OxyR-dependent expression of a novel glutathione S-transferase (Abgst01) gene in Acinetobacter baumannii DS002 and its role in biotransformation of organophosphate insecticides

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While screening a genomic library of Acinetobacter baumannii DS002 isolated from organophosphate (OP)-polluted soils, nine ORFs were identified coding for glutathione S-transferase (GST)-like proteins. These GSTs (AbGST01–AbGST09) are phylogenetically related to a number of well-characterized GST classes found in taxonomically diverse groups of organisms. Interestingly, expression of Abgst01 (GenBank accession no. KF151191) was upregulated when the bacterium was grown in the presence of an OP insecticide, methyl parathion (MeP). The gene product, AbGST01, dealkylated MeP to desMeP. An OxyR-binding motif was identified directly upstream of Abgst01. An Abgst–lacZ gene fusion lacking the OxyR-binding site showed a drastic reduction in promoter activity. Very low β-galactosidase activity levels were observed when the Abgst–lacZ fusion was mobilized into an oxyR (GenBank accession no. KF151190) null mutant of A. baumannii DS002, confirming the important role of OxyR. The OxyR-binding sites are not found upstream of other Abgst (Abgst02–Abgst09) genes. However, they contained consensus sequence motifs that can serve as possible target sites for certain well-characterized transcription factors. In support of this observation, the Abgst genes responded differentially to different oxidative stress inducers. The Abgst genes identified in A. baumannii DS002 are found to be conserved highly among all known genome sequences of A. baumannii strains. The versatile ecological adaptability of A. baumannii strains is apparent if sequence conservation is seen together with their involvement in detoxification processes.

INTRODUCTION

Glutathione transferases (EC 2.5.1.18), previously known as glutathione S-transferases (GSTs), represent a protein superfamily involved in cellular detoxification of both xenobiotics and endobiotics (Sheehan et al., 2001; Hayes et al., 2005; Oakley, 2005). GSTs metabolize a variety of electrophilic compounds by conjugating their electrophilic groups to the sulfur atom of glutathione. The conjugate thus formed is excreted from the cell (Hayes et al., 2005).

Abbreviations: 13-HPODE, 13-hydroperoxy-9,11-octadecadienoic acid; CDNB, 1-chloro-2,4-dinitrobenzene; CHP, cumene hydroperoxide; ESI, electrospray ionization; GST, glutathione S-transferase; MeP, methyl parathion; OP, organophosphate; Q-TOF, quadrupole time-of-flight; TBHP, tert-butyl hydroperoxide.

The GenBank/EMBL/DDBJ accession numbers for the Abgst01 and oxyR gene sequences of Acinetobacter baumannii DS002 are KF151191 and KF151190, respectively.

Four supplementary figures and one supplementary table are available with the online version of this paper.

Broadly, GSTs are divided into four major families: (i) cytosolic GSTs, (ii) mitochondrial GSTs, (iii) microsomal GSTs and (iv) bacterial fosfomycin-resistance proteins (Armstrong, 1997; Hayes et al., 2005). On the basis of their chemical, physical and structural properties, the cytosolic GSTs have been subgrouped into a number of divergent classes (Jakobsson et al., 1999; Allocati et al., 2009). Despite their low inter-class sequence identity, the GST monomers show a typical two-domain structural organization with a short linker of six or seven amino acids. Domain I, found at the N-terminus of the protein, contains the characteristic GSH-binding site with an α and β structural fold. The C-terminally located domain II with solely β-helices contains the typical hydrophobic binding site (H-site) (Rossjohn et al., 1998; Skopeliotou et al., 2012; Yamamoto et al., 2013). Expression of eukaryotic GSTs has been studied extensively. A number of structurally diverse chemicals have been shown to induce GST expression (Pearson et al., 1983; Rowe et al., 1997; Kim & Lee, 2007; Laborde, 2010). Although prokaryotic GST sequences are available, very little...
is known about their functions in bacterial genome sequences. The bacterial GSTs identified to date are implicated in the biodegradation of xenobiotics (Di Ilio et al., 1988; Mueller et al., 1990; Orser et al., 1993; Bader & Leisinger, 1994; Hofer et al., 1994; McCarthy et al., 1996; Nishida et al., 1994) and antibiotics (Arca et al., 1988, 1990; Piccolomini et al., 1989). Recently, bacterial GSTs have also been reported with novel catalytic properties (Wiktelius & Stenberg, 2007; Allocati et al., 2008).

