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# A proteome analysis of the response of a *Pseudomonas aeruginosa oxyR* mutant to iron limitation

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**Abstract** In *Pseudomonas aeruginosa* the response to oxidative stress is orchestrated by the LysR regulator OxyR by activation of the transcription of two catalase genes (*katA* and *katB*), of the alkyl-hydroxyperoxidases *ahpCF* and *ahpB*. Next to the expected high sensitivity to oxidative stress generated by reactive oxygen species (ROS: $H_2O_2$, $O_2^-$), the *oxyR* mutant shows a defective growth under conditions of iron limitation (Vinckx et al. 2008). Although production and uptake of the siderophore pyoverdine is not affected by the absence of *oxyR*, the mutant is unable to satisfy its need for iron when grown under iron limiting conditions. In order to get a better insight into the effects caused by iron limitation on the physiological response of the *oxyR* mutant we decided to compare the proteomes of the wild type and the mutant grown in the iron-poor casamino acids medium (CAA), in CAA plus $H_2O_2$, and in CAA plus the strong iron chelator ethylenediamine-N,N′-bis(2-hydroxyphenylacetic acid) (EDDHA). Especially in the presence of hydrogen peroxide the *oxyR* cells increase the production of stress proteins (Dps and IbpA). The superoxide dismutase SodM is produced in higher amounts in the *oxyR* mutant grown in CAA plus $H_2O_2$. The PchB protein, a isochorismate-pyruvate lyase involved in the siderophore pyochelin biosynthesis is not detectable in the extracts from the *oxyR* mutant grown in the presence of hydrogen peroxide. When cells were grown in the presence of EDDHA, we observed a reduction of the ferric uptake regulator (Fur), and an increase in the two subunits of the succinyl-CoA synthetase and the fumarase FumC1.

**Keywords** *Pseudomonas aeruginosa* · *oxyR* · Oxidative stress · Iron limitation · Proteome

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## Introduction

In aerobic microorganisms such as *Pseudomonas aeruginosa* the respiration process based on oxidative phosphorylation can result in the formation of dangerous oxygen derivatives such as the superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($OH^\bullet$), resulting in protein carbonylation, cofactor degradation, lipid peroxidation and DNA damage (Storz and Imlay 1999; Imlay 2002; Imlay 2003). Like in *E. coli*, *P. aeruginosa* regulates the expression of different reactive oxygen species defense enzymes via the LysR regulator OxyR, namely the catalase gene *katB*, and the alkyl-

hydroxyperoxidase genes *ahpCF* and *ahpB*, while partially up-regulating the major catalase KatA (Ochsner et al. 2000; Heo et al. 2010). Besides a greatly increased sensitivity to oxidative stress a *P. aeruginosa* mutant shows a decreased virulence and cytotoxicity (Hassett et al. 2000; Lau et al. 2005; Melstrom et al. 2007). We previously observed that a *P. aeruginosa oxyR* mutant growth is impaired in iron-limiting media, although the production of the siderophore pyoverdine and its uptake are not affected (Vinckx et al. 2008). The same growth impairment was also observed in media containing high concentrations of $FeCl_3$ (>20 μM) (Vinckx et al. 2008). High iron levels are known to induce the production of ROS via the Fenton reaction (Imlay 2003), while under conditions of iron limitation there could be a decrease in iron co-factored superoxide dismutase and catalases (which are heme-containing enzymes) resulting in reduced ability to fight oxidative stress (Ueda et al. 2003). Furthermore, the mutant shows other phenotypic alterations such as absence of motility, strongly reduced rhamnolipid surfactant production, and increased production of the secondary phenazine metabolite pyocyanin (Vinckx et al. 2010). These results suggest that the impact of the *oxyR* gene deletion in *P. aeruginosa* is not confined to the resistance to ROS only and/or that OxyR could regulate other genes. For this reason we decided to study the impact of the loss of OxyR by comparing the proteomes of a wild type *P. aeruginosa* and its *oxyR* mutant grown in the iron-limiting CAA medium supplemented or not with hydrogen peroxide, and under extreme iron limitation imposed by the presence of EDDHA.

