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Running Title: RCS-sensing two-component system

ABSTRACT

Two-component systems (TCS) are signalling pathways that allow bacterial cells to sense, respond and adapt to fluctuating environments. Among the classical TCS of *Escherichia coli*, HprSR has recently been shown to be involved in the regulation of *msrPQ*, which encodes the periplasmic methionine sulfoxide reductase system. In this study, we demonstrate that hypochlorous acid (HOCl) induces the expression of *msrPQ* in an HprSR-dependant manner, whereas H_2O_2, NO and paraquat (a superoxide generator) do not. Therefore, HprS appears to be an HOCl-sensing histidine kinase. Using a directed mutagenesis approach, we show that Met residues located in the periplasmic loop of HprS are important for its activity: as HOCl preferentially oxidizes Met residues, we provide evidence that HprS could be activated via the reversible oxidation of its methionine residues, meaning that MsrPQ plays a role in switching HprSR off. We propose that the activation of HprS by HOCl could occur through a Met redox switch. HprSR appears to be the first characterized TCS able to detect reactive chlorine species (RCS) in *E. coli*. This study represents an important step towards understanding the mechanisms of RCS resistance in prokaryotes.

IMPORTANCE

Understanding how bacteria respond to oxidative stress at the molecular level is crucial in the fight against pathogens. HOCl is one of the most potent industrial and physiological microbiocidal oxidants. Therefore bacteria have developed counterstrategies to survive HOCl-induced stress. Over the last decade, important insights into these bacterial protection factors have been obtained. Our work establishes HprSR as a reactive chlorine species-sensing, two-component system in *Escherichia coli* MG1655, which regulates the expression of MsrPQ, a repair system for HOCl-oxidized proteins. Moreover we provide evidence suggesting that HOCl could activate HprS through a methionine redox switch.

INTRODUCTION

During infection the host innate immune system produces high levels of reactive oxygen and chlorine species (ROS, RCS), such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), in order to kill invading pathogens[1]. One major challenge for bacteria is to overcome oxidative stress and while bacterial responses to H_2O_2 have been extensively studied, much less is known about their response to HOCl. In the cell, HOCl can damage biomolecules like DNA, lipids and proteins [2,3]. Within proteins, HOCl preferentially oxidizes sulfur-containing amino acids such as cysteine and methionine[4]. The thiol group of the cysteine can be oxidized to sulfenic (-SOH), sulfinic ($-SO_2H$) and sulfonic ($-SO_3H$) acids or to disulphide bonds (S-S)[5]. The thioether side chain of methionine can be oxidized to sulfoxide, thereby converting methionine into methionine sulfoxide (Met-O) [6].

Bacterial cells can prevent damage caused by HOCl through various mechanisms and pathways. Many of them are general ROS-responsive pathways, but others detect and respond specifically to HOCl [7]. In *Escherichia coli*, recent studies identified several regulatory factors activated by HOCl, like the transcription factors HypT (a LysR-type regulator) and RclR (an AraC-type activator). HypT detects and responds to HOCl through the oxidation of three methionine residues [8], whereas RclR is activated through the oxidation of two cysteine residues [9]. Upon activation, HypT up-regulates genes involved in Met and Cys biosynthesis while repressing genes involved in iron acquisition. The activation of RclR induces the expression of *rcl* genes that are necessary to resist HOCl treatment [9]. Similarly, NemR (a TetR-family repressor) functions as an HOCl-responsive transcription factor in *E. coli* which controls the expression of genes involved in HOCl resistance like *nemA* and *golA* [10]. Repair of HOCl damage represents another way for bacteria to survive this stress. The methionine sulfoxide reductases (MSR), which repair HOCl-oxidized proteins, are involved in HOCl resistance[11].

Two-component systems (TCS) are signal transduction pathways typically made of a membrane-bound histidine kinase sensor (HK) and its cytoplasmic response regulator (RR). They sense external signals and trigger bacterial adaptive responses to a wide range of environments, stress and growth

conditions [12]. Our previous work showed that in *E. coli* the HprSR (formerly YedVW) two-component system is involved in the induction of the *msrPQ* genes in response to HOCl[13]. The *msrPQ* encodes the periplasmic methionine sulfoxide reductase (MsrPQ), composed of a periplasmic molybdopterin-containing oxidoreductase (MsrP) and a haem-containing, membrane-bound protein (MsrQ). MsrP catalyzes the reduction of protein-bound Met-O and therefore repairs periplasmic oxidized proteins after HOCl stress [13].

In the *E. coli* genome, the *hprSR* operon is upstream of the *msrPQ* genes and transcribed in the opposite direction. The intergenic region contains *hiuH*, a gene encoding a transthyretin-like periplasmic protein: this protein hydrolyses 5-hydroxyisourate (5-HIU), a compound formed via the oxidation of urate [14]. The Ishihama and Ogasawara laboratories showed that the expression of *hiuH* was induced by H_2O_2 in an HprSR-dependent manner [15]. Moreover, they identified the oxidation of the Cys165 residue located in the transmembrane domain of HprS as being essential for the sensing of H_2O_2 [16]. Finally, they suggested renaming this system HprSR for hydrogen peroxide response sensor/regulator.

In this study, we show that *hiuH*, *msrP* and *msrQ* genes belong to the same operon and are under the control of HprSR. Our results show that HprS is predominantly an RCS and not a ROS sensing histidine kinase. Using a site-specific mutagenesis approach, we report that two methionine residues located in the periplasmic loop are critical for HprS activity and that Cys165 is more likely to be involved in signal transduction than signal detection. This report reveals for the first time a two-component system that senses RCS in *E. coli*. Interestingly, it also suggests that RCS could activate HprS through a methionine redox switch.

RESULTS

hiuH, *msrP* and *msrQ* belong to the same operon

In the genome of *E. coli*, the *hiuH* gene is located 109 bp upstream of *msrP* and 1,114 bp upstream of *msrQ* (Fig. 1A). The expression of *hiuH*, *msrP* and *msrQ* has been reported to be under the control of the HprSR two-component system [13,16], with an HprR box located upstream of *hiuH* (87 to 70 bp before the *hiuH* start codon). These observations suggest that *hiuH, msrP* and *msrQ* might belong to the same operon. This hypothesis was explored further by RT-PCR using converging pairs of primers located within each of the three genes: after RNA reverse transcription, PCR amplifications were observed between *hiuH* and *msrP* and between *msrP* and *msrQ*, showing a tri-cistronic organisation (Fig. 1B). As positive and negative controls, we used chromosomal DNA and total RNA, respectively, as templates for PCR amplifications with the same pairs of primers. Together, these experiments strongly suggest that *hiuH*, *msrP* and *msrQ* are part of the same operon.

HOCl is a better signal for HprSR than H_2O_2

To explore the physiological signals controlling the HprS-HprR regulatory system, we investigated the expression of the HprR-activated genes *hiuH, msrP, hprS and hprR,* by qRT-PCR analysis and by measuring the β-galactosidase activity originating from *hiuH-lacZ* or *msrP-lacZ* translational fusions. First, we re-evaluated the response of HprSR to H_2O_2 as previously reported by Urano and collaborators[15]. We carried out qRT-PCR analysis on RNAs isolated from wild type *E. coli* cells grown to mid-log phase and treated with H_2O_2 (Fig. 2A). The results show a 2-fold up-regulation of *hprS*, *hprR* and *msrP* and an approximately 7-fold up-regulation of *hiuH* in response to H_2O_2 treatment (6 mM as used by *Urano et al*, 2015). The induction by H_2O_2 was dependent on the presence of HprR since no up-regulation was observed in a Δ*hprR* mutant. However, using translational *hiuH-lacZ* or *msrP-lacZ* reporter fusions, no up-regulation was observed after H_2O_2 treatment in the wild-type strain (Fig. 2B). These results agree with previous observations reported by Gennaris *et al.*, showing that exposure of wild-type cells to H_2O_2 did not induce the synthesis of MsrP, despite slightly

inducing its transcription. However, Gennaris *et al.* reported an increase in the synthesis of MsrP after HOCl treatment[13]. Indeed, qRT-PCR analysis shows that HOCl treatment (4 mM) led to a 1,500-fold up-regulation of *hiuH* and a 60-fold up-regulation of *msrP* and an approximately 20-fold up-regulation of *hprR* and *hprS* in the wild-type strain (Fig. 2A). Using the translational *hiuH-lacZ* and *msrP-lacZ* reporter fusions, we found a 15-fold (*hiuH*) and 110-fold (*msrP*) up-regulation after HOCl stress (Fig. 2B). Collectively, these results indicate that the induction of the *hiuH-msrPQ* operon by HOCl was dependent on the presence of a functional HprSR system. Our results also reveal that HOCl is a better signal than H_2O_2 for HprSR activation. The concentration of H_2O_2 required to activate HprSR led to a survival rate of 33% whereas the concentration of HOCl required led to a survival rate of 92% (Fig 2C).

To overcome the lethality, different sub-lethal concentrations of H_2O_2 (0.5 to 3 mM) were tested for *msrP-lacZ* expression. The H_2O_2 was added at OD≈0.2 and the β-galactosidase activities were measured over a period of 3 hours. For all the sub-lethal concentrations tested, no induction of the *msrP-lacZ* fusion was observed (Fig. 3A) compared to the unstressed sample, whereas for the positive control, the *ahpC-lacZ* fusion, which responds to H_2O_2 via activation of OxyR, shows a 2.5-fold induction after 30 min of exposure to 0.5 and 1 mM H_2O_2 (Fig. 3B). These results challenge the relevance of the *in vivo* activation of HprSR by H_2O_2 and raise the question of the real signal detected by this system. Therefore, we decided to investigate the response of HprSR to different type of oxidants *in vivo*. We treated wild-type *E. coli* cells with various reactive oxygen, nitrogen and chlorine species at different sub-lethal concentrations. No significant expression of *msrP* was observed in response to diamide (a thiol-specific oxidant), paraquat (1,1'-dimethyl-4,4'-bipyridinium, a superoxide generator) or nitric oxide (NO) (Fig. 3C, 3E and 3G). The *ahpC-lacZ*, *soxS-lacZ* and the *hmpA-lacZ* fusions, which respond to diamide, paraquat and NO respectively, were used as positive controls (Fig. 3D, 3F and 3H). HprSR is activated by HOCl and *N*-Chlorotaurine (*N*-ChT), a weakly membrane-diffusible but long-lived oxidant produced by the reaction between HOCl and the amino acid taurine (Fig. 4A). Moreover, to overcome the problem of HOCl hyper-reactivity against LB

compounds, we performed a time course experiment in minimal media (M9) at a lower HOCl concentration (135 µM). A significant *msrP* induction was observed from 10 minutes after stress (Fig. 4B). These data show that the activation of HprSR is specific to HOCl and related RCS.

Positions Met72 and Met153 are important for HprS activity.

Our next goal was to investigate the molecular mechanism by which HprS senses HOCl. HOCl has been shown to modify proteins via the oxidation of sulfur-containing compounds, such as cysteine and methionine [17]. Both residues have the highest degree of reactivity towards HOCl. A previous study had already investigated the implication of cysteine residues in HprS activation, and it had pointed out the residue Cys165, located in the trans-membrane domain, as being important [16]. We decided to investigate the involvement of Met residues in HprS function. Analysis of the HprS sequence revealed the presence of 6 Met residues among which 4 (positions 72, 73, 100 and 153) are located in the periplasmic loop, the first segment of HprS that comes into contact with HOCl (Fig. 5A). To assess the role of each periplasmic Met residue, site directed mutagenesis with single Met-to-Ala and single Met-to-Gln substitutions was carried out.

The variant containing Met-to-Ala should inform us of the physicochemical constraints at this position, and the variant containing Gln, a mimetic of Met-O[18], should show the effect of oxidizing each Met on HprS activity. To test the effect of these substitutions, an *hprS*-less host carrying the *msrP-lacZ* fusion (strain *CH186*) was complemented with plasmids expressing mutated variants of *hprS*, as well as the plasmid carrying the WT gene. All the HprS variant proteins exhibit a similar level of production (from 1 to 2.6 fold) as revealed by western-blot analysis (Fig. 5B). Next, the strains were grown and treated with HOCl (4 mM) for 1 hour. The *msrP-lacZ* activity and the production of MsrP were monitored. As already reported, a *hprS*-null mutant has a higher basal expression of *msrP-lacZ* compared to the strain expressing the WT gene, and it cannot respond to HOCl[13] (Fig. 5C, Fig. 5D). These phenotypes can be complemented with a plasmid $hprS^{WT}$ allele. First, we found low levels

of MsrP protein and a decrease in β-galactosidase activity of the *msrP-lacZ* fusion in the absence of HOCl; then, we observed that the presence of HOCl led to a high steady-state level of MsrP and an increase in β-galactosidase activity from 20 to 230 Miller Units (Fig. 5C). We then assayed *msrP* expression in the strain *CH186* complemented with different *hprS* mutants with or without HOCl. The mutation of the Met 100 residue into either Ala or Gln had no effect on HprS activity. These mutants show similar levels of β-galactosidase activity and MsrP production compared to $HprS^{WT}$ in the absence and presence of HOCl (Fig. 5C, Fig. 5D). The mutation of the Met73 residue into Ala had no effect on HprS activity either (Fig. 5C). However, when the Met73 residue was mutated into a Met-O mimicking residue (Gln) we observed a higher basal expression of *msrP* compared to $HprS^{WT}$ in the absence of HOCl (around 20 and 280 Miller Units with $HprS^{WT}$ and $HprS^{M73Q}$ respectively). When HOCl was added, we observed an increase in *msrP* expression and β-galactosidase activity compared to those observed with the WT allele (350 Miller Units for $HprS^{WT}$ and 700 for $HprS^{M73Q}$) (Fig. 5D). Mutating residues Met72 or Met153 into either Ala or Gln resulted in an unexpected observation: these mutations shift the sensor to a "locked-on" state. Strains expressing these variants show a high level of MsrP and increased expression of *msrP-lacZ* even in the absence of HOCl (around 1,100 and 8,500 Miller Units with $HprS^{M72A}$ and $HprS^{M153A}$ and 3500 and 4900 Miller Units with $HprS^{M72Q}$ and $HprS^{M153Q}$ respectively). The β-galactosidase activity is not affected by the addition of HOCl and is maintained at high levels (around 1,600 and 5,800 Miller Units with $HprS^{M72A}$ and $HprS^{M153A}$ and 2800 and 3600 with $HprS^{M72Q}$ and $HprS^{M153Q}$ respectively) (Fig. 5C, Fig 5D). Taken together, these observations suggest that Met72 and Met153 are important for HprS activity.