Acinetobacter baumannii DS002 was isolated from agricultural soil polluted with organophosphate (OP) insecticides. It was enriched along with a Flavobacterium balustinum strain that used the OP insecticide methyl parathion (MeP) as a source of carbon and nitrogen (Somara & Siddavattam, 1995). Unlike F. balustinum, A. baumannii DS002 failed to grow using MeP as the sole source of carbon. However, when MeP was added to the culture medium, it was rapidly dealkylated. A GST-like protein was also found to be upregulated in the proteome of DS002 when MeP was added to the culture medium, it was rapidly dealkylated. A GST-like protein was also found to be upregulated in the proteome of DS002 when MeP was added to the culture medium, it was rapidly dealkylated.

**METHODS**

The oxidative stress inducers and other fine chemicals used in this study were procured from Sigma Aldrich. The Ni-Sepharose used to purify AbgstO1 was procured from GE Healthcare.

**Bacterial strains, growth conditions and DNA manipulations.**

The bacterial strains and plasmids used in this study are listed in Table 1. The primers used for performing PCR and quantitative(q)PCR are shown in Table S1 (available in Microbiology Online). Escherichia coli and A. baumannii DS002 cells were grown in LB medium at 37 and 30 °C, respectively. When necessary, the antibiotics ampicillin (100 μg ml⁻¹), kanamycin (30 μg ml⁻¹), streptomycin (20 μg ml⁻¹) and chloramphenicol (30 μg ml⁻¹) were added to the growth medium. Molecular cloning and DNA hybridization techniques were performed following procedures described elsewhere (Sambrook et al., 1989).

**Construction of the genomic DNA library.**

A genomic library of A. baumannii DS002 DNA was generated using the CopyControl Fosmid Library Production kit (EPICENTRE Biotechnologies) following the manufacturer’s protocols. The mechanically sheared genomic DNA fragments (40 kb) were end-repaired prior to their ligation to the 8.1 kb linear pCC1FOS vector. After ligation, the mixture was packaged using MaxPlax Lambda Packaging Extracts (EPICENTRE Biotechnologies) before being used to infect E. coli EP1300. The infected cells were then plated on chloramphenicol plates to obtain colonies with recombinant fosmids. The completeness of the generated DNA library was calculated following standard procedures (Sambrook et al., 1989). Colonies carrying Abgst genes were either identified by performing colony hybridization using partial Abgst sequences as a probe or amplified using primers designed based on the genome sequences of A. baumannii strains available in the database.

**DNA sequencing and analysis.**

Recombinant fosmids isolated from the Abgst-positive colonies were digested with appropriate restriction enzymes, and the fragments including complete Abgst genes were subcloned and sequenced following standard procedures (Sanger & Coulson, 1975; Sambrook et al., 1989). Sequences were analysed using online bioinformatic tools. ORF Finder (http://www.ncbi.nlm.nih.gov/orffinder) was used to predict ORFs and BPROM software (http://linux1.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb) was used for promoter predictions. The sequence similarity search and multiple sequence alignment were essentially done using BLAST (www.ncbi.nlm.nih.gov/BLAST) and ClustalW2 (www.ebi.ac.uk/Tools/msa/clustalw2/), respectively. The phylogenetic tree reconstruction was done using ITOL (http://itol.embl.de/) and Virtual Footprint (http://www.proodorico/vfp/vfp_promoter.php) was used for identification of transcription factor-binding sites.

**Promoter–lacZ fusions and assay of β-galactosidase enzyme activity.**

The strategies used to construct the AbgstO1–lacZ fusions are shown in Fig. 4(a). The upstream region of AbgstO1 with and without the putative OxyR-binding site was cloned in promoter probe vector pMP220 (Spanik et al., 1987) to generate Abgst–lacZ fusions pST2 and pST1, respectively. The generated Abgst–lacZ fusions were then mobilized into A. baumannii DS002 cells following procedures described elsewhere (Figurski & Helinski, 1979). Initially, E. coli S17-1 strain (donor), carrying the Abgst–lacZ plasmids, and A. baumannii DS002 (recipient) were grown in LB medium containing tetracycline and chloramphenicol, respectively, until the culture reached the mid-exponential phase of growth. The cells were harvested and washed thoroughly with 0.9 % NaCl. The donor and the recipient cells were then mixed in a 1:1 ratio before being spread uniformly on the surface of an LB plate. After 12 h of incubation at 30 °C, the cells were scraped from the surface using a sterile toothpick and resuspended in 0.9 % (w/v) NaCl solution. Serial dilutions made from the cell suspension were plated on an LB plate containing both tetracycline and chloramphenicol. The β-galactosidase activity was determined following established procedures (Miller, 1972).

