ImuB and ImuC contribute to UV-induced mutagenesis as part of the SOS regulon in *Pseudomonas aeruginosa*

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ABSTRACT

DNA damage-induced mutagenesis is a process governed by the SOS system that requires the activity of specialized DNA polymerases. These polymerases, which are devoid of proof-reading activity, serve to increase the probability of survival under stressful conditions in exchange for an error-prone DNA synthesis. As an opportunistic pathogen of humans, *Pseudomonas aeruginosa* employs adaptive responses that originally evolved for survival in many diverse and often stressful environmental conditions, where the action of error-prone DNA polymerases may be crucial. In this study we have investigated the role of the polymerases ImuC and ImuB in *P. aeruginosa* DNA-damage induced mutagenesis. UV irradiation of *imuB*- and *imuC*-deletion mutants showed that both genes contribute to UV-induced mutagenesis in this bacterium. Furthermore, we confirmed that UV treatment significantly increase the expression levels of the *imuB* and *imuC* genes and that they are co-transcribed as a single transcriptional unit under the control of LexA as part of the SOS regulon in *P. aeruginosa*.

INTRODUCTION

DNA damage-induced mutagenesis is, in a major extent, an active process that requires specialized DNA polymerases able to perform translesion synthesis (TLS). These DNA polymerases, which belong to the Y family of DNA polymerases and to a subfamily of the C family, diverge from the typical replicative DNA Pols I and III in their low fidelity and processivity due to the lack of 3'→5' proof-reading exonuclease activity (Goodman and Woodgate, 2013). In bacteria, the expression of these specialized polymerases is usually governed by the SOS system, a stress-inducible response that is activated when cells need to be rescued from severe DNA damage (Goodman and Woodgate, 2013). Moreover, it has been reported that the induction of these polymerases increase the probability of survival under stressful conditions in exchange for an error-prone DNA synthesis, which bestowed them the name of "mutagenic polymerases".

Pols IV and V are the best-known bacterial Y family-DNA polymerases and they had been well characterized in *Escherichia coli* (Tang et al. 1999; Wagner et al. 1999; Fuchs et al. 2004). These proteins are encoded by genes *dinB* and *umuDC*, respectively, and while *dinB* and its homologs have been identified in all three domains of life, the *umuDC* genes are confined to a group of prokaryotes (Erill et al. 2006; McHenry, 2011). Many bacterial species where Pol V is absent, contain a SOS-responsive cassette composed of four genes, *lexA2-imuA-imuB-imuC*, which is broadly distributed among Proteobacteria, frequently in the form of incomplete versions containing two or three of its genic components (Erill et al. 2006; McHenry, 2011). The first gene, *lexA2*, corresponds to a second copy of the SOS-repressor *lexA* and the second gene, *imuA*, encodes a member of the *recA*/*sulA* superfamily of still unknown function. The other two genes, *imuB* and *imuC* (also referred to as *dnaE2*), encode a Y-family DNA polymerase and a C-family homolog of the alpha subunit of DNA Pol III,

respectively (McHenry, 2011). Previous studies showed that the biological effects for ImuB and ImuC on mutagenesis can vary among bacterial species. In *Streptomyces*, ImuC was reported to be an error-prone DNA polymerase, involved in damage-inducible translesion synthesis (Tsai et al. 2012). In *Myxococcus xantus* the overexpression of ImuC is able to increase the mutation rate and cause phenotypic deficiencies in development and sporulation (Peng et al. 2017). In *Caulobacter cresentus* and *Mycobacterium tuberculosis,* both proteins act cooperatively in the mitomicyn-C- and UV-dependent mutagenic response (Boshoff et al. 2003; Galhardo et al. 2005). Importantly, it has been observed that ImuB lacks the triad of catalytic amino acids, which is conserved in its Y-family polymerase homologs, which means that it is most likely unable to polymerase DNA (Warner et al. 2010). Instead, it has been shown that ImuB bridges the interaction between ImuC and the β-clamp, facilitating its access to the replication fork, which as a consequence implies an interdependent activity of both proteins (Warner et al. 2010). However, this cooperative activity seems not to be an absolute rule. For instance, it has been reported that in *Pseudomonas putida* these two TLS factors have antagonistic effects on stationary-phase and UV-mutagenesis, where ImuB and ImuC seem to play a mutator and an antimutator role, respectively (Koorits et al. 2007).