To investigate further the roles of Met72 and Met153, we constructed mutants of HprS, each carrying a combination of two, three or four Met-to-Ala substitutions. These variant proteins exhibit a similar level of expression as revealed by western-blot analysis (Fig. 6A). The mutants were introduced into strain *CH186*. The *msrP-lacZ* activity and the production of MsrP were monitored in the presence and absence of 4 mM HOCl. We observed that simultaneously mutating Met73 and Met100 into Ala had no effect on HprS activity. Similar levels of β-galactosidase activity and MsrP

production were observed compared to $HprS^{WT}$ in the absence and presence of HOCl, showing that they are not essential for HprS activity (Fig. 6B). When we combined mutation M73A to M72A, we observed a decrease in the basal expression of *msrP* compared to that observed with $HprS^{M72A}$ (1175 and 459 Miller Units with $HprS^{M72A}$ and $HprS^{M72\text{-}73A}$ respectively). The addition of HOCl increased the expression of *msrP* in the presence of $HprS^{M72\text{-}73A}$ whereas no increase in *msrP* expression was observed with $HprS^{M72A}$ after the addition of HOCl (Fig. 6B). $HprS^{M72\text{-}100A}$ shows the same phenotype as the variant $HprS^{M72A}$: the same high basal level of *msrP* expression in the absence of HOCl (around 1180 and 1300 Miller Units with $HprS^{M72A}$ and $HprS^{M72\text{-}100A}$ respectively). The addition of HOCl did not affect the expression of *msrP* and it was maintained approximately at the same level in both mutants (around 1600 and 1900 Miller Units with $HprS^{M72A}$ and $HprS^{M72\text{-}100A}$ respectively). These results show that M72A is a dominant mutation compared to M100A (Fig. 6B). Mutations M72A or M73A seem to be recessive compared to mutation M153A: the variants $HprS^{M72\text{-}153A}$ and $HprS^{M73\text{-}153A}$ show very high levels of *msrP-lacZ* expression that are comparable to those observed with the $HprS^{M153A}$ variant (around 8550, 5550 and 6450 Miller Units with $HprS^{M153A}$, $HprS^{M72\text{-}153A}$ and $HprS^{M73\text{-}153A}$ respectively). The addition of HOCl does not affect the expression of *msrP* (around 5800, 5100 and 4760 Miller Units with $HprS^{M153A}$, $HprS^{M72\text{-}153A}$ and $HprS^{M73\text{-}153A}$ respectively) (Fig. 6B). To assess the importance of residue Met153 in HprS activity, we constructed an HprS mutant with Met72, Met73 and Met100 substituted by Ala. In this mutant, only Met153 is available to react with HOCl within the periplasmic loop. The mutant was not able to complement an *hprS* deletion in the strain *CH186*: the basal level of *msrP-lacZ* is around 608 Miller Units in the absence of HOCl. When HOCl is added, *msrP-lacZ* expression increases to around 1000 Miller Units. Nevertheless, when we compared the fold-induction between $HprS^{M72\text{-}73\text{-}100A}$ and $HprS^{WT}$ in response to HOCl, we found a 30-fold increase of *msrP-lacZ* in an $HprS^{WT}$ background and only a two-fold increase in an $HprS^{M72\text{-}73\text{-}100A}$ background. This observation suggests that the presence of Met153 alone is not sufficient for a fully functional HprS (Fig. 6C). Based on these observations, we hypothesized that all Met residues might be important for HprS activity and that substituting all Met residues by Ala might render HprS blind to the signal.

Nevertheless, the phenotype observed with $HprS^{M72\text{-}73\text{-}100\text{-}153A}$ is comparable to that observed with $HprS^{M153A}$: the variant has a "locked-on state" and the addition of HOCl does not affect the expression of *msrP-lacZ,* which is maintained at high levels. This phenotype might be due to the dominant mutation M153A (Fig. 6C).

Negative feedback of MsrP on the expression of the hiuH-msrP-msrQ operon.

The Met redox switch HprS-activation hypothesis implies that deletion of the *msrP* gene should modify the pattern of HOCl-HprSR-dependent induction of *hiuH-lacZ* expression as MsrP would be able to reduce Met-O containing HprS to turn off the activation once the HOCl signal has disappeared. To test this hypothesis, we have constructed the strains SH100 ($MsrP^+$) and SH101 ($MsrP^-$) in which the *hprRS* operon was under the control of the tetracycline resistance (TetR) promoter, in order to abolish HOCl-dependent *de novo* synthesis of the sensor. Both strains were grown in minimal medium, HOCl was added at OD = 0.2 and the β-galactosidase activity (*hiuH-lacZ)* was monitored over time. Our results show that HOCl treatment leads to a higher level of β-galactosidase activity in SH101 ($MsrP^-$) compared to SH100 ($MsrP^+$). No difference was observed in the absence of HOCl (Fig. 7A). Moreover, the over-production of MsrP using a plasmid led to a significant decrease in the induction of *hiuH-lacZ* during HOCl stress (Fig. 7B). These results show negative feedback of MsrP on HprR-regulated genes (i.e. *hiuH*) during HOCl stress, probably involving a methionine redox control process.

Residues Met72 and Met153 are highly conserved in HprS homologues.

In order to support a functional role for the periplasmic Met residues in HprS, we assessed their conservation in HprS sequences. To do so, we first established the list of HprS homologues from the STRING database. The two-component systems that have the same genetic environment as HprS of

241 *E. coli*, i.e. they are found next to *hiuH* or *msrP-msrQ* genes, were defined as homologues. Based on
242 this criterion, we selected 12 TCS including *E. coli* HprS (Fig. 8A). Ten of the selected homologues
243 were found in Gammaproteobacteria and only 2 were found in Betaproteobacteria (*Herbaspirillum*
244 *frisingense* and *Herminiimonas arsenicoxydans*). This indicates that *hprRS* might not be widely
245 distributed among prokaryotes, even though all bacteria can be stressed in their natural environment
246 by reactive chlorine species produced by photo-chemical reactions of organic matter [19]. By aligning
247 the protein sequence of the different homologues of HprS, we found that Met72 and Met153 are
248 highly conserved: Met72 is found in 92 % of the homologues (11 out of the 12 analyzed sequences)
249 and Met153 is conserved in 75% of the homologues (9 out of the 12 homologues). Conversely,
250 Met73 is less well conserved, being found in only 58% of the homologues (7 out of 12). Met100 is
251 poorly conserved and occurs only in *Shigella flexneri* and *E. coli* HprS (Fig. 8B). This analysis suggests
252 that Met72 and Met153 might be important residues for the sensor HprS.

253

254 ***The transmembrane residue Cys165 is important for signal transduction***

255 A previous mutagenesis approach, using a Cys-to-Ala substitution, highlighted the residue Cys165,
256 located in the second transmembrane domain (TM2), as important for HprS activation by H_2O_2[16]. We
257 therefore investigated the role of Cys165 in HprS activation during HOCl stress. The C165A mutant
258 was constructed and we observed a similar abundance of HprS production compared to the wild type
259 strain (Fig. 9A). We then showed that in the presence of HOCl, the expression of the *msrP-lacZ* fusion
260 and the MsrP protein levels were lower in strains expressing $HprS^{C165A}$ compared to the wild type
261 (Fig. 9B). However, this residue was only conserved in 4 HprS homologues, challenging its functional
262 role in HprS activity (Fig. 8B). We then considered the possibility that Cys165 contributes to signal
263 transmission rather than sensing. We took advantage of the M72Q variant, which caused constitutive
264 activation of HprS, to assess this question. We built the M72Q-C165A mutant, which exhibits a level
265 of HprS production similar to the WT (Fig. 9A) and by measuring the expression of the *msrP-lacZ*

fusion and the MsrP protein levels, we showed that the strain expressing HprS$^{M72Q-C165A}$ had a lower MsrP level than the strain expressing HprS^{M72Q} (Fig. 9B). These results show that the C165A mutation was dominant over M72Q and suggest that Cys165 is an important residue for signal transduction from the periplasmic sensing domain to the cytosolic kinase domain.

DISCUSSION

In bacteria, two-component systems (TCS) are widespread signaling pathways allowing cells to sense, respond and adapt to fluctuating environments and challenging conditions. Based on genome sequencing, *E. coli* MG1655 has been predicted to carry a total of 24 "classical" TCS composed of a histidine kinase and a response regulator [20]; many of them have yet to be characterized. Other well-studied TCS have been shown to regulate bacterial metabolism, nutrient acquisition and stress response to numerous stimuli such as pH, metals and envelope integrity [21,22]. Therefore, the characterization of *E. coli* TCS is important for developing complete knowledge on bacterial regulatory networks. The aim of this study was to characterize further the TCS HprSR in *E. coli* and we have found that oxidative stress mediated specifically by RCS (HOCl and *N*-ChT) can activate this system. We have also provided evidence for the molecular mechanism of signal sensing by HprS.

Until now, HprSR was poorly characterized and initial evidence for the HprSR-dependent regulon was provided by SELEX methodology[15]. Four HprR binding sites were identified in the whole genome: one between the two operons *hprRS* and *hiuH-msrPQ*; one within the *cyoABCDE* operon encoding cytochrome oxidase; one near the *qorA* gene encoding a quinone oxido-reductase; and one between the *cusRS* and the *cusCFBA* operons, encoding the copper responding TCS and the copper efflux pump, respectively [15]. However, this approach cannot exclude the possibility that some targeted genes could be regulated only by HprR and not by HprS via a cross-talk between TCS. Indeed, such a cross-talk has to be taken into account as HprR was shown to be phosphorylated by CusS, the

copper-sensing HK [21].Previous results combined with those presented in this study, show that the *hiuH-msrPQ* operon is regulated by the HprSR TCS [13,15].

As a first step towards characterizing HprSR, we tried to identify the signals sensed by the HK HprS. Previous studies reported contradictory results: it has been shown that HprSR, in response to H_2O_2 stress, increases the mRNA level of its target gene *hiuH,* suggesting that HprS senses H_2O_2 [15]. Another study showed that HprSR (formerly YedVW) induced the expression of the gene encoding the periplasmic methionine sulfoxide reductase system (MsrPQ) in response to HOCl and not to H_2O_2 [13]. This regulation was seen at both transcriptional and translational levels, by using an *msrP* reporter fusion and by assessing the MsrP protein levels [13]. These contradictory results prompted us to re-investigate the signals sensed by HprS. In this study, we assessed both mRNA and protein levels of HprSR target genes. The strength of this study is the simultaneous comparison of the effect of HOCl and H_2O_2 on HprSR and the evaluation of the expression of target genes both at the transcriptional and translational level. Our results show that HOCl is a more physiological signal for HprSR compared to H_2O_2, and that the previously described activation of HprSR by H_2O_2 might be irrelevant and non-physiological as only a slight increase in mRNA levels is detected. We also tested other ROS/RCS that might be sensed by HprS by treating cells with different compounds known to generate oxidative stress. Among all the compounds tested, we only detected an activation of HprSR in response to HOCl and *N*-ChT. However, as HOCl reacts rapidly with different compounds of the culture media, especially with LB, producing chloramines and other less toxic chlorine species [23], we suggest that HprS is a specific RCS sensor in *E. coli*.

Until now, two different names have been attributed to this TCS: HprSR for hydrogen peroxide sensor [16] and regulator and MsrVW [24] for its association with MsrPQ. We think that neither name might be suitable for this TCS in light of the present study: firstly, we have shown that H_2O_2 is not an adequate signal and secondly, the Msr denomination is misleading and should be reserved for proteins that have methionine sulfoxide reductase enzymatic activity or that are essential for Msr

activity (such as MsrQ). However, we have kept the “Hpr” terminology as it is already used in public databases like UniProt.

As a second step towards fully characterising HprSR, we investigated the molecular mechanism underlying HOCl detection by HprS. It has been shown that HOCl activates RCS-specific transcription factors through post-translational modification of Cys or Met residues. Indeed, HOCl activates RclR by a thiol-disulfide switch mechanism and NemR by the formation of a Cys-Lys sulfenamide bond, while HOCl oxidizes three Met residues in HypT, leading to its activation[9,8,10]. Interestingly, HprS contains four Cys and six Met residues: two Cys residues (Cys165 and Cys172) are located in the second transmembrane domain and the other two (Cys333 and Cys338) are in the cytoplasmic domain. In the case of the Met residues, four of them are located in the periplasmic loop of HprS (Met72, Met73, Met100 and Met153), and the other two are in the cytoplasmic domain (Met225 and Met352). The involvement of Cys residues in the HprS sensing mechanism has already been investigated and the transmembrane Cys165 residue has been identified as being important for H_2O_2 detection [16]. Met residues are also highly sensitive to HOCl and because HK usually use their periplasmic loop to sense extracellular signals [25]: we therefore focused our study on the 4 Met residues located in the periplasmic domain. We conducted a systematic Met-to-Ala and Met-to-Gln substitution of each Met residue. We also generated HprS mutants containing different combinations of two, three or four Met-to-Ala substitutions. Replacing Met with Ala prevents the sulfur atom reacting with HOCl and substituting Met with Gln mimics Met-O residues [26]. We observed that substituting Met72 or Met153 with Ala or Gln had drastic consequences on HprS, leading to constitutive expression of *msrP*. Substituting Met73 with a Gln residue led to a higher basal activity compared to $HprS^{WT}$ but this variant was not blind to the signal. Mutations of residue Met100 (M100A and M100Q) were neutral and had no effect on HprS. The analysis of HprS double mutants show that M72A and M153A are dominant compared to M73A and M100A. We also showed that residues M100 and M73 are not essential for HprS activation by HOCl. When analyzing the mutant $HprS^{M72\text{-}73\text{-}100A}$, it was clear that residue M153A alone cannot activate HprS to its full potential in

response to HOCl, showing that other residues are involved. We were not able confirm the involvement of Met residues in HprS activity since the mutant HprS$^{M72\text{-}73\text{-}100\text{-}153A}$ was constitutively active, probably due to the M153A mutation. These results show that Met72, 73 and 153 are important for HprS activity with a functional hierarchy based on the residues: Met72 and Met153 appear to be crucial for HprS whereas Met73 seems to have a secondary role. These conclusions correlate with the fact that M72 and M153 are conserved in the HprS homologues whereas M73 is less conserved.