**Real-time PCR.**

The A. baumannii DS002 cultures grown to mid-exponential growth phase were exposed to sublethal doses (below the MIC) of cumene hydroperoxide (CHP; 75 μM), tert-butyl hydroperoxide (100 μM), H₂O₂ (100 μM), MeP (300 μM) or 1-chloro-2,4-dinitrobenzene (CDNB; 100 μM) for 3 h before proceeding with RNA isolation. The total RNA (2 μg) isolated from A. baumannii DS002 cells exposed to the oxidative stress inducers was reverse transcribed using random hexamers and reverse transcriptase (Fermentas). The corresponding cDNAs (2 μl) were then used as templates in a 25 μl PCR mixture containing the SYBR Green assay mix (Bio-Rad). Primers were designed using the Primer-BLAST program (www.ncbi.nlm.nih.gov/tools/primer-blast/) and qPCR was performed using a gradient Mastercycler following the manufacturer’s universal thermal cycling conditions (Eppendorf). The total RNA isolated from A. baumannii DS002 cultures grown in the presence of CHP was used to perform primer extension (Fig. S4) (Fekete et al., 2003).

**Generation of an oxyR knockout.**

A mutant with an insertional inactivated oxyR gene was generated by inserting a kanamycin resistance gene in the coding region of oxyR. Initially, the oxyR gene was amplified using a primer set (DS00117/DS00118) and the amplified oxyR gene was then cloned into the pTZ57R/T vector. As a unique XhoI site is present in the coding region of oxyR, the kanamycin gene isolated as a XhoI fragment was cloned into this site to generate an insertional inactivated oxyR allele. The oxyR::kan

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DNA fragment was isolated by restriction digestion with EcoRI and cloned into the suicide vector pSUP202 (Simon et al., 1983). The resulting plasmid designated pSUPoxyR::kan was then mobilized into A. baumannii DS002 as described above, and the ex-conjugants were selected on chloramphenicol and kanamycin plates. The replacement of the oxyR gene with the oxyR::kan allele was identified among the ex-conjugants by performing PCR using oxyR-specific primers (DS00117/DS00118) as shown in Fig. 4(d).

**Expression and purification of AbGST01.** The Abgst01 gene was amplified from genomic DNA of A. baumannii DS002 using forward (DS00112) and reverse primers (DS00113) including Ndel and Xhol restriction sites, respectively. The PCR product was then cloned as an Ndel/Xhol fragment into pET-23b. The recombinant plasmid, designated pPAGT, codes for AbGST01 with a C-terminal His-tag. The expression and purification of recombinant AbGST01his was done essentially following procedures described elsewhere (Pandey et al., 2009).

**GST assay.** The GST assay was performed by using CDNB as a substrate essentially by following the procedures described elsewhere (Habig et al., 1974). GSH conjugation with CDNB was monitored at 340 nm using spectrophotometry. Reactions were performed in 50 mM phosphate buffer (pH 6.5) containing 1 mM CDNB and 1 mM GSH, with an appropriate amount of AbGST01his (1 μg). The reaction was initiated by adding 1 mM GSH. AbGST01his activity was measured following standard procedures using a molar extinction coefficient of 9.6 mM⁻¹ cm⁻¹ for CDNB (Habig et al., 1974). A similar assay was performed for ethacrynic acid except that the CDNB was replaced with 0.2 mM ethacrynic acid and the conjugation with GSH was measured at 270 nm. Peroxidase activity towards CHP, TBHP and 13-hydroperoxy-9,11-octadecadienoic acid (13-HPODE) was determined by using a glutathione reductase-coupled assay (Lawrence & Burk, 1976). Briefly, 0.5 ml of a reaction mixture containing 1 mM GSH, 0.2 mM NADPH and 2.265 U glutathione reductase in 100 mM sodium phosphate buffer, pH 6.5, was incubated with 50 μg of AbGST01 at 30 °C for 5 min. The reaction was initiated by the addition of CHP. The consumption of NADPH was monitored at 340 nm for 5 min at 30 °C. A no-substrate blank and a no-enzyme blank were used to correct for glutathione reductase-independent NADPH oxidation and non-enzymic peroxidase reactions.