Error-prone DNA replication or incorrect repair of base-pair mismatches may contribute directly to the unexpectedly high acquisition rates of drug resistance during chronic infections (Oliver et al. 2000; Björkholm et al. 2001; Martina et al. 2014). One of the most clear examples of such process is the genetic adaptation that *Pseudomonas aeruginosa* undergoes during respiratory chronic infections in cystic fibrosis (CF) patients (Oliver, 2000; Mena et al. 2008; Feliziani et al. 2010; Feliziani et al. 2014). Once established, *P. aeruginosa* can remain persistently associated with its host for decades due to the emergence of different phenotypes that are adapted to the CF lung environment. In this sense, factors which are able to alter the mutation rate such as the activity of mutagenic polymerases, may play an

important role in this adaptive process. Inspection of the *P. aeruginosa* genome reveals the presence of a *dinB*-enconded Pol IV and also of a three-gene cassette *imuA-imuB-imuC* (Abella et al. 2004), Pol IV being the best characterized. It has been established *in vitro* that Pol IV constitutes a LexA-dependent error-prone DNA polymerase (Sanders et al. 2006), being involved in DNA oxidative and alkylation induced mutagenesis (Jatsenko et al. 2017) and in the acquisition of prototypic CF adaptive phenotypes such as mucoidy and antibiotic resistance (Moyano et al. 2007). However, data about the role of *imuA-imuB-imuC* cassette in *P. aeruginosa* mutagenesis is still incipient and speckled in the literature. ImuC contributes to DNA alkylation (Jatsenko et al. 2017) and UV-induced mutagenesis (Sanders et al. 2006), and the transcription of both, *imuB* and *imuC*, was shown to be unevenly increased upon exposure of *P. aeruginosa* to the SOS-inducing antibiotic ciprofloxacin (Cirz et al. 2006). In this study, we have investigated the role of ImuB and ImuC in *P. aeruginosa* DNA damage induced mutagenesis. We show that both genes contribute to UV-induced mutagenesis in this bacterium, being expressed as part of a single operon regulated by LexA. The results presented here contribute to the small but growing literature investigating mutagenic DNA polymerases in *P. aeruginosa.*

MATERIALS AND METHODS

Bacterial strains and media. Strains, plasmids and oligonucleotides used in this study are described in Table 1. *P. aeruginosa* PAO1 (Holloway et al. 1979) and its *imuB* (PAOIB) and *imuC* (PAOIC) mutant strains were kindly provided by Dr Herbert Schweizer from University of Florida (USA). In order to generate PAOIB and PAOIC, internal fragments of the chromosomal *imuB* (nucleotides 327-1051 in the coding sequence) and *imuC* genes (nucleotides 370-2693 in the coding sequence) were replaced with the *aaC1* gentamicin-resistance gene, respectively, as previously described in Choi and Schweizer, 2005. To get unmarked mutants, Flp-mediated excision of the gentamicin resistance cassette in PAOIB and PAOIC strains was performed using the pFLP2 plasmid as described by Choi and Schweizer, 2005. PAODB strain was generated from PAO1 by replacing an internal fragment of the *dinB* gene with a Km^R gene, as described previously (Moyano et al. 2007). *P. aeruginosa* strains were routinely grown at 37ºC in Luria-Bertani (LB) broth or agar as the growth medium.