In addition to the mutational approach discussed earlier, we assayed the effect of MsrP on HprS activation. If the activation of HprS is linked to the oxidation of Met residues, then MsrP could participate in switching HprS off by re-reducing the Met-O residues. Our results show that the level of *msrP-lacZ* expression is dependent on the level of MsrP production. The HOCl-dependent induction of *msrP-lacZ* is regulated by HprS and so this observation is evidence for a central role of the Met redox state in HprS activity. Finally, we reinvestigated the role of the transmembrane Cys165 residue using the HprS^{M72Q} constitutive variant. First, we confirmed that an HprS^{C165A} variant is affected in its activity in agreement with a previous study [16]. However, the localization and the lack of conservation of Cys165 led us to postulate that this position (165) rather than the Cys residue itself (as it is not conserved) could be important for signal transduction to the catalytic domain and not for signal sensing. The fact that C165A has been found to be dominant over the M72Q mutation, even in the absence of the signal, supports this view. Moreover, the absence of HprS activation by diamide, a thiol-specific oxidant, led us to speculate that the weak HprS activation by H_2O_2 could be caused by Met rather Cys165 oxidation.

Based on the findings above, it appears that a Met-redox switch might play a role in an HprS sensing mechanism, like the HOCl-specific transcription factor HypT. This means that the oxido-reduction state of particular Met residues (we speculate Met72 and Met153) might participate in the activation

of HprS by HOCl. Also, MsrP might play a role in shutting down the system by reducing the oxidized Met residues, forming a negative feedback loop.

If Met residues are involved in HprS activation, this could explain why H_2O_2 cannot activate HprS properly: in terms of kinetics, the oxidizing reactivity of Met residues with H_2O_2 is much lower than with HOCl (2×10^2 $M^{-1} \cdot s^{-1}$ for H_2O_2 versus 3.4×10^7 $M^{-1} \cdot s^{-1}$ for HOCl) [17] making the reaction much slower and requiring higher concentrations of H_2O_2 that might not be physiological. However, we have no direct proof of *in vivo* Met oxidation in HprS, therefore additional studies will be required to consolidate our proposed model. Also, our data do not exclude the possibility that HprS might be activated indirectly by RCS through a Met-oxidation process of a third periplasmic component which could interact with HprS. Our model needs to be consolidated in the future by using a biochemical approach to assess the different post-translational modifications occurring in HprS during RCS stress *in vivo*.

In conclusion, we provide strong evidence that *E. coli* acquired a two-component system that detects and responds to RCS molecules and, to our knowledge, HprSR is the only RCS related TCS in *E. coli*. Unlike ROS, RCS responses in bacteria are still poorly understood. The continued characterization of HprSR is important for understanding RCS killing and resistance in prokaryotes.

MATERIAL AND METHODS

Strains and microbial techniques

The strains used in this study are listed in table 1. To construct strain SH198 (*hprR* knock-out strain), the *hprR* mutant allele from the Keio collection [27] was transferred to the *E. coli* MG1655 strain (wild-type) using a standard P1 transduction procedure [28]. Kanamycin resistant strains were selected and verified by PCR. To excise the kanamycin cassette, we used the pCP20 plasmid [27,29]. The *hiuH-lacZ* fusion in strain CH184 was constructed using the protocol described by Mandin and Gottesman [30]. First, the promoter region of *hiuH* located between nucleotides -274 and the ATG initiation triplet was amplified using the primers lacI-hiuH-For and lacZ-hiuH-Rev. Using mini lambda recombination, the PCR product was recombined in the chromosome of an *E. coli* strain carrying a P_{BAD}-cat-SacB cassette inserted in front of the gene *lacZ* (strain PM1205). Recombinants which had lost the cat-sacB cassette were selected and verified by PCR: these clones carried the translational fusion *hiuH-lacZ*. Strain LL1700 containing a CmR cassette was constructed to transfer the *hiuH-lacZ* fusion into an *E. coli* MG1655 (Wild-type) background. To do so, we inserted a resistance cassette close to the fusion enabling its transduction: a Cat cassette was amplified using primers 5deI and 3deI. The resulting product carried on its 5' end, a 40 bp homologous sequence to *lacI* and on its 3' end, a 41 bp sequence homologous to the *hiuH* promoter (located between the nucleotide -133 and -92, before the HprR boxes). The amplicon was then electroporated into the strain CH184 carrying the pKD46 plasmid. Cat resistant clones were selected and tested by PCR. The *lacI-cat-hiuH-lacZ* construct was then transferred into MG1655 cells using a standard P1 transduction procedure. Chloramphenicol resistant clones were selected and verified by PCR. Strain SH100 was constructed as follows: (i) a TetR-Kan sequence composed of the promoter TetR and the kanamycin cassette was amplified using the plasmid pBbS2K as the template and the primers YedW_TetR_F and Kan_Boxyedx_R. The resulting PCR product shared a 48 bp sequence homologous to *hprR* and a 41 bp sequence homologous to the intergenic region between *hprR* and *hiuH* located upstream of the

HprR boxes. (ii) After purification, the PCR product was transformed by electroporation into *E. coli* BW25113 cells (WT) carrying the plasmid pKD46 [29]. Kan resistant clones were selected and verified by PCR. (iii) This construction was then transferred into LL1700 cells expressing *hiuH-lacZ* fusion using standard P1 transduction procedures. Selected clones were verified by PCR.

The same procedure was followed to construct strain SH101, except that the TetR-Kan amplicon was electroporated into an *E. coli ΔmsrP* strain.

Plasmid constructions

The plasmids and primers used in this study are listed in table 2 and 3 respectively. The pHprS plasmid was constructed as follows: (i) *hprS* was amplified from the chromosome of *E. coli* (MG1655) using primers XhoI_YedV_atg_F and HindIII_YedV_R. (ii) The PCR product was cloned into the pBBRMCS1 plasmid using XhoI and HindIII restriction sites, generating plasmid pHprS. In this plasmid, the expression of HprS is under the control of the P_{lac} promoter which is inducible by IPTG. Plasmids pHprSM72A, pHprSM73A, pHprSM100A, pHprSM153A, pHprSM72Q, pHprSM73Q, pHprSM100Q, pHprSM153Q and pHprSC165A were derived from pHprS and carry single mutations of the *hprS* gene. Each mutant was constructed by site directed mutagenesis using a two-step PCR method: two overlapped PCR products were generated from pHprS using one external primer (either XhoI_YedV_atg_F or HindIII_YedV_R) and an internal primer carrying the desired mutation. After purification, the PCR products were used as templates for the second PCR carried out with the external primers only: XhoI_YedV_atg_F and HindIII_YedV_R. The resulting PCR fragment was digested with XhoI and HindIII restriction enzymes (Biolabs) then cloned into pBBRMCS1. The same procedure was used to construct pYedVM72Q/C165 using pYedVM72Q as a template and YedV_C165A_2_F and YedV_C165A_2_R as internal primers. *hprS* mutants carrying different combinations of double Met-to-Ala substitutions were constructed using the two-step PCR method described earlier: $pHprS^{M72\text{-}73A}$, $pHprS^{M72\text{-}100A}$, $pHprS^{M72\text{-}153A}$, $pHprS^{M73\text{-}100A}$, $pHprS^{M73\text{-}153A}$. For this,

pHprS plasmids carrying one of the two mutations were used as a template, and the internal primers used for the PCR reaction carried the second mutation to be introduced. All double mutants were cloned into pBBRMCS1. Mutant *hprS*$^{M72\text{-}73\text{-}100A}$ was constructed using pHprS$^{M72\text{-}73A}$ as the template and YedV_M100A_F and YedV_M100A_R as internal primers. Mutant *hprS*$^{M72\text{-}73\text{-}100\text{-}153A}$ was constructed using pHprS$^{M72\text{-}73\text{-}100A}$ as the template and YedV_M153A_F and YedV_M153A_R as internal primers. Both mutants *hprS*$^{M72\text{-}73\text{-}100A}$ and *hprS*$^{M72\text{-}73\text{-}100\text{-}153A}$ were cloned into pBBRMCS1. All PCR reactions were carried out using Phusion high fidelity Taq polymerase (Thermo Fisher).

Operonic organization analysis

E. coli cells (MG1655) were grown to OD_{600}≈ 2 in the presence of 2 mM HOCl added at the beginning of the culture. Total bacterial RNA was extracted with a Maxwell® 16 LEV miRNA Tissue Kit (Promega) according to the manufacturer's instructions. Contaminating chromosomal DNA was digested using Turbo DNase (Invitrogen). RNA quality was assessed using a Tape station system (Agilent) then quantified at 260 nm using a NanoDrop 1000 (Thermo Fisher scientific). cDNA was synthesized with 1 µg of total RNA, 0.5 µg random primers (Promega) and GoScript™ reverse transcriptase (Promega). To analyze the *hiuH-msrP-msrQ* operon structure, two sets of primers, 5QTyedX-3QTmsrP and 5QTmsrP-3QTmsrQ, were used to amplify the possible transcripts. As a negative control, RNA samples were amplified using the same sets of primers in order to verify the absence of chromosomal contamination. As a positive control, chromosomal DNA was amplified using EconoTaq DNA polymerase (Promega). Results were visualized on a 1 % agarose gel prepared with TBE (Tris-Borate-EDTA) and stained with ethidium bromide.

qRT-PCR analysis

Total RNA was extracted from wild type *E. coli* (MG1655) cells and *hprS* mutants (SH198) either cultured to OD_{600}≈ 0.6 and subjected to 6 mM H_2O_2 for 30 min or cultured to OD_{600}≈ 1 and stressed with 4 mM HOCl for 1 h. mRNA levels of *hprS*, *hprR*, *msrP* and *msrQ* were quantified by qRT-PCR analysis using the incorporation of EvaGreen (Bio-rad) in a CFX96 Real Time system (Bio-Rad) according to the manufacturer's instructions. For these reactions, primers 5QT-3QT yedX, 5QT-3QT msrP, 5QT-3QT yedV, and 5QT-3QT yedW were used. For accurate evaluation of gene expression, RNA were quantified and normalized to the 16S rRNA housekeeping gene. qRT-PCR technical triplicates for each condition were carried out. All biological repeats were selected and reported.

HOCl induction assays

E. coli cells expressing *msrP-lacZ* or *hiuH-lacZ* (CH183, CH184 or CH186 carrying pYedV or pYedV derived plasmids) were grown aerobically in LB medium at 37 °C. At OD_{600}≈ 1, the culture was divided into two 15 mL falcon tubes each containing 5 mL of this culture: 4 mM HOCl (NaOCl, 10-15% AC Acros Organics) was added to one of these tubes. Cultures were then incubated at 37 °C. To assess the activity of HprS mutants, β-galactosidase activity was measured after 1 h of incubation. To follow the kinetics of *msrP-lacZ* or *hiuH-lacZ* expression in response to HOCl, β-galactosidase activity was measured at different time points. The kinetics of *msrP-lacZ* expression in response to HOCl were also evaluated in minimal medium (M9) supplied with 0.2 % glucose and 0.2 % CASA. For this, strain CH183 expressing the *msrP-lacZ* fusion was cultured to OD_{600}≈ 0.2. The culture was then divided into two 50 mL falcon tube each containing 5 mL of the culture. To one of the tubes, 135 µM of HOCl were added and the β-galactosidase activity was measured at different time points. For each protocol, the reaction mixture was prepared as follows: 200 µL of bacteria were added to 800 µL of β-galactosidase buffer. β-galactosidase was measured as previously described by Miller *et al*. [31].

H_2O_2 induction assays

To test the induction of *msrP-lacZ* or *hiuH-lacZ* (CH183 and CH184) in response to H_2O_2, two different protocols were tested: first, as described by Urano and his collaborators [16], *E. coli* cells were cultured aerobically in LB medium at 37 °C. At OD_{600}≈ 0.6, cultures were split into two 50 mL falcon tubes each containing 5 mL of the culture; 6 mM of H_2O_2 was added to one of them and after 30 min of incubation at 37°C, β-galactosidase activity was measured. For the second protocol, *E. coli* cells were aerobically cultured in LB medium at 37°C. At OD_{600}≈ 0.2, the culture was divided into 5 mL aliquots in 50mL falcon tubes. Different sub-lethal concentrations of H_2O_2 were added for each (500 µM, 1 mM, 2 mM and 3 mM of H_2O_2). 200 µL of culture were harvested at different time points and β-galactosidase assays were conducted. As a positive control for this protocol, strain BE1001 was used to follow the expression of *ahpC-lacZ* under the tested conditions.

Bacteria survival assay in vitro.

To test the effect of H_2O_2 and HOCl on cell viability, overnight cultures of *E. coli* (MG1655) were diluted in LB medium to OD_{600}≈ 0.4. Cells were then subjected to 6 mM H_2O_2 and 4 mM HOCl using the protocols described earlier. Cultures were serially-diluted in PBS. 5 µL of each dilution were spotted onto LB agar plates; after 18h at 37 °C, CFU were counted.

Chlorotaurine synthesis

Chlorotaurine (*N*-ChT) is produced by the reaction between HOCl and the amino acid taurine. An identical volume of 10 mM NaOCl and 10 mM taurine (Sigma) were mixed and incubated for 10 min at room temperature. Chlorotaurine concentration was then determined by measuring the solution

absorbance at 252 nm (ε=429 $M^{-1}cm^{-1}$). Solutions of HOCl and taurine were prepared in 0.1 M phosphate potassium buffer (pH 7.4).

Chlorotaurine induction assays

To test the induction of *msrP-lacZ* by *N*-ChT, *E. coli* CH183 cells were grown aerobically in LB medium to OD_{600}≈ 1. Then, 1 mM of *N*-ChT was added. 200 µL of culture were harvested at different time points and β-galactosidase activities were measured.

NO induction assays

To test the induction of *msrP-lacZ by* NO, CH183 cells were grown in Hungate tubes under strictly anaerobic conditions in 10 mL LB medium at 37 °C. At OD_{600}≈ 0.2, different concentrations of NO were applied (500 nM, 1 µM, 5 µM and 50 µM NO from an NO water-saturated solution injected into the anaerobic cultures and followed by incubation at 37 °C. 200 µL of culture were harvested with a gas-tight Hamilton syringe at different time points and β-galactosidase activities were measured. The DV1301 strain expressing the *hmpA-lacZ* fusion was used as a positive control.