## Materials and methods

### Bacterial strains and growth conditions

Bacterial cultures were grown with aeration at 37°C for *P. aeruginosa* strains in iron-poor casamino acids medium (CAA). The *P. aeruginosa* strains used in this study are wild type PAO1 (ATCC 15692) and its gentamicin (Gm)-resistant *oxyR* mutant (Ochsner et al. 2000). To create oxidizing conditions, 100 μl of a 30% (v/v) $H_2O_2$ solution was added to the culture (250 ml) during exponential phase.

### Preparation of soluble protein extracts

Cells were grown to stationary phase. After centrifugation in a Sorvall RC5B SLA rotor (15 min, 9,000 rpm, 4°C), the pellet was re-suspended in Tris 25 mM pH 8 and washed twice. Next, the pellet was re-suspended in 3 ml Tris 25 mM pH 8 containing 40 μl 0.1 M EDTA (pH 8), protease inhibitor mix (Sigma) and DNase I (Sigma). The suspension was sonicated till the solution was clear (Bioblock Scientific). Centrifugation was performed (2 h, 28,000 rpm) to remove cell debris. The remaining supernatant is the soluble protein fraction which was quantified using the QuantiPro bicinchoninic acid assay kit (Sigma).

### Two-dimensional gel electrophoresis (2D)

Immobilized linear pH gradients (11 cm, pH 4–7, GE Healthcare) were used for the first dimension:isoelectric focusing (IEF). One mg of proteins was treated with the 2D clean-up kit (GE Healthcare) to prepare the samples for 2D electrophoresis, removing interfering, non-protein substances. The resulting pellet was resolved in a rehydration solution containing 7 M urea, 2 M thiourea, 2% (w/v) 3[(3-Cholamidopropyl) dimethylammonio]-propanesulfonic acid (CHAPS), carrier ampholyte IPG-buffer (0.5%, v/v) and 1% bromophenol blue (BPB) (0.002%, w/v). Approximately 1 mg proteins were loaded on an immobilized pH-gradient, range 4–7 (GE Healthcare). Next, these IPG strips were rehydrated overnight at 20°C in a swelling tray. The first dimension was performed with the Ettan IPGphor III (GE Healthcare).

After the first dimension, the IPG strips were reduced and alkylated in equilibration buffers (15 min shaking in 10 ml) composed of 6 M urea, 75 mM Tris–HCl pH 8.8, 2% SDS, 29.3% glycerol, 0.002% BPB and 1% (w/v) dithiothreitol (DTT) for reduction or 2.5% (w/v) iodoacetamide for alkylation.

Second dimension was accomplished with self-made gels with a final gel concentration of 12.5% acrylamide using the DALTsix electrophoresis system (GE Healthcare). After the second dimension, the gels were fixed overnight and stained with Coomassie G-250 staining.

Gel images were captured using a GS-800 densitometer (Bio-Rad Laboratories). Image analyses were performed using PDQuest 2D-analyis software (Bio-

Rad Laboratories). Spots displaying a different intensity were selected for further analysis.

Proteins were digested according to the protocol of Shevchenko et al. 2006. In a first step, the spots were de-stained with 25 mM ammonium carbonate in 50% acetonitrile followed by a drying step in vacuum conditions. Next, 30 µl of 10 mM DTT in 100 mM ammonium carbonate was added to the dried spots, which were then incubated for 45 min in the dark at room temperature. After centrifugation, the supernatant was discarded and the samples were incubated in 100 µl of 100 mM ammonium carbonate for 10 min. After centrifugation, the supernatant was discarded, 100 µl acetonitrile was added and the incubation was repeated. Again, both steps were repeated. Ten µl of digestion buffer containing 12.5 ng trypsin $ml^{-1}$ were added to the sample and incubated 45 min in an ice bath. Finally, 30 µl of 50 mM ammonium carbonate was added and the sample incubated overnight at 37°C. Following overnight incubation, the supernatant was collected in two steps which were repeated three times. In a first step, 20 µl 20 mM ammonium carbonate was added and the sample was sonicated during 20 min (Bioblock Scientific). In a second step, 50 µl 5% formic acid in 50% acetonitrile were added, followed by a sonication step. Each time, the supernatant was collected and kept for further analysis.

