treated cultures of P. aeruginosa PAO1 and S. aureus 8325 in which CIP exposure resulted in the upregulation of several prophage genes (23, 29). Likewise, MMC treatment of Pseudomonas fluorescens strain DC206 resulted in the upregulation of a prophage that shares homology to the B. thailandensis lambdoid phage /H9278 E125 (25). How-

FIG 3 CIP and MMC cause the induction of prophage PI E264-3 in B. thailandensis DW503 in a RecA-dependent manner. (A) PFU/ml in mid-log-phase cultures (120 min after chemical treatment) of B. thailandensis DW503 and RU0643 treated with either CIP (6.5 μg/ml) or MMC (4 μg/ml). (B) Prophage plaque PCR (4 of 10 plaques analyzed) for phage identification and differentiation using the techniques described in Materials and Methods. Primer pairs were designed specifically for PI E264-1 (G11), PI E264-2 (G13), and PI E264-3 (G12) islands encoded by B. thailandensis E264 described in Table 1. Lanes: 1, M-PCR 100-bp Low Ladder (Sigma); 2 to 4, control PCRs performed using B. thailandensis DW503 genomic DNA; 5 to 7, template DNA obtained from plaque 1; 8 to 10, plaque DNA from prophage 2; 11 to 13, DNA from plaque 3; 14 to 16, PCR amplicons obtained using plaque 4 DNA. PCR products were separated on a 2.0% agarose gel containing ethidium bromide.
ever, and by using plaque assays and transmission electron microscopy, the latter study was not able to detect phage particles in culture supernatants after MMC exposure.

COG categories of \textit{B. thailandensis} DW503 and RU0643 DT genes in response to CIP and MMC. With the magnitude of the number of DT genes, we categorized their biological roles based on COG groups, which categorize genes based on function (45, 46). The majority of COGs identified for both strains after CIP and MMC treatment are not in a GOG category and are likely a result of the incomplete annotation of the \textit{B. thailandensis} genome (Fig. 4). The gene induction patterns for \textit{B. thailandensis} in response to CIP and MMC vary and are more pronounced for CIP (Fig. 4A and C). The most highly upregulated genes in \textit{B. thailandensis} after CIP exposure are in the energy production and conversion, carbohydrate transport and metabolism, lipid transport and metabolism, transcription, cell wall/membrane biogenesis, cell motility, inorganic ion transport and metabolism, and general function prediction groups only (Fig. 4A). Similar results were observed for RU0643, with the exception of the cell wall/membrane biogenesis COG group (Fig. 4A). As expected, we observed an overall reduction in gene expression for both strains to CIP and MMC, which suggests that \textit{B. thailandensis} reduces transcription and translation to compensate for drug exposure (Fig. 4C and D).

**In silico analysis of the \textit{B. thailandensis} genome for LexA binding boxes.** To complement our RNA sequencing results and to identify additional genes potentially involved in the SOS response, we searched the \textit{B. thailandensis} genome for LexA binding sites using the Gram-negative bacterium consensus LexA binding motif (CTGT-N8-ACAG) (47) or the \textit{P. aeruginosa} motif CTGTA TAAATACAG (100% conserved residues are represented in boldface) LexA consensus sequence (23). Our search was limited to 400 bp upstream from putative start codons, and a total of 5,265 promoters were identified and searched for LexA binding sites. Using a 75% stringency analysis with the \textit{P. aeruginosa} SOS binding site, we identified 22 putative LexA binding motifs within the \textit{B. thailandensis} genome (Table 6). Similar results were obtained when the Gram-negative bacterial LexA binding consensus motif was used as the query search (data not shown). We next performed a reverse search that entailed looking for putative LexA binding sites across the whole \textit{B. thailandensis} genome and then testing whether the identified promoters are enriched among the genes upregulated in response to CIP and MMC in a RecA-dependent fashion. The \textit{P. aeruginosa} and Gram-negative SOS consensus boxes were combined, and a genome-wide search of the \textit{B. thailandensis} putative promoters was performed. We identified 11 genes whose promoters contain LexA binding sites that were upregulated in response to either CIP or MMC (Table 6). As expected, several well-characterized genes involved in the SOS response containing LexA binding motifs were identified, including \textit{dinB} (DNA polymerase IV), \textit{recA} (DNA recombination and repair), and \textit{dnaE} (replicative DNA polymerase) (Table 6), thus demonstrating that our \textit{in silico} search methods correlate with our \textit{in vitro} methods for gene transcription profiling. The \textit{B. thailandensis} conserved SOS box logo is depicted in Fig. 5. However,
LexA binding sites were also identified in promoters upstream from genes with no known role in DNA repair or recombination (i.e., tRNA-Ala) (Table 6). The presence of an SOS box within the promoter region of a gene does not necessarily mean the allele is regulated by LexA or involved in the bacterial SOS response.

**Conclusion.** We characterized here the transcriptome response of *B. thailandensis* and a recA mutant during CIP and MMC exposure. The two compounds had similar effects on both cellular and metabolic pathways. We observed an overall downward trend in gene expression, in particular amino acid transporters and permeases. As anticipated, the *B. thailandensis* SOS response included dinB, dnaE, lexA, uvrA, and recA, which have been identified and characterized in the SOS pathways in other bacteria. Interestingly, we also observed the upregulation of protein encoding genes that are required for flagellum biosynthesis and 6E264 production. Using plaque assays in parallel with PCR, we showed that both CIP and MMC induce the production of viable phage particles in *B. thailandensis* DW503 culture supernatants in a RecA-dependent manner. The core *B. thailandensis* SOS regulon is not as large as that reported for *Bacillus*, *E. coli*, or *Pseudomonas* spp. and is likely the result of the compounds utilized for SOS induction and the environmental niches that each bacterial species occupies. Our findings provide the first comprehensive report on the characterization of a *Burkholderia* SOS regulon and lay the framework for deciphering the global effect of CIP and MMC on *B. thailandensis*. Given the genomic similarity and drug resistance and sensitivity profiles between *B. thailandensis* and *B. pseudomallei*, our findings will facilitate rational drug design for treating disease(s) caused by infectious *Burkholderia* spp.

**ACKNOWLEDGMENTS**

We thank Mariano Alvarez and Jennifer Ojeda for assistance with the RNA-Seq and RT-PCR analysis. This study was funded by the Department of Defense Chemical Biological Defense Program through the Defense Threat Reduction Agency (JSTO-CBM.THERB.02.11.RD.041 [to R.G.P.]). The opinions, interpretations, conclusions, and recommendations ex-

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**TABLE 6** Potential LexA binding boxes in the *B. thailandensis* genome identified *in silico* and DT *B. thailandensis* genes in response to either CIP or MMC found in this study

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* Additional genes detected in this study following DNA damage.

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**FIG 5** *B. thailandensis* LexA binding box logo. A consensus LexA binding sequence logo obtained from an unbiased search of the *B. thailandensis* E264 chromosomes.
pressed here are those of the authors and are not necessarily endorsed by the U.S. Army.

REFERENCES