UV mutagenesis assay. UV-induced mutagenesis assay was performed as previously described (Le Chatelier et al. 2004) with minor modifications. Briefly, cells of *P. aeruginosa* PAO1 strain as well as its *imuB, imuC,* and *dinB* deletion mutants were grown on LB medium to mid-exponential growth phase (OD_{600}=0.4). Cells from 2,5-ml aliquots were pelleted by centrifugation and resuspended in 2,5 ml of minimal salts. Each aliquot was transferred to a petri dish (5 cm) and subjected to UV-C irradiation at 10 J/m^2 dose using a UV Crosslinker 500 (Hoefer). After irradiation, bacteria were cultivated in LB for 1 h at 37ºC 220 r.p.m to allow mutation fixation. UV-treated and control cultures (mock-irradiated aliquots) were submitted to serial dilutions and plated on LB agar to quantify total CFU. Then, the whole culture volume was concentrated by centrifugation and plated in LB agar plated supplemented with 100 µg ml^{-1} of rifampicin. Rif^R colonies were scored after 48 h of incubation at 37°C. UV-induced mutant frequencies were calculated by dividing the numbers

of Rif^R mutants by the total number of CFU. At least 40 independent measurements were performed for each strain.

Analysis of *rpoB* sequence in rifampicin resistant cells. *rpoB* gene was sequenced from independent Rif^R colonies isolated from UV-irradiated and non-irradiated cultures from PAO1, PAOIB and PAOIC strains. The region corresponding to cluster I and II of *rpoB* gene was PCR amplified using *rpoB* F and *rpoB* R primers (Table 1). The PCR products were cleaned with a Gel Purification kit (FERMENTAS) and directly sequenced by the DNA Sequencing Service of the University of Chicago. To identify mutations in the *rpoB* gene, the sequences obtained from the Rif^R variants were compared with the *rpoB* PAO1 reference sequence (Stover et al. 2000) by CLUSTALW analysis (Larkin et al. 2007). For each strain, ~30 independent Rif^R colonies were analyzed.

RNA harvest and semi quantitative RT-PCR. Reverse transcriptase PCR assays were carried out in order to investigate the expression of *imuB* and *dnaE*2 genes in response to UV-induced DNA damage. *P. aeruginosa* PAO1, PAOIB and PAOIC mid-exponential phase cultures (OD_{600}=0.4) were irradiated with UV-C light as previously described. The *P. aeruginosa* lexA G86V mutant (PAOL86), a mutation that renders cells unable to induce the SOS response (Table 1), was used as a negative control. Treated and untreated cultures were used to extract total RNA using the RNA Purification Kit (Fermentas). RNA was quantified by UV spectrophotometry, and its integrity was checked by electrophoresis in 1.5% (w/v) agarose gels. Then, 1 µg of total RNA was reverse-transcribed using the QuantiTect Reverse Transcription Kit (QIAGEN). PCR primers were manually designed with the assistance of the Netprimer software (PREMIER Biosoft International, Palo Alto, CA) and evaluated for their specificity with the BLAST program at the NCBI Web site. Specific transcripts were semi-quantitatively measured by RT-PCR using primers ImuB-RT-F and ImuB-RT-R (for *imuB*), ImuC-RT-F and ImuC-RT-R (for *imuC*). Transcripts of the *rpoD* gene were

amplified with primers RpoD-RT-F and RpoD-RT-R and served as housekeeping controls. All primer sequences are described in Table 1. The optimal number of cycles was determined in advance to evaluate expression in the exponential phase of amplification. Final cycling conditions included a hot start at 95°C for 2 min, followed by 28 cycles of 30 sec at 94°C, 30 sec at 56°C, 30 sec at 72°C, and a final extension cycle of 5 min at 72 °C. Specificity was verified by agarose gel electrophoresis. Band intensities were then measured with the Gel-Pro Analyzer Software. For the calculation of fold change in gene expression, band intensities of *imuB* and *imuC* genes were previously normalized using band intensities of their respective *rpoD* housekeeping controls. No amplification was observed in PCR reactions containing non-reverse transcribed RNA as template.

Characterization of *imuB* and *imuC* transcriptional organization. Primers were designed in order to amplify PCR products containing regions of both, *imuB* and *imuC* genes, which share the same orientation and are located downstream from *imuA*. The cDNA to be used as a template for PCR was obtained by reverse transcription of purified total RNA as described above. Thus, primers imuC-imuB-F and imuC-imuB-R (Table 1) were employed to determine the co-expression of genes *imuB* and *imuC* .