Superoxide radical induction assay

To test the induction of *msrP-lacZ* by the superoxide radical, CH183 cells were grown in LB medium at 37 °C. At OD_{600}≈ 0.2, the culture was split into 5 mL aliquots in 50 mL falcon tubes. Each culture was treated with a different concentration of paraquat (300 µM, 500 µM and 1 mM) followed by incubation at 37 °C. 200 µL of culture were harvested at different time points and β-galactosidase activities were measured. The BE1000 strain carrying *soxS-lacZ* was used as a positive control.

Diamide induction assay

To test the induction of *msrP-lacZ* by diamide, CH183 cells were grown in LB medium at 37 °C. At OD_{600}≈ 0.2, the culture was split into 5 mL aliquots in 50 mL falcon tubes. Each culture was treated with a different concentration of diamide (0.2, 0.3 and 0.4 mM diamide) followed by incubation at 37 °C. 200 µL of culture were harvested at different time points and β-galactosidase activities were measured. The BE1001 strain carrying *ahpC-lacZ* was used as a positive control.

Immunoblot analysis of MsrP expression

To assess MsrP induction by HOCl, overnight cultures of the CH186 strain expressing different plasmid alleles of *hprS* were diluted to OD_{600}≈ 0.04 in LB medium. Cells were grown aerobically to OD_{600}≈ 1. The culture was divided into two 15 mL falcon tubes each containing 5 mL of the culture; 4 mM HOCl was added to one tube. Cultures were then incubated at 37 °C for 1 h. Samples were diluted in sample buffer (Laemmli: 2 % SDS, 10 % glycerol, 60 mM Tris-HCl, pH 7.4, 0.01 % bromophenol blue). The amount of protein loaded onto the gel was standardized for each culture based on their A_{600} values. Samples were then heated for 10 min at 95 °C and loaded onto an SDS-PAGE gel for immunoblot analysis. Immunoblot analysis was performed according to standard procedures: the primary antibody is a guinea pig anti-MsrP antibody (provided by Jean-François Collet Lab, De Duve Institute, Belgium). The secondary antibody is anti-guinea pig IgG conjugated to horseradish peroxidase (HRP) (Promega). An ImageQuant LAS4000 camera (GE Healthcare Life Sciences) was used for image Chemiluminescence.

Immunoblot analysis of HprS production

To analyze the expression level of *hprS* mutants, overnight cultures of the CH186 strain expressing different plasmid alleles of *hprS* were diluted to OD_{600}≈ 0.04 in LB medium. Cells were cultured for 4 hours at 37 °C. 1 mM of isopropyl β-D-1-thiogalactopyranoside (IPTG) was then added and cultures were incubated overnight at 37 °C. Samples preparation was carried out as described above. For immunoblot analysis, rabbit anti-HprS was used as a primary antibody (synthesized for Benjamin Ezraty's Lab by Agro-bio) and an anti-rabbit IgG conjugated to horseradish peroxidase (HRP) (Promega) was used as a secondary-antibody. Chemiluminescence signals were visualized using an ImageQuant LAS4000 camera (GE Healthcare Life Sciences).

Analysis of the effect of MsrP on hiuH expression

Overnight cultures of SH100 and SH101 with or without plasmids pAG117 and pAG195 were diluted to OD_{600}≈ 0.04 in M9 minimal media supplemented with 0.2 % Casaminoacids (Bio Basic Canada). Cultures were incubated at 37 °C. At OD_{600}≈ 0.2, 135 µM of HOCl was added and β-galactosidase activity was measured at different time points as described above.

Table 1- Strains used in this study.

This table contains information regarding the strains used in this study, including their name, their genotype and their source.

Strain	Genotype	Source
MG1655	Wilde-Type	Laboratory collection
PM1205	MG1655 *mal*::*lacI*^{q}, *ΔaraBAD araC*$^{+}$, *lacI'*::P_{BAD}-*cat-sacB-lacZ*, *mini λ tet*^{R}	Mandin *et al*, 2009[30]
CH183	PM1205 *msrP-lacZ*	Gennaris *et al*, 2015[13]
CH184	PM1205 *hiuH-lacZ*	This study
CH186	PM1205 *msrP-lacZ* Δ*hprS::Kan*^{R}	Gennaris *et al,* 2015[13]
SH198	MG1655 *ΔhprR*	This study
BE1001	PM1205 *ahpC-lacZ*	Ezraty *et al*, 2014[32]
DV1301	*lacI poZ*Δ(*Mlu*) *hmpA-lacZ*	Vinella *et al*, 2013[33]
BE1000	PM1205 *soxS-lacZ*	Ezraty *et al*, 2014[32]
SH100	LL1700 P_{Tet}-*hprSR cat-hiuH-lacZ*	This study
SH101	LL1700 P_{Tet}-*hprSR ΔmsrP cat-hiuH-lacZ*	This study
LL1700	*MG1655 cat-hiuH-lacZ*	This study

Table 2- Plasmids used in this study.

This table contains information regarding the plasmids used in this study, including their name, their genotype and their source.

Plasmid	Genotype and description	Source
pBBR-MCS1	P_{lac} promoter, IPTG inducible, Cm^R selection	Laboratory collection
pAG177	pTAC-MAT-Tag-2 containing an NdeI restriction site (pTAC-MAT-Tag-2-NdeI)	Gennaris *et al*, 2015[13]
pAG195	pTAC-MAT-Tag-2-NdeI MsrP-MsrQ	Gennaris *et al,* 2015[13]
pBbS2K	pBbS2K-RFP *ΔRFP1*, selfligated	Germain *et al*, 2019[34]
pHprS	pBBR-MCS1-*XhoI*-HprS ^{WT}	This study
pHprSM72A	pBBR-MCS1-*XhoI*-HprS ^{M72A}	This study
pHprSM73A	pBBR-MCS1-*XhoI*-HprS ^{M73A}	This study
pHprSM100A	pBBR-MCS1-*XhoI*-HprS ^{M100A}	This study
pHprSM153A	pBBR-MCS1-*XhoI*-HprS ^{M153A}	This study
pHprSM72Q	pBBR-MCS1-*XhoI*-HprS ^{M72Q}	This study
pHprSM73Q	pBBR-MCS1-*XhoI*-HprS ^{M73Q}	This study
pHprSM100Q	pBBR-MCS1-*XhoI*-HprS ^{M100Q}	This study
pHprSM153Q	pBBR-MCS1-*XhoI*-HprS ^{M153Q}	This study
pHprSC165A	pBBR-MCS1-*XhoI*-HprS ^{C165A}	This study
pHprSM72Q-C165A	pBBR-MCS1-*XhoI*-HprS $^{M72Q-C165A}$	This study
pHprSM72-73A	pBBR-MCS1-*XhoI*-HprS $^{M72A-M73A}$	This study
pHprSM72-100A	pBBR-MCS1-*XhoI*-HprS $^{M72A-M100A}$	This study
pHprSM72-153A	pBBR-MCS1-*XhoI*-HprS $^{M72A-M153A}$	This study
pHprSM73-100A	pBBR-MCS1-*XhoI*-HprS $^{M73A-M100A}$	This study
pHprSM73-153A	pBBR-MCS1-*XhoI*-HprS $^{M73A-M153A}$	This study
pHprSM72-73-100A	pBBR-MCS1-*XhoI*-HprS $^{M72A-M73A-M100A}$	This study
pHprSM72-73-100-153A	pBBR-MCS1-*XhoI*-HprS $^{M72A-M73A-M100A-M153A}$	This study

Table 3- Primers used in this study.

This table contains information regarding the primers used in this study, including their names and their sequence.

Name	Sequence (5' to 3')
5QTyedX	TTGGTTAATGCCGCACAACA
3QTyedX	GCCACAGTGCCTTAATTCGT
5QTmsrP	TGATGACTTAACCCGTCGCT
3QTmsrP	GCATCTGTTCCGGTGCATAA
5QTmsrQ	TCGCCGCCTGTTAGGATTAT
3QTmsrQ	AGTGAACGCTAAAGCAAGCA
5QT YedV	CGGATGATGGATGTTAGTCAG
3QT YedV	TGAAGCCAATTTAGCCACAG
5QT YedW	AACAGCAAAGCAAACCCC
3QT YedW	TCCCTGCTCACACTATGAC
XhoI_YedV_atg_F	GGCCTCTCGAGATGAAGATTCTACTTATTGAAGAT
HindIII_YedV_R	CCGGAAAAGCTTTAATTTATGGCGTACTGAAGCCCTATG
YedV_M72A_F	CCTGTGTACTTTAACCGGGCGATGGATGTTAGTCAGGATATC
YedV_M72A_R	GATATCCTGACTAACATCCATCGCCCGGTTAAAGTACACAGG
YedV_M73A_F	CCTGTGTACTTTAACCGGATGGCGGATGTTAGTCAGGATATC
YedV_M73A_R	GATATCCTGACTAACATCCGCCATCCGGTTAAAGTACACAGG
YedV_M100A_F	CAAATGTCAGTGATGGCGCGTTAAATAACATACCTGCTAGTG
YedV_M100A_R	CACTAGCAGGTATGTTATTTAACGCGCCATCACTGACATTTG
YedV_M153A_F	GGC TAA ATT GGC TTC AGC CAG ACA TAAC GCG CT TGA ACA G
YedV_M153A_F	C TGT TCA AG CGC GTTA TGT CTG GCT GAA GCC AAT TTA GCC
YedV_M72Q _F	GTG TAC TTT AAC CGG CAG ATG GAT GTT AGT CAG GAT ATC TTG ATT ATT CAT GGT GAT
YedV_M72Q_R	GATATCCTGACTAACATCCATCTGCCGGTT AAAGTACACAGGTAACGTATCTGGATT
YedVM73Q_F	GTGTACTTTAACCGGATGCAGGATGTT AGTCAGGATATCTTGATTATTCATGGTGAT
YedV_M73Q_R	ATCACCATGAATAATCAAGATATCC TGACTAACATCCTGCATCCGGTTAAAGTACAC
YedV_M100Q_F	CAAATGTCAGTGATGGCGCGTTAAATAACATACCTGCTAGTG
YedV_M100Q_R	CACTAGCAGGTATGTTATTTAACGCGCCATCACTGACATTTG
YedV_M153Q_F	GGC TAA ATT GGC TTC AGC CAG ACA TAAC CAG CT TGA ACA G
YedV_M153Q_R	C TGT TCA AG CTG GTTA TGT CTG GCT GAA GCC AAT TTA GCC
YedV_C165A_2_R	GCA AAG TAC AAT GGC GAC AAT CGC AAT TAT AAT GCT
YedV_C165A_2_F	AGC ATT ATA ATT GCG ATT GTC GCC ATT GTA CTT TGC
YedW_TetR_F	TACCCATTCCTGGGTCCTTTGATTATCTTCAATAAGTAGAATCTTCATATGT ATATCTCCTTCTTAAAAGATCTTTTGAATTCTTTTCTCTATCACTGATAGG
Kan_Boxyedx_R	GGCTGTTTCTATAACATATGATTTATGGCATATT ATTTTCATCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGC
lacI-HiuH-for	CGAAGCGGCATGCATTTACGTTGACACCA TCGAATGGCGCATCAATGCATAATCATCCTTC

lacZ-HiuH-rev	TAACGCCAGGGTTTTCCCAGTCACGACGTT GTAAAACGACCATGTTTATATCCTTGTCATG

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FIGURE LEGENDS

Figure 1- Genetic organization of *hiuH*, *msrP* and *msrQ* in *Escherichia coli* K12.

A) Schematic representation of the *hprRS* operon, *hiuH, msrP* and *msrQ* genes. In the *E. coli* genome, *hiuH, msrP* and *msrQ* are adjacent: they are located in proximity to the *hprR-hprS* operon. The intergenic region between *hiuH* and *hprR* contains the HprR box (CATTACAAAATTGTAATG)[15]. Primer positions used for the following experiments are indicated on the diagram: the first set of primers amplify the region of 1030 bp between *hiuH* and *msrP* (1). The second set amplify the region of 1024 bp between *msrP* and *msrQ* (2) **B)** RT-PCR analysis of *hiuH*, *msrP* and *msrQ*: *E. coli* cells MG1655 (WT) were cultured in the presence of 2 mM HOCl in LB medium. Total RNA was extracted and retro-transcribed into cDNA. PCR reactions were carried out on total extracted RNA, chromosomal DNA and on cDNA using the primers depicted in panel (A).

Figure 2- Influence of H_2O_2 and HOCl on the expression of the *hiuH-msrPQ* and *hprRS* operons.

A) Fold-expression level of *hprS*, *hprR*, *hiuH* and *msrP* transcripts under H_2O_2 and HOCl stresses in MG1655 (WT) and SH198 (*ΔhprR*) cells. Both strains were cultured in LB and subjected to either 6 mM H_2O_2 for 30 min, as described by Urano and his collaborators[15], or to 4 mM HOCl for 1 hour. For each condition, quantitative real-time PCR (RT-qPCR) was performed to amplify *hprS*, *hprR*, *hiuH* and *msrP* cDNA. Values are expressed as means ± SEM of three independent experiments. ND: not detemined. **B)** Evaluation of *msrP* and *hiuH* expression under HOCl or H_2O_2 stress. The expression of *msrP-lacZ* and *hiuH-lacZ* fusions, in strains CH183 and CH184 respectively, were used as a proxy for *hiuH* and *msrP* expression. Strains CH183 and CH184 were cultured in LB and subjected to H_2O_2 or HOCl stress, followed by ß-galactosidase assays. Results are expressed as means ± SD, n=3. **C)** Effect of 6 mM H_2O_2 and 4 mM HOCl on the viability of WT *E. coli* cells. *E. coli* MG1655 (wild-type) strain was grown in LB medium, each culture was subjected to either 6 mM H_2O_2 for 30 min[15] or to 4 mM HOCl for 1h. Cultures were then serially diluted in PBS and spotted onto LB agar plates. After 18 h of incubation, CFU (colony forming unit) were counted and compared to untreated cells. Values shown represent the ratio between the CFU of treated cells and that of untreated cells at a dilution of $1x10^{-7}$. The results are expressed as means ± SD of three independent experiments.