## Liquid chromatography and tandem mass spectrometry (LC–MS–MS)

Protein digests were analyzed by liquid chromatography-electrospray ionization-tandem mass spectrometry, as described previously (Dumont et al. 2007). Each digest was taken to dryness by in vacuo centrifugal evaporation and re-suspended in 5% (v/v) acetonitrile in 100 mM acetic acid (20 µl). This solution contained 4 pg $\mu l^{-1}$ of cortisone as an internal analytical standard to monitor flow stability of the chromatographic system (by retention time) and overall performance (by peak height as derived from the selected ion chromatogram for $[M + H]^+ = m/z$ 361.2) of the ion trap mass spectrometer (LCQ Classic, Thermo Electron Corporation, San Jose, CA, USA). Of each digest, 10 µl was injected (autoinjector AS3000, Thermo Electron) and trapped peptides were separated on an analytical column (Biosphere C18, 200 mm × 0.05 mm., 5 µm, NanoSeparations) using a linear gradient from 5 to 60% (v/v) acetonitrile in water-containing 100 mM acetic acid in 55 min. The eluate from the analytical column was sprayed from a gold-coated fused silica emitter (5 µm i.d., NanoSeparations). The LCQ automated protocols were used to optimize the ion optics and calibration parameters. The mass spectrometer was operated in a data-dependent acquisition mode to automatically switch between MS ($m/z$ 300–1,500 in centroid mode at a maximum injection time of 150 ms) and MS/MS acquisition on the three most intense precursor ions, controlled by Xcalibur 1.3 software.

## Database search

Peak lists in DTA file format were generated from MS/MS raw data files using the CreateDTA tool available in Sequest v27 within BioWorks v3.0 (Thermo Electron). DTA files were examined with the search engines Sequest v27 and Mascot 2.2 (Matrix Science) using the database NCBInr (subset *P. aeruginosa*, 28.328 entries). Sequest parameters were set as follows: $X_{correlation} \geq 1.8 \geq 2.5$ or $\geq 3.5$ for singly, doubly or triply charged peptide ions; $\Delta C_n > 0.08$; precursor and product ion mass tolerance ±3 and ±1 Da; enzyme: trypsin; one missed cleavage allowed; static chemical modification: cysteine-carbamidomethylation; dynamic chemical modification: oxidation of methionine, histidine, and tryptophan. Mascot search parameters were: parent and peptide ion mass tolerance ±1.5 and ±1 Da, enzyme: trypsin/P, one missed cleavage allowed and peptide expect value $< 0.05$. For each protein identified, at least two sibling peptides were detected by both search engines.

## OxyBlot™ protein modification determination

Soluble proteins were extracted by sonication. Briefly, overnight bacterial cultures were collected by centrifugation and then washed with 25 mM Tris–HCl (pH 8.0) once. Cells were suspended in 25 mM Tris–HCl (pH 8.0) containing protease inhibitor cocktails (Sigma), and sonication was performed to obtain a clear cell lysate. Finally, the soluble protein fraction was collected after centrifugation and concentration measured by the BCA (bicinchronic acid) method using QuantPro Kit (Sigma). Subsequently, protein carbonylation levels were detected with the help of an OxyBlot™ Protein Oxidation Detection Kit

(Millipore Corporation), which measures carbonyl groups of proteins generated by oxidative reactions. All procedures were performed according to the manufacturer's instructions. Carbonyl groups in the air-exposed proteins were derivatized with 2,4-dinitrophenyl hydrazine (DNPH), and 40 μg of each DNPH-derivatized protein were loaded on a 10% SDS-PAGE gel and the electrophoresis run using a Bio-Rad gel system. After electrophoresis, derivatized proteins together with non-derivatized controls were electroblotted to nitrocellulose membranes, and immunodetection of DNPH-derivatized proteins was done using a rabbit anti-DNP antibody and, as secondary antibody, a goat anti-rabbit IgG conjugated with horseradish peroxidase (HRP). Antibody bindings were detected by enhanced chemiluminescence (ECL, Pierce) detection and blots were exposed to X-ray films (Kodak) for different time intervals to obtain optimal results and then quantified by densitometry. Carbonylation levels are expressed as the relative intensity compared to wild type level.