Statistical analysis. Statistical significance in UV-induced mutagenesis assays within strains was evaluated using a non-parametric Wilcoxon matched-pairs signed rank test. .*P* values less than or equal to 0.05 were considered statistically significant. The statistical significance of differences between Rif^R mutational spectra was determined by using the hypergeometric test algorithm previously described (Adams and Skopek 1987; Cariello et al. 1997). All *p* values were based on 30000 iterations. A *p*-value of <0.05 means that the spectra are different in a pairwise comparison.

RESULTS

ImuB and ImuC contribute to UV-induced mutagenesis.

As mentioned, it has been previously shown that ImuC is involved in UV-induced mutagenesis in *P. aeruginosa* (Sanders et al. 2006). However, the role of ImuB in this process remains still unexplored. Thus, we examined the contribution of ImuB and ImuC in DNA-damage induced mutagenesis in *P. aeruginosa*. To do this, we performed UV mutagenesis assays using the *P. aeruginosa* PAO1 strain which expresses all five known *P. aeruginosa* polymerases, and its isogenic PAOIB and PAOIC strains carrying deletions in the *imuB* and *imuC* genes, respectively. As a control, we also included a *dinB* deficient strain (PAODB), which, according to the literature, is not involved in UV mutagenesis (Sanders et al. 2006). It is important to note that in-frame non-polar deletion of *imuB* did not affect the expression of *imuC* and vice versa, as the expression of the reciprocal transcripts was checked in each of the mutant strains by RT-PCR (Figure S1). Table 2 shows the frequencies of Rif^R mutants for control and UV-induced cultures in all the analyzed strains. Following UV irradiation, PAO1 and PAODB displayed a significant increase in its mutant frequency, confirming that *P. aeruginosa* displays a modest UV-induced mutator phenotype and that DinB is not playing an important role in the generation of these mutants. However, this increase was not further observed in strains PAOIC and PAOIB as determined by their mutant frequency to Rif, indicating that both gene products participate and are necessary for UV-induced mutagenesis in this species. Evaluation of the effect of UV-treatment on cell viability showed ~30% of surviving cells after UV exposure, with no significant differences observed among the different strains (Figure S2, $p=0.086$). Furthermore, UV treatment did not produce any significant filamentation of dividing cells (Figure S3), which is one potential problem of cells exposed to UV that may generate artifacts in CFU counts (Rudolph et al. 2007). Interestingly, PAOIB showed the highest mutant frequency among mock-irradiated

controls (Table 2). However, further mutation fluctuation tests indicated that no significant differences were observed in spontaneous mutagenesis between PAO1 wild type and PAOIB (*p=0.23*), PAOIC (*p=0.*39) and PAODB (*p=0.12)* mutants (Figure 1).

UV-induced mutational spectra in PAO1, PAOIB and PAOI strains.

The rifampicin mutation assay detects point mutations in the *rpoB* gene, which encodes the β-subunit of bacterial RNA polymerase, and is very useful due to the high conservation of the target gene, allowing its utilization in a wide variety of bacterial species (Garibyan et al. 2003). So, we next analyzed the nature of UV-induced mutations in the *rpoB* gene of PAO1 and its isogenic deletion mutants PAOIB and PAOIC by obtaining randomly selected rifampicin-resistant (Rif^R) mutants from UV-irradiated cultures and further sequencing of their *rpoB* clusters (Material and Methods). Rif^R mutants from mock-irradiated cultures were used as controls to obtain spontaneous mutations in *rpoB*. Interestingly, there were no differences in the distribution of transitions and transversions observed after UV treatment in the different strains (hypergeometric test, pairwise comparisons mock-irradiated: PAO1: *p*= 0.776; PAOIC: *p*= 0.172; PAOIB: *p*= 0.467). Transition mutations dominated the mutational spectra of UV-treated and control PAO1, PAOIB and PAOIC strains, constituting ~80% of the total mutations found in the *rpoB* gene (Figure 2). Among these, G:C to A:T was the most common type of mutation seen in the PAO1 strain irradiated with UV. This dominance of G:C to A:T was not seen in the PAOIB and PAOIC mutant strains, which is in agreement with the lack of UV mutagenesis. Since PAOIB and PAOIC strains did not show any increase in mutagenesis after UV irradiation (Table 2), all the mutations observed in these strains are likely to be spontaneous mutations.