Figure 3- Effect of different types of oxidative stress on HprSR activation. The induction of the *msrP-lacZ* fusion in strain CH183 was used as a proxy for HprSR activation. **A-B)** Effect of H_2O_2 on *msrP-lacZ* and *ahpC-lacZ* expression. Strain CH183 was subjected to sub-lethal concentrations of H_2O_2, ranging from 0.5 to 3 mM, and *msrP-lacZ* expression was followed at different time points. Strain BE1001 carrying the *ahpC-lacZ* fusion, known to respond to H_2O_2, was used as a positive control. Results are expressed as means ± SD (n=5). **C-D)** Effect of diamide on *msrP-lacZ* and *ahpC-lacZ* expression. Strain CH183 was subjected to sub-lethal concentrations of diamide (0.2, 0.3 and 0.4 mM), and *msrP-lacZ* expression was followed at different time points. Strain BE1001 carrying the *ahpC-lacZ* fusion, known to respond to diamide, was used as a positive control. Results are expressed

as means ± SD (n=3). **E-F)** Effect of the superoxide radical on *msrP-lacZ* and *soxS-lacZ* expression. Paraquat was used to produce superoxide radical in the cells. Strain CH183 was subjected to sub-lethal concentrations of paraquat (0.3 mM, 0.5 mM and 1 mM), and *msrP-lacZ* expression was followed at different time points. Strain BE1000 carrying the *soxS-lacZ* fusion, known to respond to the superoxide radical, was used as a positive control. Results are expressed as means ± SD (n=3). **G-H)** Effect of NO on *msrP-lacZ* and *hmpA-lacZ* expression. Strain CH183 was cultured anaerobically and subjected to different concentrations of NO (0.5 µM, 1 µM, 5 µM and 50 µM). *msrP-lacZ* expression was followed at different time points. The strain DV1301 carrying the fusion *hmpA-lacZ,* known to respond to NO, was used as a positive control. Results are expressed as means ± SD (n=3).

Figure 4- Effect of Reactive Chlorine Species on HprSR activation in rich and minimal medium. The induction of the *msrP-lacZ* fusion in strain CH183 is used as a proxy for HprSR activation. **A)** Effect of HOCl and Chlorotaurine (*N*-ChT) on *msrP-lacZ* expression in rich medium (LB). Strain CH183 was cultured in LB and subjected to 4 mM HOCl or 1 mM *N*-ChT. ß-galactosidase assays were conducted at different time points for each condition. Results are expressed as means ± SD (n=3). **B)** Effect of HOCl on *msrP-lacZ* expression in minimal medium (M9). Strain CH183 was cultured in M9 and subjected to 135 µM HOCl. ß-galactosidase assays were conducted at different time points. Results are expressed as means ± SD (n=3).

Figure 5- Role of each individual Met residue of the periplasmic loop of HprS.

A) Schematic view of HprS sub-domains. HprS is a transmembrane protein comprised of a small cytosolic N-Ter domain and two transmembrane (TM) helices bordering a periplasmic domain. The periplasmic domain contains 4 Met residues and the second TM (TM2) contains the Cys165 identified by *Urano et al.*[16] as being important for HprS. The C-ter cytosolic domain is formed by the HAMP domain and by the catalytic domain harboring the kinase activity. **B)** Evaluation of the expression of different plasmid alleles of *hprS* in strain CH186. CH186 cells carrying the empty plasmid pBBRMCS1

or expressing a variant of *hprS* (Wild type or mutants) were cultured overnight at 37 °C in LB medium in the presence of 1 mM IPTG. Immunoblot analysis was carried out using rabbit anti-HprS as a primary antibody. The abundance of each HprS variant was quantified and the relative HprS amount was calculated and normalized to $HprS^{WT}$. **C-D)** Effect of single Met mutations on *msrP* expression. The expression of *msrP* is used as a proxy for HprSR activity. MsrP production was assessed by immunoblot analysis using anti-MsrP as a primary antibody and by measuring *msrP-lacZ* expression. Both analyses were carried out on CH186 cells carrying either the empty plasmid pBBRMCS1 (negative control), expressing wild-type *hprS* (positive control) or *hprS* mutants. *msrP* expression was analyzed in each strain after treatment with 4 mM HOCl for 1 hour in LB medium. Values of the ß-galactosidase assays are expressed as means ± SD (n=3). **C)** Effect of Met-to-Ala substitution on HprS activity: M72A, M73A, M100A, M153A **D)** Effect of Met-to-Gln substitution on HprS activity: M72Q, M73Q, M100Q, M153Q.

Figure 6- Effect of combining two, three or four Met-to-Ala mutations on HprS activity

A) Immunoblot analysis showing the expression of different plasmid *hprS* alleles (WT and double Ala-to Met mutations) in CH186 cells. CH186 cells carrying the empty plasmid pBBRMCS1 or expressing a variant of *hprS* (WT or mutants) were cultured overnight at 37 °C in LB medium in the presence of 1 mM IPTG. Immunoblot analysis was carried out using rabbit anti-HprS as a primary antibody. The abundance of each HprS variant was quantified and the relative HprS amount was calculated and normalized to $HprS^{WT}$. **B)** Effect of combining two Met-to-Ala mutations on HprS activity. The expression of *msrP* is used as a proxy for HprSR activity. MsrP production is assessed by immunoblot analysis using an anti-MsrP antibody and by measuring *msrP-lacZ* expression. Both analyses were carried out on CH186 cells carrying either the empty plasmid pBBRMCS1 (negative control), expressing wild-type *hprS* (positive control) or *hprS* mutants: M72-73A, M72-100A, M72-153A, M73-100A, M73-153A. *msrP* expression was analyzed in each strain after treatment with HOCl. Values of the ß-galactosidase assays are expressed as mean ± SD (n =3). **C)** Effect of combining three or four

682 Met-to-Ala mutations on HprS activity. The expression of *msrP* is used as a proxy for HprSR activity.
683 MsrP production is assessed by immunoblot analysis using an anti-MsrP antibody and by measuring
684 *msrP-lacZ* expression. Both analyses were carried out on CH186 cells carrying either the empty
685 plasmid pBBRMCS1 (negative control), expressing wild-type *hprS* (positive control) or the *hprS*
686 mutants: M72-73-100A or M72-73-100-153A. *msrP* expression was analyzed in each strain after
687 treatment with 4 mM HOCl. Values of the ß-galactosidase assays are expressed as mean ± SD (n =3).
688
689 **Figure 7- Effect of MsrP on *hiuH-lacZ* expression. A)** Effect of *msrP* deletion on *hiuH-lacZ* expression.
690 Strains SH100 (P_{TetR}-*hprRS cat-hiuH-lacZ*) and SH101 (P_{TetR}-*hprRS ΔmsrP cat-hiuH-lacZ*) were cultured
691 in M9 minimal medium. 135 µM of HOCl was added to each strain at OD_{600}=0.2, and *hiuH-lacZ*
692 expression was followed for 4 hours. Values of the ß-galactosidase assays are expressed as means ±
693 SD (n=4). An asterisk indicates a statistically significant difference between the ß-galactosidase
694 activity of different strains. **B)** Effect of complementing the *msrP* deletion on *hiuH-lacZ* expression.
695 SH100 carrying an empty plasmid and SH101 carrying either an empty plasmid or pMsrPQ were
696 cultured in M9 minimal medium. 135 µM HOCl was added to each strain at OD_{600}=0.2 and *hiuH-lacZ*
697 expression was followed for 4 hours. Values of the ß-galactosidase assays are expressed as means ±
698 SD (n=3). An asterisk indicates a statistically significant difference between ß-galactosidase activity of
699 different strains. *P ≤ 0.05 (Mann-Whitney U test).
700
701 **Figure 8- Protein sequence comparison of HprS homologues.** HprS homologues were selected based
702 on their genetic environment (close to an *msrP-msrQ gene* or an *hiuH* gene) and on their orthology
703 with *E. coli hprS*. **A)** Diagram representing the genetic environment of each selected *hprS* homologue.
704 **B)** Protein sequence comparison of different HprS homologues. The alignment shown in this figure
705 represents three different regions of HprS each containing one or two residue(s) of interest: Met72,
706 Met73, Met100, Met153 and Cys165. Sequence comparison was carried-out using Clustal Omega
707 (Whitworth and Cock 2009). The code attributed to each homologue in the STRING database is the

following: *b1968* (*Escherichia coli K12 MG1655*), *SF2015* (*Shigella flexneri*), *BBNB01000011_gene586* (*Citrobacter sedlakii*), *CY43_05595* (*Salmonella enterica Typhimurium*), *BN137_3921* (*Cronobacter condimenti*), *EBL_c13160* (*Shimwellia blattae*), *HEAR0605* (*Herminiimonas arsenicoxydans*), *B597_003020* (*Pseudomonas stutzeri KOS6*), *HFRIS_015940* (*Herbaspirillum frisingense*), *PROVALCAL_02806* (*Providencia alcalifaciens*), *MU9_2071* (*Morganella morganii*), *HW45_14750* (*Vibrio sp. ER1A*).

Figure 9- Evaluation of the role of Cys165 in HprS activity. A) Immunoblot analysis showing the expression of different plasmid *hprS* alleles: WT, M72Q, C165A and M72Q-C165A in CH186 cells. CH186 cells carrying the empty plasmid pBBRMCS1 or expressing a variant of *hprS* (WT or mutants) were cultured overnight at 37 °C in LB medium in the presence of 1 mM IPTG. Immunoblot analysis was carried out using rabbit anti-HprS as a primary antibody. The abundance of each HprS variant was quantified and the relative HprS amount was calculated and normalized to $HprS^{WT}$. **B)** Effect of Cys165A mutation on HprS activity. The expression of *msrP* is used as a proxy for HprSR activity. MsrP production was assessed by immunoblot analysis using an anti-MsrP antibody and by measuring *msrP-lacZ* expression. Both analyses were carried out on CH186 cells carrying either the empty plasmid pBBRMCS1 (negative control), expressing wild-type *hprS* (positive control) or *hprS* mutants: M72Q, C165A or M72Q-C165A. *msrP* expression was analyzed in each strain after treatment with 4 mM HOCl for 1 hour in LB medium. Values of the ß-galactosidase assays are expressed as means ± SD (n =3).

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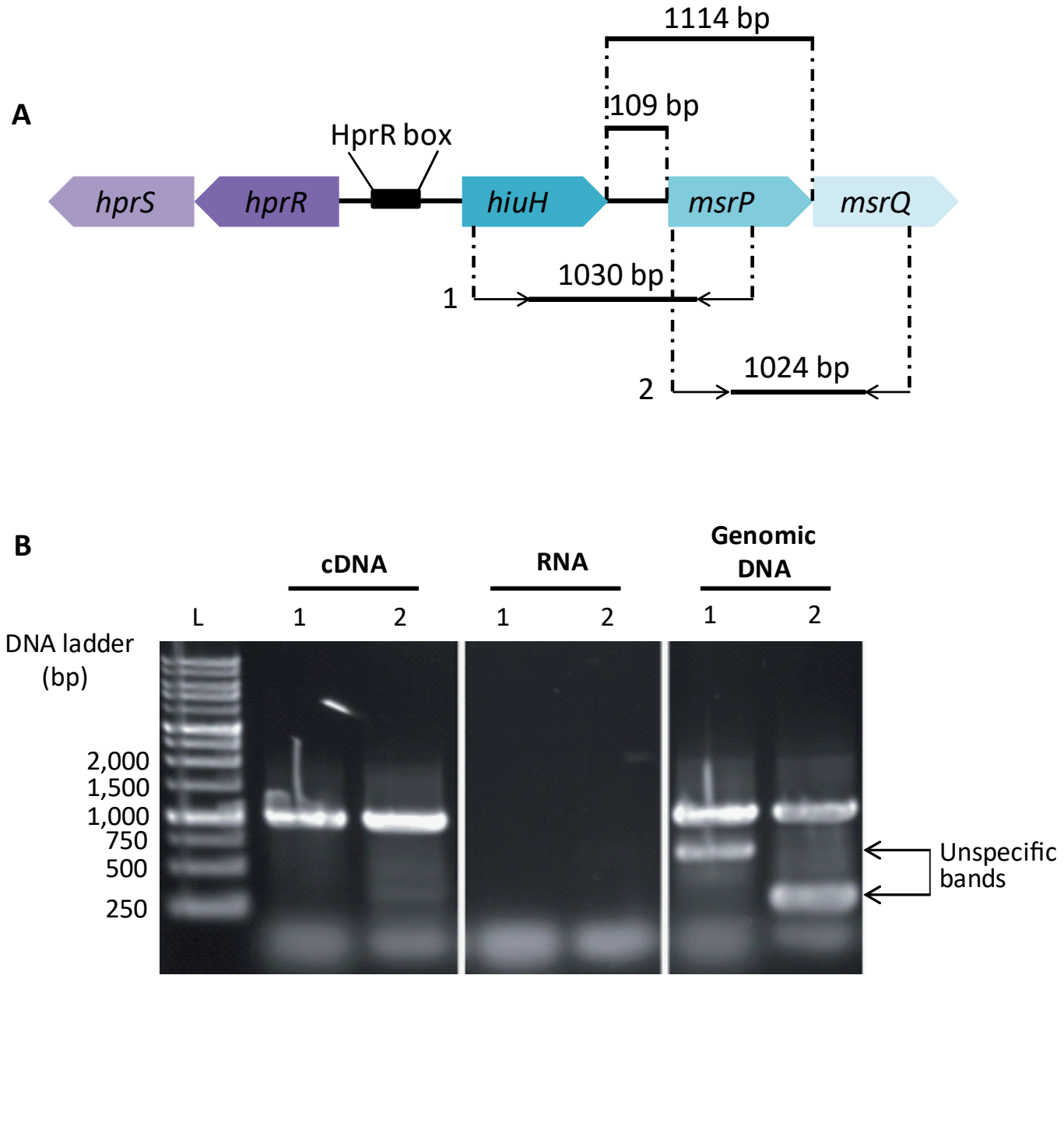
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hprS
hprR
hiuH
msrP
msrQ
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1
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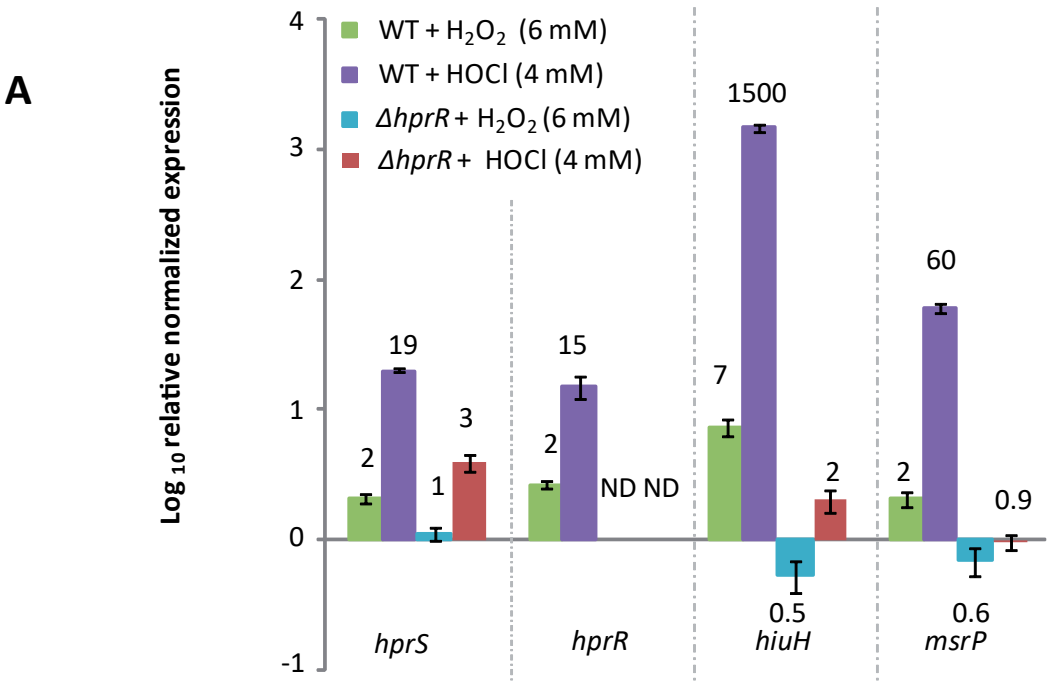
2
B
cDNA
RNA
Genomic DNA
L
1
2
1
2
1
2
DNA ladder (bp)
2,000
1,500
1,000
750
500
250
Unspecific bands