**Dealkylation of MeP.** The GST-mediated biotransformation of MeP was performed following standard procedures (Anderson et al., 1992). In a 1.5 ml microfuge tube, 100 μg purified GST was added to 145 μl incubation buffer (100 mM Tris, pH 7.4) with glutathione (5 mM). The mixture was gently vortexed and prewarmed (37 °C) for 5 min. Following pre-incubation, MeP (300 μM) was added and the reaction mix returned to 37 °C for a further 30 min. Protein concentration and incubation times were modified to ensure that the reaction proceeded within the linear range for the entire incubation period. After the incubation period, the enzyme reaction was stopped by the addition of 200 μl ice-cold methanol containing 250 μM p-phenylphenol. The samples were stored at −20 °C until further use. A reaction in which GST was replaced with a similar amount of BSA served as a negative control. When necessary the reaction mixture was centrifuged (10000 g) for 3 min and 200 μl of the clear supernatant was used for HPLC analysis (Anderson et al., 1992). For electrospray ionization (ESI) quadrupole time-of-flight (Q-TOF) measurements, the supernatant (200 μl) was mixed with an equal volume of the solvent system (25% of 80% acetonitrile in 0.1% ammonium formate) and injected into the injection port of an ESI micro Q-TOF instrument (Agilent Technologies) and MS was performed as described previously (Pandeeti et al., 2009).

**RESULTS**

**Multiple GSTs exist in A. baumannii DS002**

The present study was designed to gain further insights into the biotransformation of OP insecticides such as MeP in A. baumannii DS002. In this process we examined the proteome of A. baumannii DS002 derived from cultures grown in the presence and absence of MeP, and analysed them using 2D electrophoresis. In the proteome extracted...
from cultures grown in the presence of MeP we identified a GST-like protein as a significantly upregulated polypeptide. A partial DNA sequence encoding this GST-like protein was amplified using degenerate primers designed based on the de novo sequence generated for the protein spot (data not shown). When we used the ampiclon as a probe to screen the genomic library of A. baumannii DS002 by performing colony hybridization, a large number of colonies yielded positive signals. Surprisingly, the recombinant fomspids isolated from the gst-positive clones gave unique restriction profiles and in some cases even partial similarity was not observed among these recombinant fomspids. A close examination of the available A. baumannii genome sequences revealed the presence of multiple copies of gst-like genes. Total genome sequences are available in the database for five A. baumannii strains and in all of them nine highly conserved gst genes were identified. Anticipating similar conservation of these gst genes in A. baumannii DS002, we designed appropriate primers and successfully amplified and cloned all nine gst genes from A. baumannii DS002. These gst genes were named following established convention (Wagner et al., 2002) as Abgst01, Abgst02, Abgst03, Abgst04, Abgst05, Abgst06, Abgst07, Abgst08 and Abgst09. Interestingly, when the proteins encoded by these Abgst genes were aligned to identify structural similarities among AbGSTs (Fig. S1), only poor amino acid sequence similarities were found among AbGSTs (<22 %) and the sequence identity was only seen in certain regions, especially in the region comprising the N’N’N-cap-N1-N2-N3-N4 domain (Allocati et al., 2006). Considering such structural diversity among AbGSTs (Figs S1 and S2), a phylogenetic tree was reconstructed to establish their relationship with known GST classes (Fig. 1). The phylogenetic tree reconstructed depicted the existence of tau (AbGST01 and AbGST06), zeta (AbGST02 and AbGST08), pi (AbGST03 and AbGST04), beta (AbGST05), chi (AbGST09), and non-mammalian pi and chi GST classes (AbGST07) in A. baumannii DS002.