UV-treatment induces the co-expression of *imuB* and *imuC* under the control of *lexA* as components of a single operon.

The genomic organization of *P. aeruginosa imuA* (PA0671), *imuB* (PA0670) and *imuC* (PA0669) genes indicate that they constitute an operon (Figure 3) (operon id: 12177 (Mao et al. 2009)). We subsequently analyzed the transcriptional organization of *imuB* and *imuC* in order to confirm whether these genes behave as a single transcriptional unit, thereby constituting an operon. For that, we carried out PCRs using cDNA as template and primers designed to amplify fragments containing regions of the two neighboring genes which share the same orientation. As shown in Figure 3, we were able to amplify a fragment between *imuB* and *imuC* following UV irradiation suggesting that *imuB* and *imuC* were co-expressed. This result depicts a transcriptional organization of these genes structured in one polycistronic operon, whose expression is increased upon UV-induced DNA-damage.

Previous studies reported an upregulation of the *imuABC* operon expression after ciprofloxacin treatment (Cirz et al. 2006). To evaluate the effect of UV-induced DNA damage on *imuB* and *imuC* expression, we analyzed transcripts abundances of both genes using *rpoD* as a housekeeping control in mid-log phase cultures of *P. aeruginosa* PAO1 that were treated with UV or left untreated. Consistent with the results shown in Figure 3, UV treatment induced a significant 6.2- and 4.5-fold increase in the expression levels of *imuB* and *imuC* transcripts, respectively (Figure 4, $p<0.05$), indicating that the stress induced by UV triggered a similar transcriptional response of both genes.

The presence of a LexA binding domain 137 bp upstream from the translational start codon of the *imuA* gene (Cirz et al. 2006) indicates that in *P. aeruginosa* the *imuABC* operon is probably part of the SOS regulon, as it has been described in other Proteobacteria (Abella et al. 2004). In fact, a recent study have shown that the LexA1 protein from *P. putida* is able to bind this regulatory motif in *P. aeruginosa* (Abella et al. 2004). To confirm whether these genes form part of the SOS regulon, the expression of *imuB* and *imuC* were also analyzed in a *P. aeruginosa lexA* mutant strain (PAOL86) in which the SOS response is impaired (Table 1).

As shown in Figure 4, the UV-induction of *imuB* and *imuC* transcripts was drastically reduced in the PAOL86 strain, indicating that they are co-ordinately regulated by the SOS response. In contrast to the PAO1 wild type strain, the *lexA* mutant showed no significant differences in the transcript levels of *imuB* and *imuC* after UV treatment (Figure 4, $p<0.05$). These results confirm the prediction that both *imuB* and *imuC* are expressed in response to DNA damage and are controlled by the SOS regulon in *P. aeruginosa*.

DISCUSSION

Bacteria present alternative ways to face stress that can injure DNA. Among these, TLS is carried out by specialized DNA polymerases that allow cell survival at a cost of increasing mutagenesis (Goodman and Woodgate, 2013). This mutagenesis may in turn serve as a supply of the genetic resources needed in the adaptation process to new or hostile environments (Rosenberg, 2001; Foster, 2007). In *E. coli*, this function is fulfilled by three well-known TLS DNA polymerases, Pol II (*polB*), Pol IV (*dinB*) and Pol V (*umuDC*). Particularly in the case of UV-induced DNA damage, Pol V but not Pol IV is able to bypass the UV-lesions with further error-prone synthesis (Kato and Shinoura, 1977). Moreover, among *E. coli* TLS polymerases, only Pol V contributes to the survival of UV-irradiated cells (Courcelle et al. 2004). Consistent with this, and with the findings of Sanders et al. (Sanders et al. 2006), we evaluated a PAO1 *dinB*-deficient mutant and confirmed that Pol IV is not involved in UV-induced mutagenesis in *P. aeruginosa*. Instead, in *P. aeruginosa*, the role of Pol V seems to be replaced by a "mutagenic cassette", generally constituted by the *imuA*-*imuB*-*imuC* genes, which is widely distributed among Proteobacteria (Abella et al. 2004). However, the role of these TLS-associated enzymes in this bacterial species is still poorly known. In order to get insights on their contribution to the mechanisms of adaptive mutagenesis in *P. aeruginosa*, we constructed deletion mutants of the *imuB* and *imuC* genes and evaluated their role in UV-induced mutagenesis. The results presented here suggest that ImuC and ImuB are responsible for most of the induced mutagenesis in *P. aeruginosa* upon UV light exposure, functioning as components of the SOS regulon under the control of the master regulator LexA.