## Results and discussion

The proteins showing a difference in intensities in wild type and in the *oxyR* mutant are presented in Table 1.

**Table 1** proteins showing a difference in expression between wild type and *oxyR* mutant of *P. aeruginosa* grown in CAA, CAA + $H_2O_2$, and CAA + EDDHA

| | WT/*oxyR* | Protein | gi number | Signal | Matching peptides | Mw (Da) |
|---|---|---|---|---|---|---|
| CAA | | | | | | |
| PA3326 | WT | ClpP | 15599687 | No | 7 | 22,128 |
| PA0059 | WT | OsmC | 15595257 | No | 6 | 15,714 |
| PA4739 | WT | OsmY | 15599933 | Yes | 1 | 11,753 |
| PA3347 | WT | Hypothetical | 15598543 | No | 4 | 11,276 |
| PA3332 | *oxyR* | Hypothetical | 15598528 | No | 4 | 15,967 |
| PA0962 | *oxyR* | Dps | 15596159 | No | 5 | 17,482 |
| PA5289 | *oxyR* | Hypothetical | 15600482 | No | 1 | 9,527 |
| CAA + $H_2O_2$ | | | | | | |
| PA4495 | WT | Hypothetical | 15599691 | Yes | 5 | 24,921 |
| PA4230 | WT | PchB | 15599426 | No | 5 | 11,482 |
| PA5489 | *oxyR* | DsbA | 15600682 | Yes | 10 | 23,360 |
| PA4468 | *oxyR* | SodM | 15599664 | No | 2 | 20,492 |
| PA1579 | *oxyR* | Hypothetical | 15596776 | Yes | 7 | 22,211 |
| PA0962 | *oxyR* | Dps | 15596159 | No | 6 | 17,482 |
| PA3126 | *oxyR* | IbpA | 15598322 | No | 4 | 16,573 |
| PA0941 | *oxyR* | Hypothetical | 15596138 | No | 3 | 8,393 |
| CAA + EDDHA | | | | | | |
| PA0139 | WT | AhpC | 15595337 | No | 7 | 20,541 |
| PA0423 | WT | PasP | 15595620 | Yes | 8 | 20,777 |
| PA4764 | WT | Fur | 15599978 | No | 4 | 15,234 |
| PA3807 | WT | Ndk | 15599002 | No | 2 | 15,605 |
| PA2570 | WT | LecA | 15597766 | No | 2 | 11,846 |
| PA4470 | *oxyR* | FumC | 15599666 | No | 13 | 48,696 |
| PA0291 | *oxyR* | OprE | 15595488 | Yes | 9 | 49,667 |
| PA1588 | *oxyR* | SucC | 15596785 | No | 14 | 41,543 |
| PA1589 | *oxyR* | SucD | 15596786 | No | 23 | 30,266 |
| PA0660 | *oxyR* | Hypothetical | 15595857 | No | 7 | 34,815 |
| PA0085 | *oxyR* | Hcp1 | 15595283 | No | 5 | 17,404 |