Expression profiling of Abgst genes
Consistent with their structural diversity, the promoter regions of Abgst genes have shown the presence of sequence motifs that serve as target sites for a number of well-characterized transcription factors (Fig. S2). As the existence of such sequence motifs indicates differential expression of Abgst genes, their expression levels were quantified in the presence of various oxidative stress inducers and the OP insecticide MeP (Fig. 2). As expected, CHP treatment influenced positively the expression of Abgst01, Abgst02, Abgst03 and Abgst07, with fold changes of 1.8, 2.6, 1.9 and 2.5, respectively. Similarly, the transcript levels of Abgst01, Abgst02, Abgst07 and Abgst09 were increased by 1.8-, 1.8-, 2.5- and 2.4-fold, respectively, in the presence of TBHP. The rest of the Abgst genes showed no significant increase in the presence of these two organic hydroperoxides. Inorganic hydroperoxide, H2O2, moderately enhanced the expression of Abgst02 (1.6-fold) and Abgst07 (1.6-fold), while the expression levels of the other Abgst genes were unaffected by the presence of H2O2.

Interestingly, the expression of both Abgst01 and Abgst02 was moderately increased (1.4-fold) in the presence of MeP. The common GST substrate CDNB showed a moderate influence on Abgst01 (1.7-fold). Surprisingly, the transcript levels of other Abgst genes (Abgst02, Abgst06, Abgst07, Abgst08 and Abgst09) either decreased (~0.5-fold) or remained unaltered (Abgst03, Abgst04 and Abgst05) in the presence of CDNB. Out of all the Abgst genes, only the expression of Abgst01 showed a marginal increase in the presence of both MeP and CDNB, suggesting an influence of MeP on expression of Abgst01 (Figs 2 and 3a). Reconfirming the qPCR data, the promoter activity of Abgst01 also showed a marginal increase in the presence of MeP. The A. baumannii DS002 (pST2) cells showed more β-galactosidase activity in the presence of MeP (Fig. 3b).

OxyR-dependent expression of Abgst01
Since the aim of the study was to understand the biotransformation of OPs, the Abgst01 promoter region was examined to identify reasons for its upregulation in the presence of MeP. Initially, primer extension was performed to determine the precise transcription start point of Abgst01 (Ying et al., 2007). The size of the extended product was found to be 165 nt, which indicated that the 5’ end of the transcript corresponded to an A residue located 121 bp upstream of the translational start site AUG (Fig. S4). The sequence found upstream of the transcription start point revealed the existence of a σ70-dependent promoter and a putative OxyR-binding site. The OxyR-binding site was found 124 bp upstream of the promoter motif (Fig. 4a). In order to analyse the role of OxyR in expression of Abgst01, Abgst01−lacZ fusions were constructed with (pST2) and without (pST1) the OxyR-binding site. These Abgst01−lacZ fusions were then mobilized into A. baumannii DS002 cells. As revealed through β-galactosidase activity, existence of the OxyR-binding site showed a significant influence on promoter activity of Abgst01. In particular, the presence of the OxyR-binding site enhanced promoter activity in the presence of organic peroxides and MeP. Such an increase was not observed in A. baumannii DS002 carrying plasmid pST1 (Fig. 4b). Only a basal level of β-galactosidase activity was observed in the oxyR null mutant of A. baumannii DS002 and no influence of either organic peroxides (CHP, TBHP) or MeP was observed on induction of Abgst01 in the oxyR-negative background (Fig. 4c). These results confirm the important role of OxyR in induction of Abgst01 expression.

Abgst01 dealkylates MeP
After establishing the influence of MeP on the expression of Abgst01, further experiments were conducted to assess the influence of Abgst01 on MeP. Initially, AbGST01 was expressed in E. coli without any affinity tag under the assumption that the recombinant AbGST01 could be
purified using a GSH-affinity column. Surprisingly, the overproduced protein, despite being soluble and active, failed to bind to the GSH-affinity column. Therefore, AbGST01 was synthesized with a C-terminal His-tag and the affinity-purified recombinant AbGST01His was used for assaying its activity. Purified AbGST01His was found to be active and showed a specific activity of 3.26 mmol min^{-1} mg^{-1} when CDNB was used as substrate. It also exhibited GSH-dependent peroxidase activity. The peroxidase activity towards CHP, TBHP and 13-HPODE was found to be 1.33, 0.669 and 2.4 μmol min^{-1} mg^{-1}, respectively. However, AbGST014His showed no activity against other conventional bacterial GST substrates, such as H_2O_2, dichloromethane, tetrachlorohydroquinone or the antibacterial fosfomycin.