It has been reported that ImuB and ImuC act cooperatively during TLS in several bacterial species (Boshoff et al. 2003; Le Chatelier et al. 2004; Galhardo et al. 2005; Warner et al. 2010; Alves et al. 2017). Based on sequence analyses, Warner et al. (2010) suggested that

ImuB is devoid of DNA polymerase activity because it lacks the highly conserved residues of the active site required to polymerize DNA. Instead, ImuB has a conserved peptide sequence motif able to interact with the β-clamp and mediate the access of the *bona fide* polymerase ImuC to the replication fork (Warner et al. 2010). On the other hand, a study in *P. putida* described antagonistic functions between ImuB and ImuC during stationary-phase and UV-induced mutagenesis: while ImuB shows an error-prone activity, ImuC seems to play an anti-mutator role (Koorits et al. 2007). Thus, both factors may probably fulfil different roles in different bacterial species. Particularly in *P. aeruginosa*, ImuC has been reported to be involved in UV-induced mutagenesis (Sanders et al. 2006) and in coping with DNA alkylation damage, along with ImuA and ImuB (Jatsenko et al. 2017). Consistent with previous reports, our observations indicate that ImuC significantly contribute to the UV-induced mutator phenotype in *P.* aeruginosa. However, the role of the putative polymerase ImuB, which is part of the same polycistronic operon than ImuC, remained still unexplored. Here we demonstrate that ImuB plays an essential role in UV-induced mutagenesis in *P. aeruginosa.* Importantly, it has been described that the *P. aeruginosa* ImuB harbors a β-clamp binding domain but lacks the active site to polymerase DNA which is highly conserved in the Y-familiy of DNA polymenrases (Warner et al. 2010). Although we have not evaluated any physical interaction between ImuB and ImuC, or with the β-clamp, our results in *P. aeruginosa* fit well with an interdependency of roles for ImuB and ImuC, as it was observed in other bacteria. Thus, the *P. aeruginosa*'s mutasome complex resembles that proposed for *M. tuberculosis* in which ImuB seems to constitute an accessory factor functioning as a nexus between ImuC. The fact that ImuB and ImuC form part of an operon which has been widely spread by horizontal transfer (Abella et al. 2004) opens the question of whether this reported "bridge" role of ImuB is limited to its operon partner ImuC or is, in turn, able to interact with other DNA polymerases,

Because of its contribution to pathogenesis, understanding the different mechanisms of adaptive mutagenesis in *P. aeruginosa* is important and timely. Our results constitute a new step to comprehend the adaptive repertory of *P. aeruginosa* by positioning ImuB and ImuC as relevant contributors to thrive in stressful environments.

STATEMENT OF AUTHOR CONTRIBUTION

A.M.L. and A.M.S conceived the study. A.M.L., A.J.M and A.M.S designed the experiments, supervised the study and wrote the manuscript. A.M.L., A.J.M., R.A.M., S.F. and M.U. performed the experiments. All authors discussed, edited and agreed on the final manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

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FIGURE LEGENDS

Figure 1. Spontaneous mutation rates to Rif^R for PAO1, PAOIB, PAOIC and PAODB strains. The rates of mutation resulting in rifampicin resistance (Rif^R) were determined by the modified Luria fluctuation tests (Luria et al. 1943). rSalvador (Zheng et al. 2017) was used to estimate mutation rate (*m*) in each strain, accounting for plating efficiency (e=0.3) and using the likelihood ratio test when comparing estimates from different strains. These analyses were conducted in R 3.5.2 (R Core Team, 2018). Error bars represent the upper and lower 95% confidence intervals.