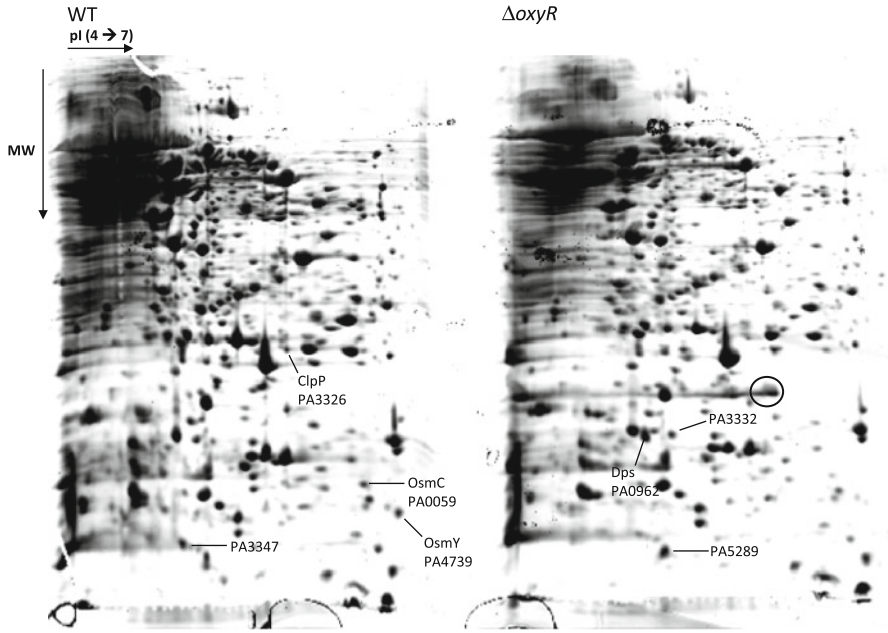


**Fig. 1** 2D gel separation of soluble proteins of *P. aeruginosa* wild type (*left*) and *oxyR* mutant (*right*) grown in CAA medium. The proteins are described in Table 1. See text for details

Down-regulated proteins in *oxyR* mutant

*CAA*

Only four proteins were clearly down-regulated in the *oxyR* mutant, ClpP (PA3326), OsmC (PA0059), OsmY (PA4739), and PA3347 (Fig. 1). ClpP is a serine protease which has been shown to be involved in resistance to oxidative and nitrosative stress in different bacteria, for antibiotic tolerance, and for virulence (Michel et al. 2006; Loughlin et al. 2009; Chattoraj et al. 2010; Li et al. 2010; Park et al. 2010). Interestingly, in a *P. fluorescens* strain, ClpP was found to be indispensable for the production of cyclic lipopeptides, hence for motility (de Bruijn and Raaijmakers 2009). OsmC is a homologue of the OhR protein involved in resistance to organic peroxides (Atichartpongkul et al. 2001; Lesniak et al. 2003). However, in *P. aeruginosa*, OhR, but not OsmC is involved in such resistance, while OsmC is needed for growth under high salt conditions (Atichartpongkul et al. 2001). Likewise, *osmY* encodes a periplasmic protein involved in the resistance to osmotic stress in *E. coli* and its transcription, like *osmC* is under the control of the sigma factor RpoS (Yim and Villarejo 1992; Yim et al. 1994). The product of PA3347 is a protein involved in a multiple phosphorelay cascade involving the HptB protein and the PA3346 phosphatase; a PA3347 mutant shows higher swarming activity compared to the wild type (Hsu et al. 2008).

*CAA* + $H_2O_2$

Two proteins are clearly produced in higher amounts in the wild-type, PchB and PA4495 (Fig. 2). The protein encoded by PA4495 is hypothetical, but is annotated as a short chain dehydrogenase. PchB is an isochorismate-pyruvate lyase involved in the biosynthesis of salicylate, the precursor of the second siderophore of *P. aeruginosa*, pyochelin (Serino et al. 1995; Gaille et al. 2002). Pyochelin is known to generate ROS, especially in conjunction with pyocyanin (Britigan et al. 1992). Some pyochelin-metal complexes can also be very toxic for *P. aeruginosa* (Baysse et al. 2000). Down-regulation of pyochelin production in the *oxyR* mutant with weakened oxidative stress defense responses could therefore be a way to protect cells from the generation of ROS via the Fenton reaction (Teitzel et al. 2006).