Considering the positive influence of MeP on expression of Abgst01, we also tested whether MeP serves as a substrate for AbGST01His. Approximately 1 mg purified AbGST01His (Fig. 5a) was incubated with MeP before analysing the reaction mixture using HPLC and ESI Q-TOF (Fig. 5b–f).
Interestingly, during HPLC analysis the MeP concentration decreased strongly only in samples incubated with AbGST01_His. Simultaneously, a new peak appeared at a retention time of 0.9 min and the size of the peak increased with incubation time, suggesting strongly that the appearance of the new peak was due to AbGST01 activity on MeP (Fig. 5b, c). Further, the ESI Q-TOF analysis of the reaction mixture identified the new mass peak as desMeP, which was found only in the test sample (Fig. 5d–f). This indicated clearly the involvement of AbGST01 in the biotransformation of MeP via dealkylation.

**DISCUSSION**

The involvement of GSTs in transformation of xenobiotics is well documented in eukaryotes. GSTs isolated from mammals, especially from humans (Radulovic *et al.*, 1986, 1987) and rats (Benke & Murphy, 1975), have dealkylated...
MeP to desMeP. Prokaryotic GSTs have been discovered very recently (Vuilleumier, 1997). Unlike their mammalian counterparts, which primarily are involved in detoxification processes, bacterial GSTs have been shown to play an active role in central metabolism (Allocati et al., 2009). The physiological substrates have been identified for certain bacterial GSTs. GST enzymes, such as dichloromethane dehalogenases and the aromatic dehalogenase PcpC, have been shown to be synthesized at high levels in the presence of their cognate substrates. They also generate metabolites and conserve energy required for cell growth (Scholtz et al., 1988; La Roche & Leisinger, 1990; Bader & Leisinger, 1994; Vuilleumier et al., 2001). Bacterial GSTs have also been shown to be active on model epoxides (Di Ilio et al., 1988), herbicides (Laura et al., 1996) and isothiocyanates (Wiktelius & Stenberg, 2007).

Despite identifying multiple copies of genes encoding GST in bacterial genome sequences, very little is known about their functions (Vuilleumier & Pagni, 2002; Rife et al., 2003). The available genome sequences revealed the existence of nine gst homologues in A. baumannii strains, all of which were identified in A. baumannii DS002. With regard to the structural diversity of Abgst promoters, we have tried to assess their expression profiles in the presence of various oxidative stress inducers. The Abgst genes responded differently to various oxidative stress inducers. The organic peroxides seem to have a wider influence than the inorganic peroxides. Abgst02, Abgst07 and Abgst09 showed maximum induction in the presence of organic hydroperoxides, whereas the inorganic peroxide tested had a minimal effect on the expression of these Abgst genes. Abgst01 showed moderate upregulation of expression in the presence of the OP insecticide MeP, which is consistent with our observations where the induction of GST was seen in MeP-grown cultures.

Fig. 4. OxyR-dependent expression of Abgst01. (a) Construction of the Abgst–lacZ fusions. The putative OxyR-binding site is shown with an open box; pST1 and pST2 represent Abgst–lacZ fusions constructed without and with a putative OxyR-binding site. (b, c) CHP influence on Abgst–lacZ fusions in an OxyR-positive (b) or -negative (c) background. (d) Agarose gel showing amplification of oxyR::kan from OxyR null mutants of A. baumannii DS002. Lane 1, DNA ladder; lane 2, amplification of oxyR::kan; lanes 3 and 4, control PCRs performed without primers and template DNA, respectively; lanes 5 and 6, amplification of oxyR::kan from plasmid pSUPoxyR::kan and WT A. baumannii genomic DNA, respectively.
oxidized SoxR induces the expression of a second transcription factor, SoxS. SoxS has a direct influence on the transcription of several genes, including sodA in *E. coli* (Wu & Weiss, 1991; Li & Demple, 1994; Jair et al., 1996). Similarly, the inorganic peroxide, H$_2$O$_2$, has been shown to induce at least 30 genes in *E. coli*. The response of a subset of these genes is OxyR-dependent (Christman et al., 1989; Storz et al., 1990). In the presence of H$_2$O$_2$, the tetrameric OxyR becomes active due to the formation of an intramolecular disulfide bond between two cysteine residues (Cys199 and Cys208). OxyR is deactivated when the disulfide bond is re-reduced by thioredoxin or glutathione, which accumulate upon relief of oxidative stress (Aslund et al., 1999; Zheng et al., 1998).