Fig.1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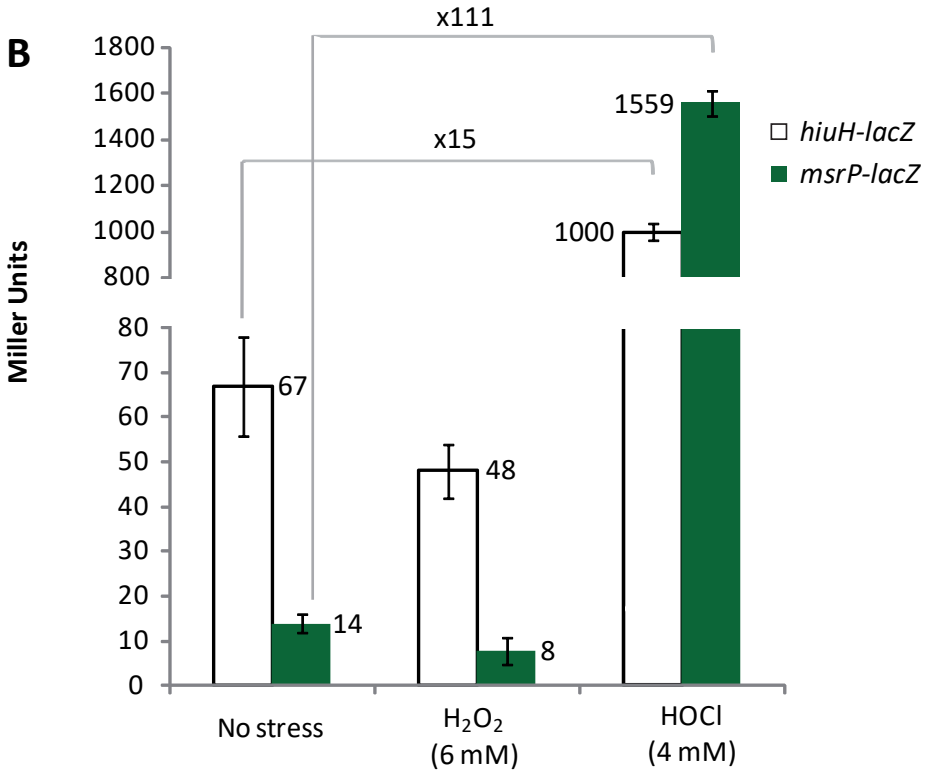
WT + HOCl (4 mM)
ΔhprR + H2O2 (6 mM)
ΔhprR + HOCl (4 mM)
4
3
2
1
0
-1
2
19
1
3
2
15
ND ND
7
1500
0.5
2
2
60
0.6
0.9
hprS
hprR
hiuH
msrP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Miller Units
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x15
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1600
1400
1200
1000
800
80
70
60
50
40
30
20
10
0
1559
1000
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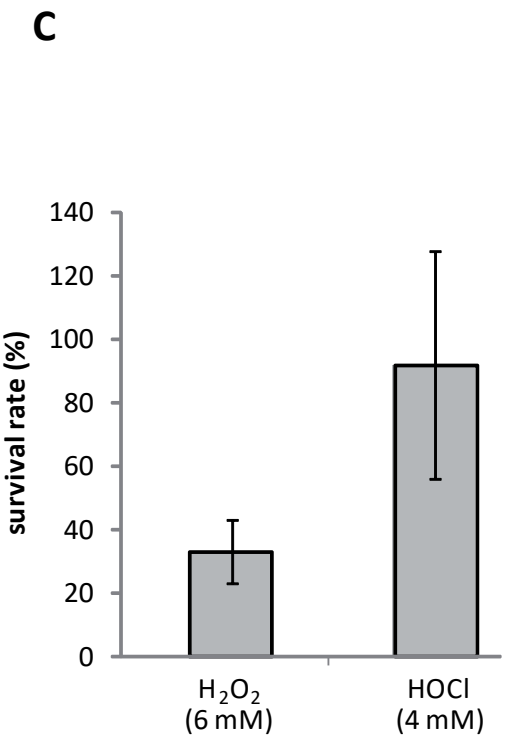
14
48
8
hiuH-lacZ
msrP-lacZ
No stress
H2O2 (6 mM)
HOCl (4 mM)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40
20
0
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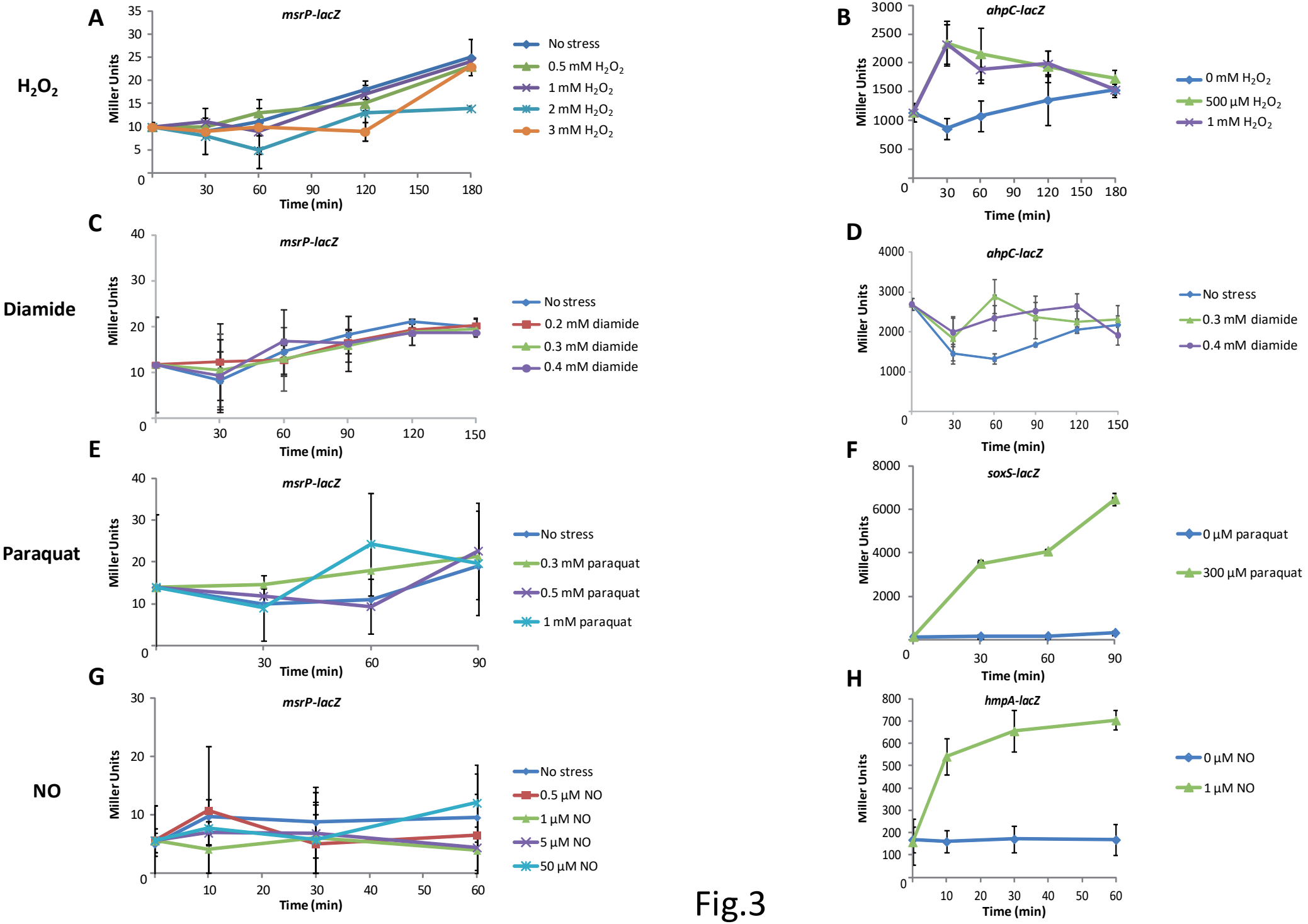
HOCl (4 mM)

Fig.2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H2O2
B
ahpC-lacZ
Miller Units
Time (min)
0 mM H2O2
500 µM H2O2
1 mM H2O2
C
msrP-lacZ
Miller Units
Time (min)
No stress
0.2 mM diamide
0.3 mM diamide
0.4 mM diamide
Diamide
D
ahpC-lacZ
Miller Units
Time (min)
No stress
0.3 mM diamide
0.4 mM diamide
E
msrP-lacZ
Miller Units
Time (min)
No stress
0.3 mM paraquat
0.5 mM paraquat
1 mM paraquat
Paraquat
F
soxS-lacZ
Miller Units
Time (min)
0 µM paraquat
300 µM paraquat
G
msrP-lacZ
Miller Units
Time (min)
No stress
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NO
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Time (min)
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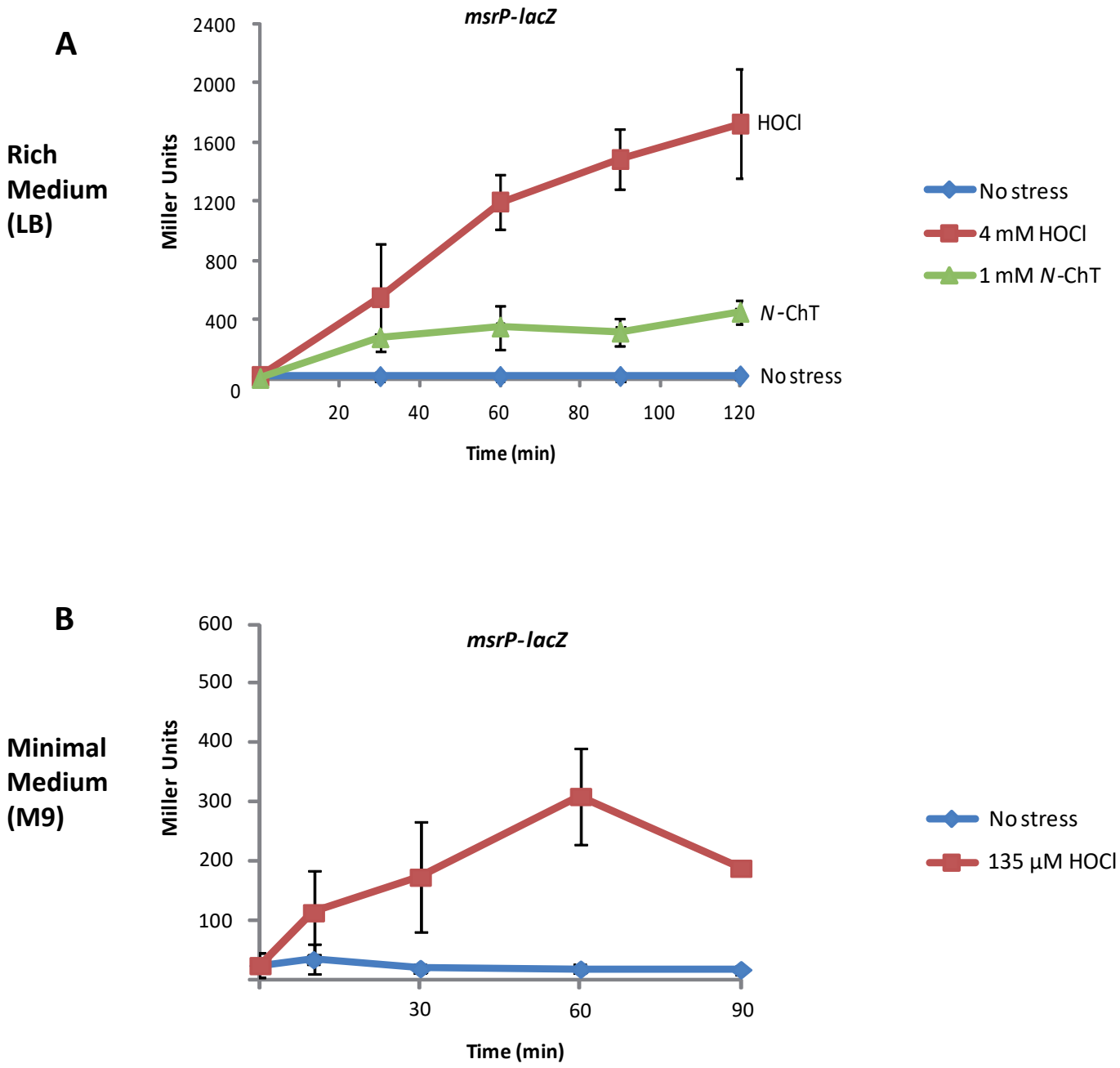
1 µM NO