*CAA* + *EDDHA*

The production of five proteins is down-regulated in the *oxyR* mutant: AhpC, Fur, Ndk, LecA, and PasP

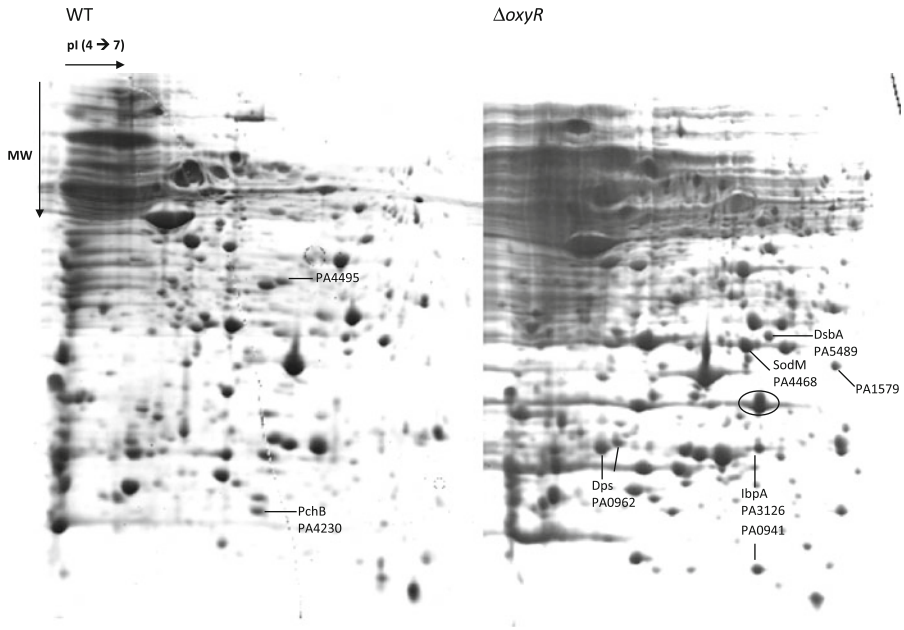


**Fig. 2** 2D gel separation of soluble proteins of *P. aeruginosa* wild type (*left*) and *oxyR* mutant (*right*) grown in CAA medium with addition of $H_2O_2$. The proteins are described in Table 1. See text for details

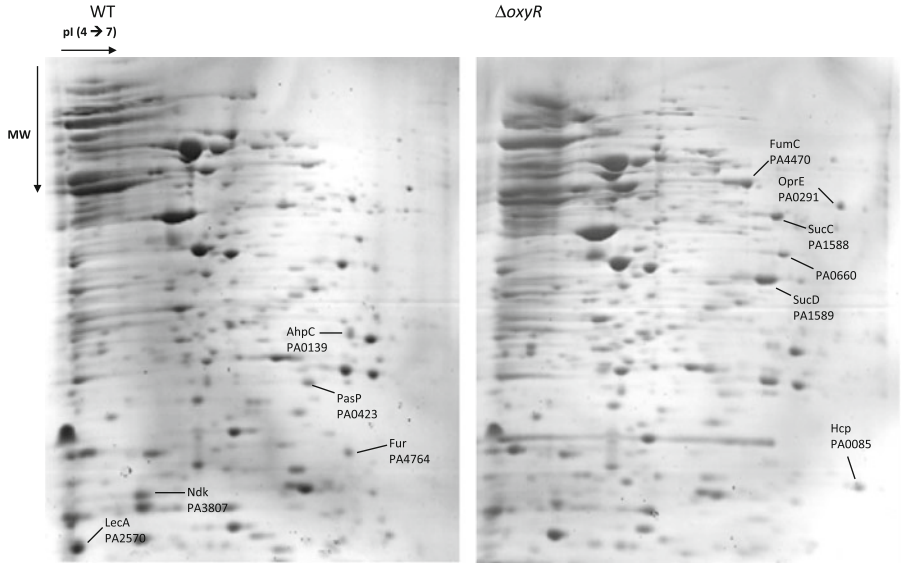


**Fig. 3** 2D gel separation of soluble proteins of *P. aeruginosa* wild type (*left*) and *oxyR* mutant (*right*) grown in CAA medium with addition of EDDHA. The proteins are described in Table 1. See text for details