OxyR involvement has been shown to control the expression of bacterial catalase (*katB*) and alkyl hydroperoxide reductase (*ahpB*) genes in *Pseudomonas aeruginosa* (Ochsner et al., 2000). However, no such dependency has been reported so far for the expression of bacterial *gst* genes. The present study provides convincing evidence to show the requirement of OxyR for expression of *Abgst01*. However, the OxyR-dependent activation of *Abgst01* expression is only seen in the presence of organic peroxides such as CHP, TBHP and 13-HPODE. Ironically, inorganic oxidative stress inducers such as H$_2$O$_2$ and paraquat showed no influence on the expression of *Abgst01*. However, MeP induced *Abgst01* in an OxyR-dependent manner (Fig. 3a). Although the influence of MeP on *Abgst01* expression is clear, the level of expression is low when compared with its expression in the presence of organic peroxides (Fig. 2). MeP has been shown to induce oxidative stress in a variety of animal models (Edwards et al., 2013). If these reports are taken into consideration, MeP’s effect on *Abgst01* expression appears to be indirect. The MeP-induced oxidative stress in *A. baumannii* DS002 might be the direct cause of increased expression of...
Abgst01, as well as other OxyR-dependent genes. The observation most difficult to explain, however, is the influence of H$_2$O$_2$ on Abgst01 expression. Expression of Abgst01 remained unaltered in the presence of H$_2$O$_2$. Both qPCR experiments and promoter activity assays gave no indication of an activation of Abgst01 in the presence of H$_2$O$_2$ (Fig. 2). Normally, OxyR acquires its active conformation in the presence of H$_2$O$_2$ and then can interact with the consensus OxyR-binding site found upstream of OxyR-dependent promoters (Tartaglia et al., 1989; Aslund et al., 1999). The existence of an OxyR-binding site and requirement of OxyR for expression of Abgst01 is evident from our promoter fusion studies and the oxyR knockout experiments. At this stage it is unclear why H$_2$O$_2$ does not show an influence on Abgst01 expression. It may be due to the existence of structural differences in OxyR, which means it responds to a yet-identified signal in A. baumannii DS002. Unusual behaviour of OxyR is not uncommon among bacteria. A number of OxyR homologues identified among pathogenic and free-living bacteria have been shown to have properties that are significantly different from those of E. coli OxyR (LeBlanc et al., 2008; Whitby et al., 2012; Parti et al., 2013). Their transcriptional regulation pattern, hydroperoxide-sensing behaviour and conserved cysteine composition have been shown to differ (Wei et al., 2012).

As an opportunistic pathogen, A. baumannii causes severe and acute nosocomial infections in patients admitted to intensive care units (Towner, 2009). Eradication of this bacterium from infection sites is highly problematic due to its intrinsic antibiotic resistance and versatile ecological adaptability (Woodford et al., 2011). While adapting to a pathogenic and a free-living lifestyle, A. baumannii strains face an onslaught of both endogenous and exogenous reactive oxygen species. Their adaptability to these harsh conditions points towards the existence of an efficient mechanism to counter oxidative stress. However, to date limited efforts have been made to understand the defence system against oxidative stress in A. baumannii. The work reported in this study explains, we believe for the first time, an OxyR-dependent expression of Abgst01 and its protective role against classical oxidative stress inducers such as H$_2$O$_2$ and TBHP. During oxidative metabolism in mammals, degradation of linoleic acid produces a number of bioactive products, including 13-HPODE, 13-hydroxyoctadeca-9,11-dienoic acid (13-HODE) and 2,4-dienone 13-oxooctadeca-9,11-dienoic acid, a bioactive endogenous substrate for mammalian glutathione transferase (Antonov et al., 2007). Their transcriptional regulation pattern, hydroperoxide-sensing behaviour and conserved cysteine composition have been shown to differ (Wei et al., 2012).

**REFERENCES**