Figure 2. Characterization of the *rpoB* mutational spectra before and after UV-treatment. The percentage of total mutations found for PAO1, PAOIB and PAOIC strains treated or non-treated with UV (10 J/m^2) are shown. Mutations are classified among the different substitution and insertion/deletion (indel) mutations. For each strain, ~30 independent Rif^R colonies were analyzed.

Figure 3. Transcriptional organization of *imuB* and *imuC*. The *imuC* gene is co-expressed with gene *imuB* as a transcriptional unit, thereby belonging to the same operon. The gel below represents typical PCR amplifications obtained using cDNA from UV treated (10 J/m^2) or untreated cultures, as templates. The arrow marks the position of *imuC-ImuB* F and R primers (see Table 1) that delimitate a 300 bp region between both genes.

Figure 4. UV damage and SOS dependent expression of *imuB* and *imuC* in *P.*

***aeruginosa*.** A) Semiquantitative RT–PCR analysis of UV-induced gene expression. RNAs were extracted from exponentially growing *lexA* (PAOL86) and wild-type (PAO1) cells before and after irradiation with 10 J/m^2 UVC. B) Relative levels of expression of *imuB* (light grey bars) and *imuC* (black bars) shown represent the average of three independent experiments done in triplicate. Significant differences are shown respect to untreated controls. *$p<0.05$ *t*-test.

Table 1. Strains and oligonucleotides used in this study.

Strains	Description	Reference
PAO1	Wild type	Holloway *et al.*, 1979
PAOIB	In-frame deletion mutant of *imuB*; PAO1 derivative	Provided by H. Schweizer
PAOIC	In-frame deletion mutant of *imuC*; PAO1 derivative	Provided by H. Schweizer
PAODB	*dinB*::Km^R; PAO1 derivative	This study
PAOL86	*lexA* G86V. SOS-non-inducible PAO1 mutant	Boles and Singh, 2008
Primers		
rpoB F	5'-AATGGCCGAGAACCAGTTCCG-3'	This study
rpoB R	5'-AAGCCTGGGCGATGACGTGG-3'	This study
imuC-RT-For	5'-GGTGAGTTCGGCGGTGG-3	This study
imuC-RT-Rev	5'-CGACAGCAGCGACAGCG-3'	This study
imuB-RT-For	5'-CAACTGTTCCCAGCCGAGATAC-3'	This study
imuB-RT-Rev	5'-GGCCATGCTCTTCGAACTGAC-3'	This study
rpoD-RT-For	5'-TCGATCTGCTTGCGGACCTTG-3'	This study
rpoD-RT-Rev	5'-CTGCTGGCGGAAACCCTGAA-3'	This study
imuC-imuB F	5' TGGTGGGATGGCGAAGACA-3'	This study
imuC-imuB R	5'- GCTGCCGACGATCAGTTGT-3'	This study

Table 2. Role of *imuB* and *imuC* in UV-mutagenesis in *P. aeruginosa*.

Strains[a]	Rif^R mutants/10^8 cells[b]		Ratio of UV-induced/spontaneous	Median of differences (*p*)
	Control	UV-induced		
PAO1	1.76 ± 0.24	4.9 ± 1.04	2.78	0.92 (<0.0001)
PAOIB	3.16 ± 0.42	2.18 ± 0.42	0.68	-0.84 (0.0509)
PAOIC	1.36 ± 0.20	1.37 ± 0.26	1.00	-0.29 (0.3157)
PAODB	1.67 ± 0.65	3.04 ± 0.68	1.82	1.93 (0.0273)

[a] *P. aeruginosa* strains are described in Table 1.

[b] Induced mutation rates were determined after UV irradiation (10 J/m^2) of mid-log phase cultures of *P. aeruginosa* PAO1 (wt), PAOIB (Δ*imuB*), PAOIC (Δ*imuC*) and PAODB (Δ*dinB*) strains. Control mutation rates were determined in the same manner using mock non-irradiated cultures. Values presented for spontaneous and UV-induced Rif^R mutant frequencies represent the mean from 40 independent experiments ± SEM. Statistically significant differences UV-treated and non- treated samples for PAO1 and PAODB (Wilcoxon matched-pairs signed rank test).