(Fig. 3). AhpC is a subunit of the alkylhydroperoxide reductase encoded by *ahpC*, which is activated by OxyR (Ochsner et al. 2000). In other experiments using LB medium we found the same repression of AhpC (results not shown). Fur is a general regulator of iron uptake in many Gram-negative bacteria (Escolar et al. 1999). In *P. aeruginosa* the Fur repressor controls many genes, including other regulators (van Oeffelen et al. 2008; Cornelis et al. 2009). In *E. coli fur* is controlled by OxyR and it has been shown that $H_2O_2$ in submicromolar concentrations can oxidize Fur and inactivate it (Varghese et al. 2007). Ndk is a diphosphate nucleoside kinase, which has a broad specificity, generating the different triphosphate nucleotides (Kapatral et al. 2000). The LecA galactophilic lectin is a surface-exposed protein contributing to the formation of biofilms and its gene is under the control of quorum sensing (Diggle et al. 2006; Kirkeby et al. 2006). PasP is a small protease secreted by *P. aeruginosa*, which was detected in the

extracellular proteome of PAO1 (Marquart et al. 2005; Tang et al. 2009).

Up-regulated proteins in the *oxyR* mutant

*CAA*

The most highly induced protein in the *oxyR* mutant is Dps (PA0962) and it is found both in CAA and CAA + $H_2O_2$ grown cells (Figs. 1, 2). Dps (DNA-binding proteins from starved cells) belongs to the ferritin family of proteins and sequesters 12 Fe atoms (Andrews 2010). Dps proteins protect DNA against Fenton-mediated oxidative stress since they use $H_2O_2$ to catalyze the oxidation of $Fe^{2+}$ at ferroxidase centers (Andrews 2010; Bellapadrona et al. 2010). Dps also protects DNA from hydroxyl radical-mediated damage by binding to it, shielding the molecule (Chiancone and Ceci 2010). Two other proteins are present in higher abundance in the protein extracts of the *oxyR* mutant: the products of PA3332 and PA5289. Both genes encode a hypothetical protein of unknown function.

*CAA + $H_2O_2$*

Next to the already mentioned Dps, five other proteins were found to be highly expressed in the *oxyR* mutant when grown in the presence of hydrogen peroxide (Fig. 2), DsbA, SodM, IbpA, and two hypothetical proteins encoded by PA1579 and PA0941.

The *sodM* (*sodA*) gene is not part of the OxyR regulon and encodes a manganese-co-factored superoxide dismutase (Ochsner et al. 2000). The *sodA* and *fumC* genes are both regulated by Fur in *P. aeruginosa* and SodA is produced under condition of iron limitation to replace the iron co-factored SodB superoxide dismutase (Hassett et al. 1995, 1997). Another protein showing higher abundance in the *oxyR* mutant extracts is the periplasmic DsbA thiol-disulfide interchange protein (Shouldice et al. 2010). PA1579 encodes a hypothetical protein with a signal peptide and a START lipid binding domain. IbpA was also detected in the proteome fraction of the *oxyR* mutant (Fig. 2). In *E. coli*, IbpA and IbpB are known to be small heat-shock proteins involved in the refolding of protein aggregates formed when cells are exposed to heat stress (Laskowska et al. 1996; Kitagawa et al. 2002; Ratajczak et al. 2009). Interestingly, this protein has also been shown to be involved in the resistance to oxidative stress induced by exposure to copper (Matuszewska et al. 2008) or by treatment with superoxide (Kitagawa et al. 2000). In another study involving proteome analysis it was found that exposure of *P. aeruginosa* cells to arsenite (AsIII) generates an oxidative stress and the induction of both IbpA and Dps (Parvatiyar et al. 2005), suggesting a role of these two proteins in defense against oxidative stress like in *E. coli* (Kitagawa et al. 2002; Matuszewska et al. 2008). PA0941 encodes a small hypothetical protein annotated as a glutaredoxin. Interestingly, glutaredoxins have been shown to play a role in the defence of *E. coli* against oxidative stress and the *grx1* gene is under the control of OxyR in this bacterium (Tao 1997; Fernandes and Holmgren 2004).