Fig.3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1600
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800
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20
40
60
80
100
120
Time (min)
HOCl
N-ChT
No stress
No stress
4 mM HOCl
1 mM N-ChT
B
Minimal Medium (M9)
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500
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300
200
100
30
60
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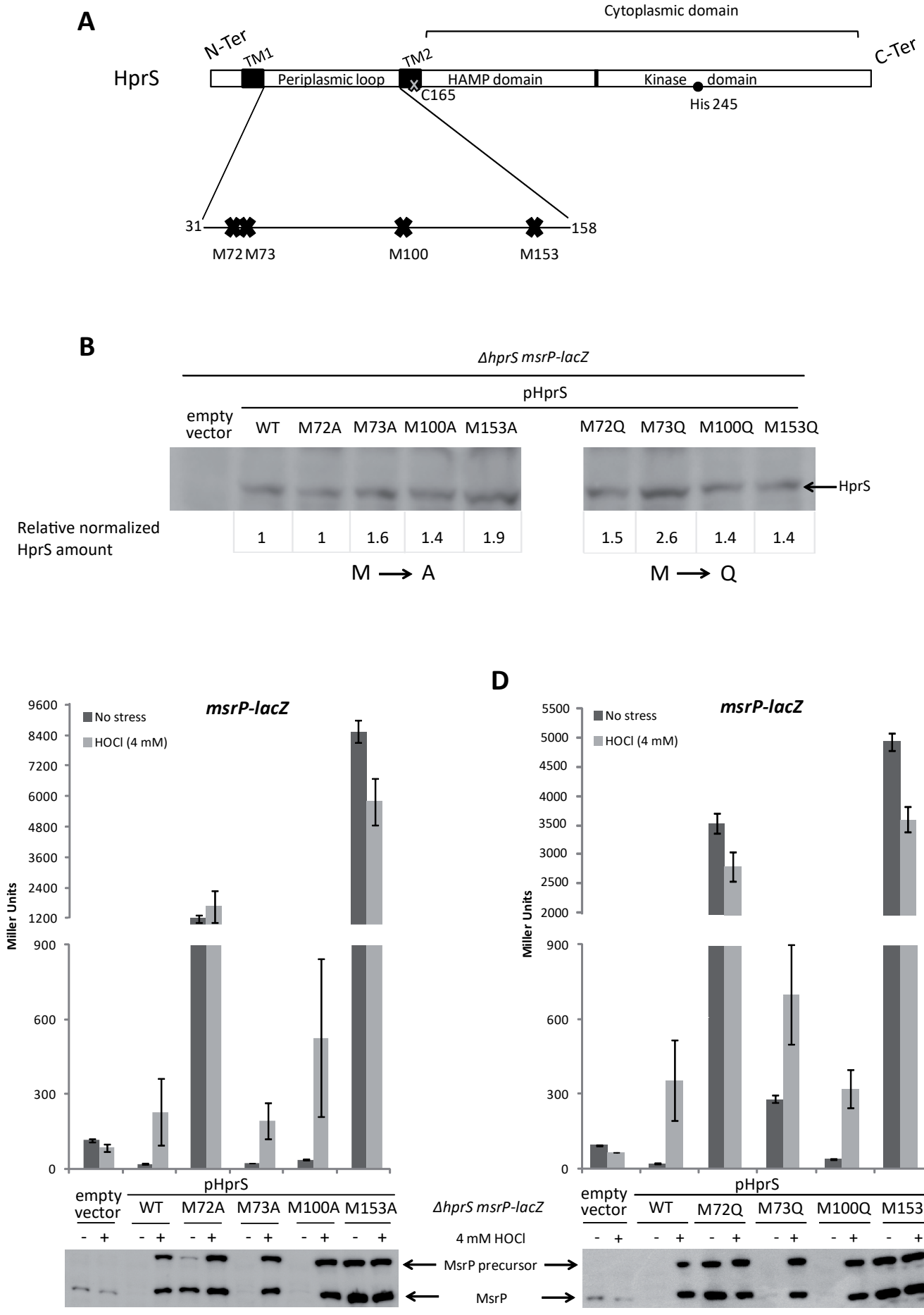
Fig.4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N-Ter
TM1
TM2
C-Ter
HprS
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HAMP domain
Kinase domain
C165
His 245
31
158
M72 M73
M100
M153
B
ΔhprS msrP-lacZ
pHprS
empty vector
WT
M72A
M73A
M100A
M153A
M72Q
M73Q
M100Q
M153Q
HprS
Relative normalized HprS amount
1
1
1.6
1.4
1.9
1.5
2.6
1.4
1.4
M → A
M → Q
C
msrP-lacZ
No stress
HOCl (4 mM)
Miller Units
9600
8400
7200
6000
4800
3600
2400
1200
900
600
300
0
pHprS
empty vector
WT
M72A
M73A
M100A
M153A
ΔhprS msrP-lacZ
4 mM HOCl
MsrP precursor
MsrP
D
msrP-lacZ
No stress
HOCl (4 mM)
Miller Units
5500
5000
4500
4000
3500
3000
2500
2000
900
600
300
0
pHprS
empty vector
WT
M72Q
M73Q
M100Q
M153Q

Fig.5

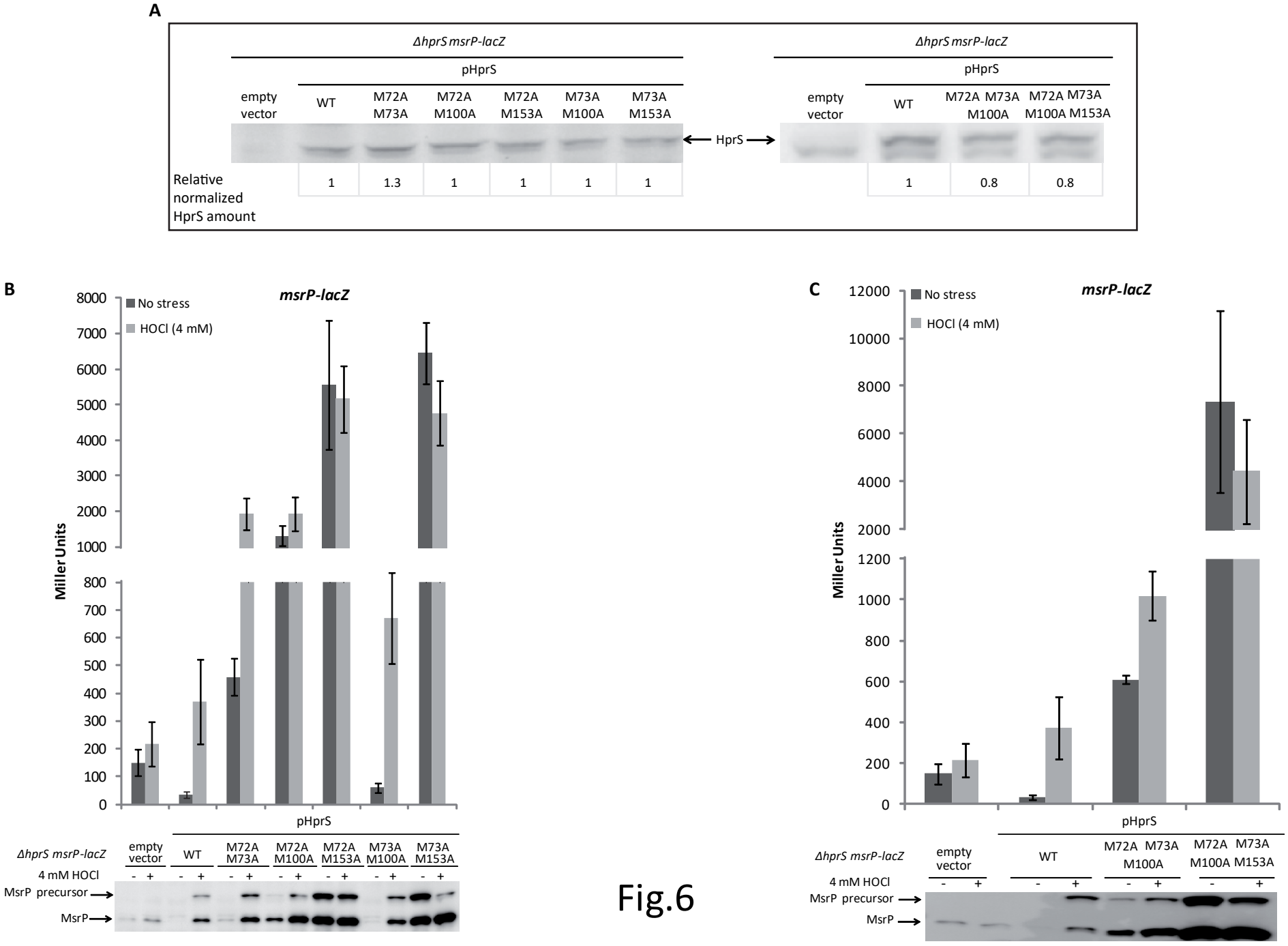
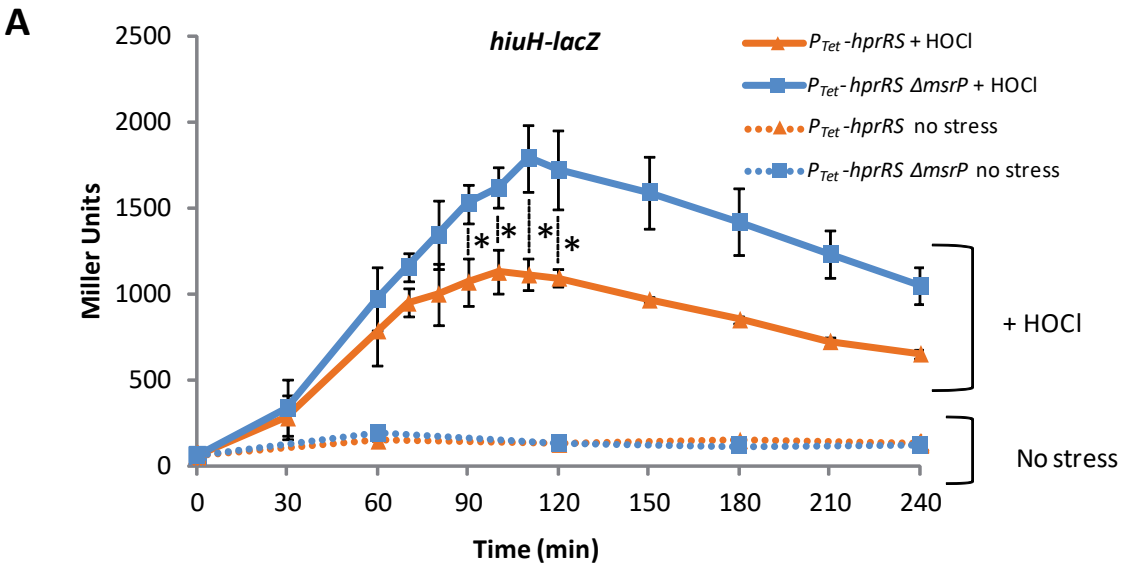

A
ΔhprS msrP-lacZ
pHprS
empty vector
WT
M72A M73A
M72A M100A
M72A M153A
M73A M100A
M73A M153A
HprS
ΔhprS msrP-lacZ
pHprS
empty vector
WT
M72A M73A M100A
M72A M73A M100A M153A
Relative normalized HprS amount
1
1.3
1
1
1
1
1
0.8
0.8
B
msrP-lacZ
No stress
HOCl (4 mM)
Miller Units
8000
7000
6000
5000
4000
3000
2000
1000
800
700
600
500
400
300
200
100
0
pHprS
ΔhprS msrP-lacZ
empty vector
WT
M72A M73A
M72A M100A
M72A M153A
M73A M100A
M73A M153A
4 mM HOCl
- + - + - + - + - + - +
MsrP precursor
MsrP
C
msrP-lacZ
No stress
HOCl (4 mM)
Miller Units
12000
10000
8000
6000
4000
2000
1200
1000
800
600
400
200
0
pHprS
ΔhprS msrP-lacZ
empty vector
WT
M72A M73A M100A
M72A M73A M100A M153A
4 mM HOCl
- + - + - + - +
MsrP precursor
MsrP

Fig.6


A
hiuH-lacZ
P_{Tet}-hprRS + HOCl
P_{Tet}-hprRS ΔmsrP + HOCl
P_{Tet}-hprRS no stress
P_{Tet}-hprRS ΔmsrP no stress
Miller Units
2500
2000
1500
1000
500
0
0
30
60
90
120
150
180
210
240
Time (min)
+ HOCl
No stress

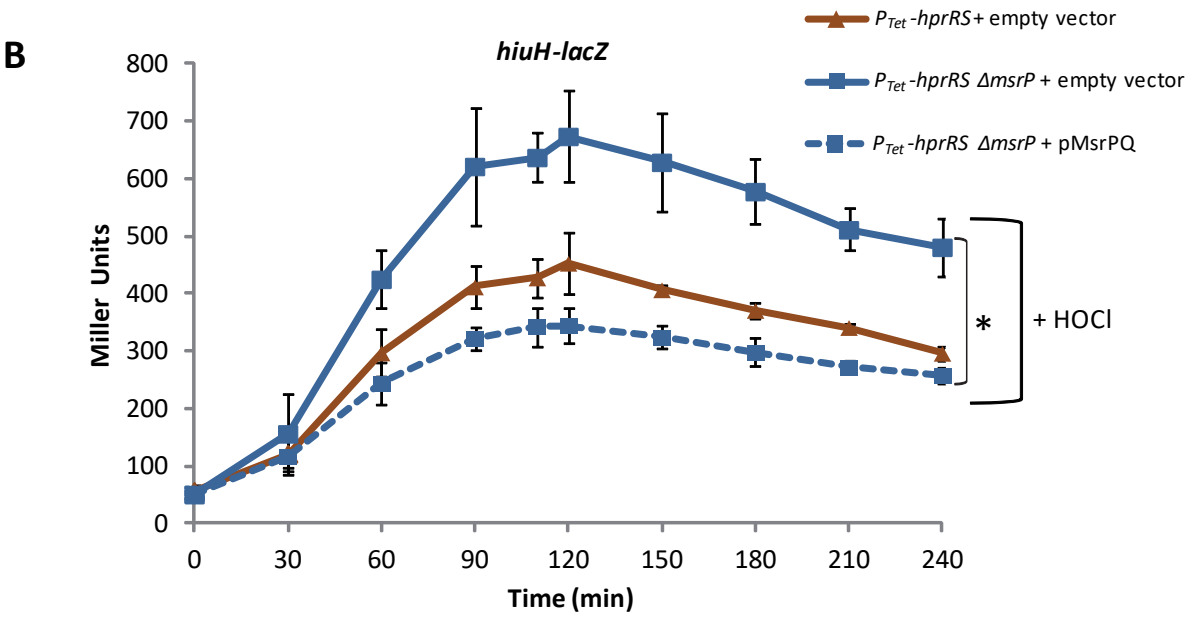
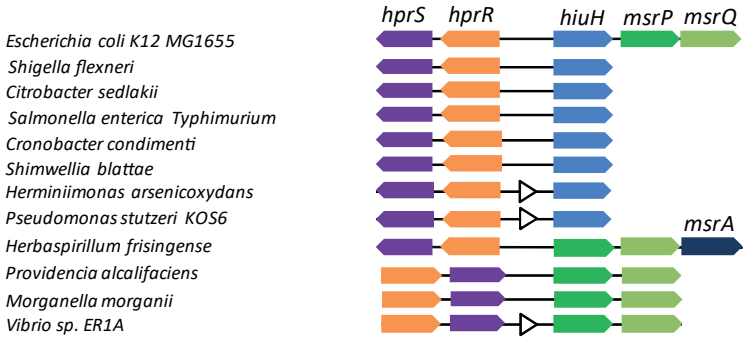