*CAA + EDDHA*

Six proteins are more abundant in the extracts from *oxyR* cells grown in CAA medium in the presence of the strong iron chelator EDDHA (Fig. 3). Although we have shown previously that the *oxyR* mutant cannot grow in this medium, using large inocula (>$10^8$ cells $ml^{-1}$) growth is possible (Vinckx et al. 2008). Interestingly, three proteins involved in the TCA cycle are overproduced by the *oxyR* mutant: FumC, SucC and SucD. FumC is a fumarase and the *fumC1* gene is repressed by Fur (Hassett et al. 1997), which is to be put in relation with the observed decrease in the Fur protein compared to the wild type. FumC is a hydratase devoid of Fe S center, hence insensitive to oxidative damage (Lemire et al. 2010). SucC and SucD are the two subunits of the succinyl coenzyme A synthase (Kapatral et al. 2000). This enzyme is an iron sulphur protein which can be inactivated by ROS (Imlay 2003). Overproduction of the two subunits could a way to compensate for the loss of Fe S clusters due to oxidative damage as demonstrated in the case of *P. fluorescens* cells exposed to aluminium (Lemire et al. 2010). In the same study the authors demonstrated the concomitant overproduction of FumC, SucC and SucD in Al-stressed cells. The OprE porin is more abundant in the extracts of the *oxyR* mutant. The *oprE* gene is known to be induced by anaerobiosis in *P. aeruginosa* (Yamano et al. 1998) and has been shown to participate in the resistance to chromate in this bacterium (Rivera et al. 2008). PA0085 encodes

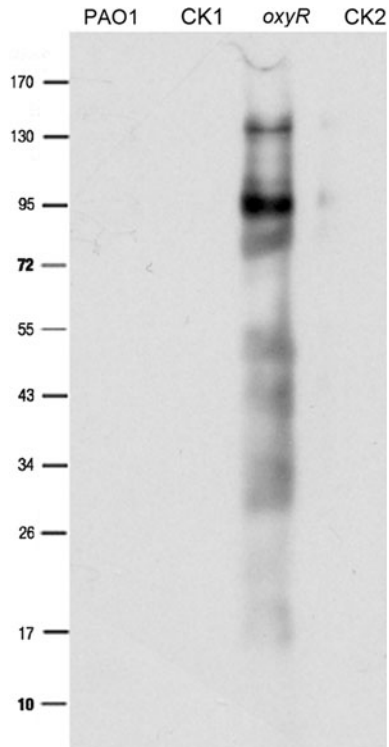


**Fig. 4** Detection of carbonylated proteins by Western blot after derivatization. The CK1 and CK2 lanes correspond to non-derivatized proteins from wild type and *oxyR* cells grown in CAA medium, respectively. See text for details

Hcp1, a small ring-like protein which is part of the type VI secretion system in *P. aeruginosa* (Mougous et al. 2006).

PA0660 is a hypothetical protein which is annotated as a nitropropane dioxygenase, the function of which is not known.

Protein carbonylation

As mentioned in the Introduction, ROS can oxidize proteins resulting in protein carbonylation (Nystrom 2005). As shown in Fig. 4, more proteins are detected as being carbonylated in the extract of *oxyR* cells grown in CAA medium, in agreement with their higher exposure to ROS.

## Conclusion

The here presented proteomic comparison between wild-type and *oxyR* mutant *P. aeruginosa* cells is certainly not exhaustive, but brings some interesting observations. A previous study realized by Panmanee et al. 2008 compared the proteomes of cells grown in the $H_2O_2$-genarting LB medium (Ochsner et al. 2000). In this study we used the casamino acids medium, which is known to be iron-limiting (Cornelis et al. 1992) and is generating much less $H_2O_2$ compared to LB (0.15 μM $min^{-1}$ compared to 0.8 μM $min^{-1}$ for LB, unpublished results). Furthermore, in their study, Panmanee et al. looked only at the oxidized proteins in the *oxyR* mutant, explaining the absence of correlation between the two studies.

One surprising result from this study is the response to oxidative stress in the *oxyR* mutant by induction of stress proteins including chaperones and by the production of the manganese co-factored superoxide dismutase, suggesting that in *P. aeruginosa* other regulator(s) could control the response to oxidative stress, such as OhrR which is a negative regulator of *ohr*, a gene encoding a thiol peroxidase (Atichartpongkul et al. 2010).

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