B
hiuH-lacZ
P_{Tet}-hprRS+ empty vector
P_{Tet}-hprRS ΔmsrP + empty vector
P_{Tet}-hprRS ΔmsrP + pMsrPQ
Miller Units
800
700
600
500
400
300
200
100
0
0
30
60
90
120
150
180
210
240
Time (min)
*
+ HOCl

Fig.7

A


hprS
hprR
hiuH
msrP
msrQ
msrA
Escherichia coli K12 MG1655
Shigella flexneri
Citrobacter sedlakii
Salmonella enterica Typhimurium
Cronobacter condimenti
Shimwellia blattae
Herminiimonas arsenicoxydans
Pseudomonas stutzeri KOS6
Herbaspirillum frisingense
Providencia alcalifaciens
Morganella morganii
Vibrio sp. ER1A

B

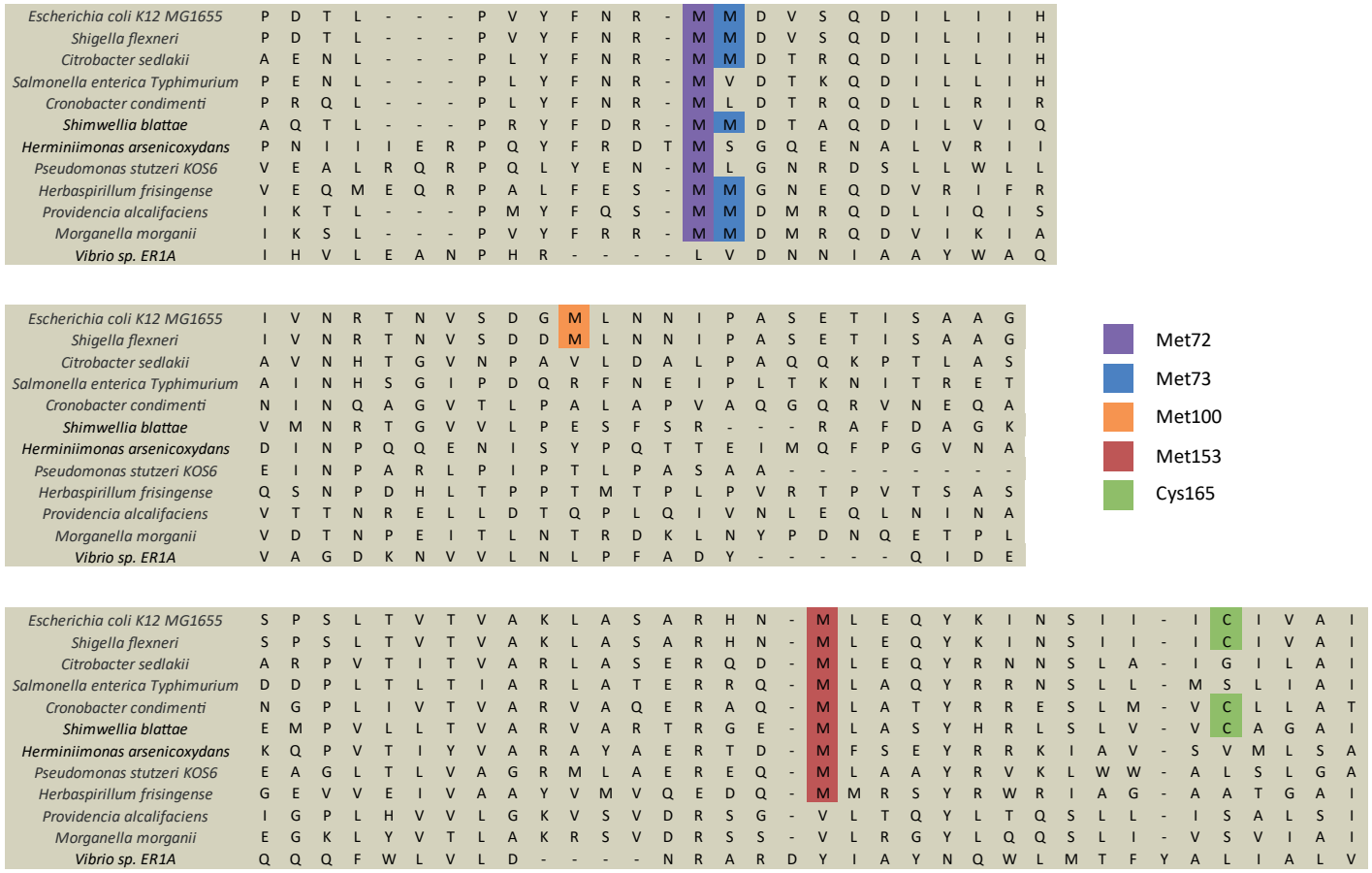

Escherichia coli K12 MG1655 P D T L - - - P V Y F N R - M M D V S Q D I L I I H
Shigella flexneri P D T L - - - P V Y F N R - M M D V S Q D I L I I H
Citrobacter sedlakii A E N L - - - P L Y F N R - M M D T R Q D I L L I H
Salmonella enterica Typhimurium P E N L - - - P L Y F N R - M V D T K Q D I L L I H
Cronobacter condimenti P R Q L - - - P L Y F N R - M L D T R Q D L L R I R
Shimwellia blattae A Q T L - - - P R Y F D R - M M D T A Q D I L V I Q
Herminiimonas arsenicoxydans P N I I I E R P Q Y F R D T M S G Q E N A L V R I I
Pseudomonas stutzeri KOS6 V E A L R Q R P Q L Y E N - M L G N R D S L L W L L
Herbaspirillum frisingense V E Q M E Q R P A L F E S - M M G N E Q D V R I F R
Providencia alcalifaciens I K T L - - - P M Y F Q S - M M D M R Q D L I Q I S
Morganella morganii I K S L - - - P V Y F R R - M M D M R Q D V I K I A
Vibrio sp. ER1A I H V L E A N P H R - - - - L V D N N I A A Y W A Q
Escherichia coli K12 MG1655 I V N R T N V S D G M L N N I P A S E T I S A A G
Shigella flexneri I V N R T N V S D D M L N N I P A S E T I S A A G
Citrobacter sedlakii A V N H T G V N P A V L D A L P A Q Q K P T L A S
Salmonella enterica Typhimurium A I N H S G I P D Q R F N E I P L T K N I T R E T
Cronobacter condimenti N I N Q A G V T L P A L A P V A Q G Q R V N E Q A
Shimwellia blattae V M N R T G V V L P E S F S R - - - R A F D A G K
Herminiimonas arsenicoxydans D I N P Q Q E N I S Y P Q T T E I M Q F P G V N A
Pseudomonas stutzeri KOS6 E I N P A R L P I P T L P A S A A - - - - - - - -
Herbaspirillum frisingense Q S N P D H L T P P T M T P L P V R T P V T S A S
Providencia alcalifaciens V T T N R E L L D T Q P L Q I V N L E Q L N I N A
Morganella morganii V D T N P E I T L N T R D K L N Y P D N Q E T P L
Vibrio sp. ER1A V A G D K N V V L N L P F A D Y - - - - - Q I D E
Escherichia coli K12 MG1655 S P S L T V T V A K L A S A R H N - M L E Q Y K I N S I I - I C I V A I
Shigella flexneri S P S L T V T V A K L A S A R H N - M L E Q Y K I N S I I - I C I V A I
Citrobacter sedlakii A R P V T I T V A R L A S E R Q D - M L E Q Y R N N S L A - I G I L A I
Salmonella enterica Typhimurium D D P L T L T I A R L A T E R R Q - M L A Q Y R R N S L L - M S L I A I
Cronobacter condimenti N G P L I V T V A R V A Q E R A Q - M L A T Y R R E S L M - V C L L A T
Shimwellia blattae E M P V L L T V A R V A R T R G E - M L A S Y H R L S L V - V C A G A I
Herminiimonas arsenicoxydans K Q P V T I Y V A R A Y A E R T D - M F S E Y R R K I A V - S V M L S A
Pseudomonas stutzeri KOS6 E A G L T L V A G R M L A E R E Q - M L A A Y R V K L W W - A L S L G A
Herbaspirillum frisingense G E V V E I V A A Y V M V Q E D Q - M M R S Y R W R I A G - A A T G A I
Providencia alcalifaciens I G P L H V V L G K V S V D R S G - V L T Q Y L T Q S L L - I S A L S I
Morganella morganii E G K L Y V T L A K R S V D R S S - V L R G Y L Q Q S L I - V S V I A I
Vibrio sp. ER1A Q Q Q F W L V L D - - - - N R A R D Y I A Y N Q W L M T F Y A L I A L V
Met72
Met73
Met100
Met153
Cys165

Fig.8

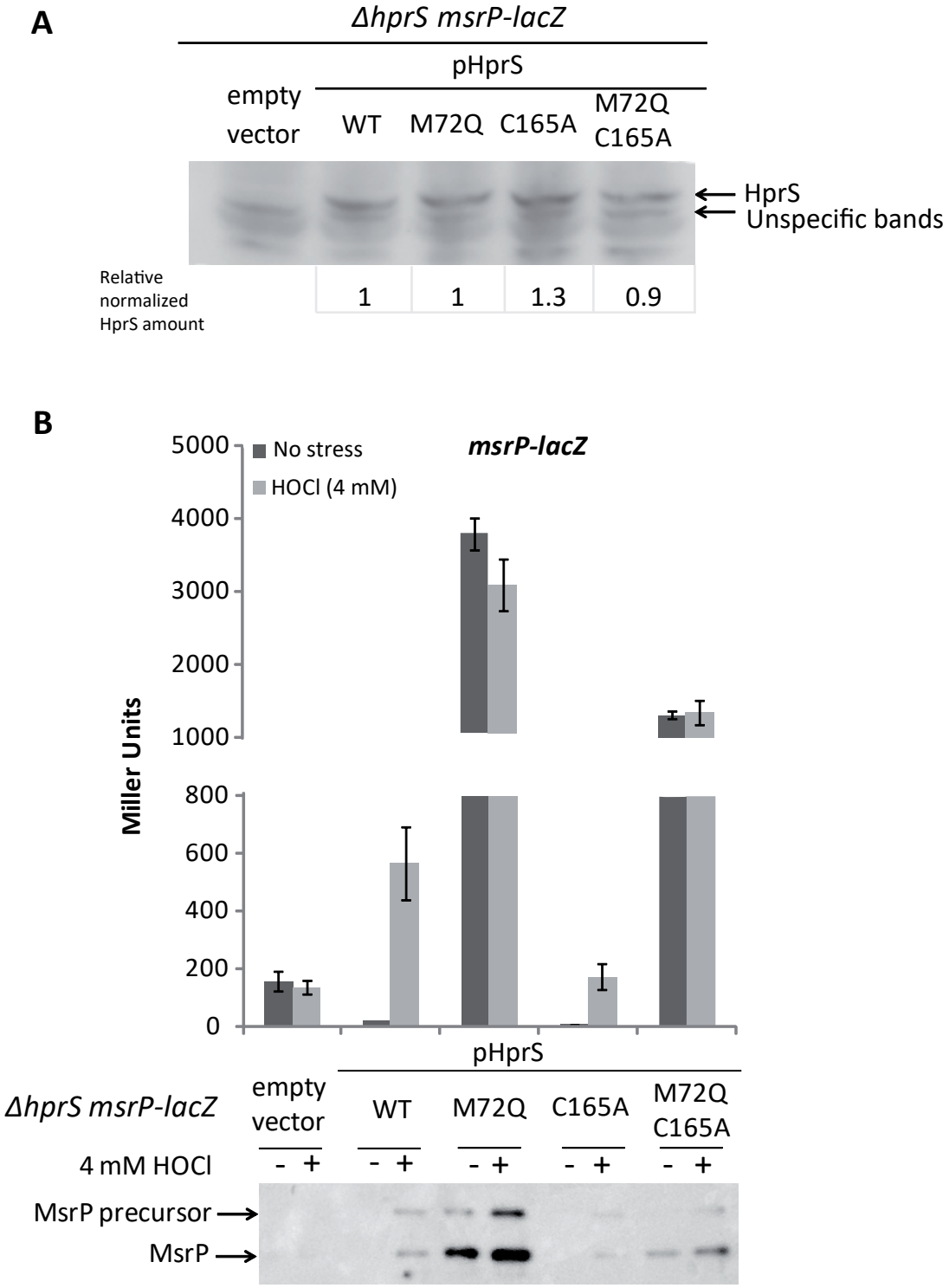

A
ΔhprS msrP-lacZ
pHprS
empty vector
WT
M72Q
C165A
M72Q C165A
HprS
Unspecific bands
Relative normalized HprS amount
1
1
1.3
0.9
B
msrP-lacZ
No stress
HOCl (4 mM)
Miller Units
5000
4000
3000
2000
1000
800
600
400
200
0
pHprS
ΔhprS msrP-lacZ
empty vector
WT
M72Q
C165A
M72Q C165A
4 mM HOCl
- + - + - + - + - +
MsrP precursor
MsrP

Fig.9