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1 **HprSR is a Reactive Chlorine Species-Sensing, Two-Component System in**
2 ***Escherichia coli***

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14 **Running Title:** RCS-sensing two-component system

15

16 **ABSTRACT**

17 Two-component systems (TCS) are signalling pathways that allow bacterial cells to sense, respond
18 and adapt to fluctuating environments. Among the classical TCS of *Escherichia coli*, HprSR has
19 recently been shown to be involved in the regulation of *msrPQ*, which encodes the periplasmic
20 methionine sulfoxide reductase system. In this study, we demonstrate that hypochlorous acid (HOCl)
21 induces the expression of *msrPQ* in an HprSR-dependant manner, whereas H₂O₂, NO and paraquat (a
22 superoxide generator) do not. Therefore, HprS appears to be an HOCl-sensing histidine kinase. Using
23 a directed mutagenesis approach, we show that Met residues located in the periplasmic loop of HprS
24 are important for its activity: as HOCl preferentially oxidizes Met residues, we provide evidence that
25 HprS could be activated via the reversible oxidation of its methionine residues, meaning that MsrPQ
26 plays a role in switching HprSR off. We propose that the activation of HprS by HOCl could occur
27 through a Met redox switch. HprSR appears to be the first characterized TCS able to detect reactive
28 chlorine species (RCS) in *E. coli*. This study represents an important step towards understanding the
29 mechanisms of RCS resistance in prokaryotes.

30

31 **IMPORTANCE**

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

40 INTRODUCTION

41 During infection the host innate immune system produces high levels of reactive oxygen and chlorine
42 species (ROS, RCS), such as hydrogen peroxide (H₂O₂) and hypochlorous acid (HOCl), in order to kill
43 invading pathogens¹. One major challenge for bacteria is to overcome oxidative stress and while
44 bacterial responses to H₂O₂ have been extensively studied, much less is known about their response
45 to HOCl. In the cell, HOCl can damage biomolecules like DNA, lipids and proteins^{2,3}. Within proteins,
46 HOCl preferentially oxidizes sulfur-containing amino acids such as cysteine and methionine⁴. The
47 thiol group of the cysteine can be oxidized to sulfenic (-SOH), sulfinic (-SO₂H) and sulfonic (-SO₃H)
48 acids or to disulphide bonds (S-S)⁵. The thioether side chain of methionine can be oxidized to
49 sulfoxide, thereby converting methionine into methionine sulfoxide (Met-O)⁶.

50 Bacterial cells can prevent damage caused by HOCl through various mechanisms and pathways.
51 Many of them are general ROS-responsive pathways, but others detect and respond specifically to
52 HOCl⁷. In *Escherichia coli*, recent studies identified several regulatory factors activated by HOCl, like
53 the transcription factors HypT (a LysR-type regulator) and RclR (an AraC-type activator). HypT detects
54 and responds to HOCl through the oxidation of three methionine residues⁸, whereas RclR is
55 activated through the oxidation of two cysteine residues⁹. Upon activation, HypT up-regulates genes
56 involved in Met and Cys biosynthesis while repressing genes involved in iron acquisition. The
57 activation of RclR induces the expression of *rcI* genes that are necessary to resist HOCl treatment⁹.
58 Similarly, NemR (a TetR-family repressor) functions as an HOCl-responsive transcription factor in *E.*
59 *coli* which controls the expression of genes involved in HOCl resistance like *nemA* and *goIA*¹⁰. Repair
60 of HOCl damage represents another way for bacteria to survive this stress. The methionine sulfoxide
61 reductases (MSR), which repair HOCl-oxidized proteins, are involved in HOCl resistance¹¹.

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

65 conditions ¹². Our previous work showed that in *E. coli* the HprSR (formerly YedVW) two-component
66 system is involved in the induction of the *msrPQ* genes in response to HOCl¹³. The *msrPQ* encodes the
67 periplasmic methionine sulfoxide reductase (MsrPQ), composed of a periplasmic molybdopterin-
68 containing oxidoreductase (MsrP) and a haem-containing, membrane-bound protein (MsrQ). MsrP
69 catalyzes the reduction of protein-bound Met-O and therefore repairs periplasmic oxidized proteins
70 after HOCl stress ¹³.

71 In the *E. coli* genome, the *hprSR* operon is upstream of the *msrPQ* genes and transcribed in the
72 opposite direction. The intergenic region contains *hiuH*, a gene encoding a transthyretin-like
73 periplasmic protein: this protein hydrolyses 5-hydroxyisourate (5-HIU), a compound formed via the
74 oxidation of urate ¹⁴. The Ishihama and Ogasawara laboratories showed that the expression of *hiuH*
75 was induced by H₂O₂ in an HprSR-dependent manner ¹⁵. Moreover, they identified the oxidation of
76 the Cys165 residue located in the transmembrane domain of HprS as being essential for the sensing
77 of H₂O₂ ¹⁶. Finally, they suggested renaming this system HprSR for hydrogen peroxide response
78 sensor/regulator.

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

86

87

88 RESULTS

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

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

100

101 *HOCl* is a better signal for HprSR than H_2O_2

102 To explore the physiological signals controlling the HprS-HprR regulatory system, we investigated the
103 expression of the HprR-activated genes *hiuH*, *msrP*, *hprS* and *hprR*, by qRT-PCR analysis and by
104 measuring the β -galactosidase activity originating from *hiuH-lacZ* or *msrP-lacZ* translational fusions.
105 First, we re-evaluated the response of HprSR to H_2O_2 as previously reported by Urano and
106 collaborators¹⁵. We carried out qRT-PCR analysis on RNAs isolated from wild type *E. coli* cells grown
107 to mid-log phase and treated with H_2O_2 (Fig. 2A). The results show a 2-fold up-regulation of *hprS*,
108 *hprR* and *msrP* and an approximately 7-fold up-regulation of *hiuH* in response to H_2O_2 treatment (6
109 mM as used by Urano *et al*, 2015). The induction by H_2O_2 was dependent on the presence of HprR
110 since no up-regulation was observed in a $\Delta hprR$ mutant. However, using translational *hiuH-lacZ* or
111 *msrP-lacZ* reporter fusions, no up-regulation was observed after H_2O_2 treatment in the wild-type
112 strain (Fig. 2B). These results agree with previous observations reported by Gennaris *et al.*, showing
113 that exposure of wild-type cells to H_2O_2 did not induce the synthesis of MsrP, despite slightly

114 inducing its transcription. However, Gennaris *et al.* reported an increase in the synthesis of MsrP
115 after HOCl treatment¹³. Indeed, qRT-PCR analysis shows that HOCl treatment (4 mM) led to a 1,500-
116 fold up-regulation of *hiuH* and a 60-fold up-regulation of *msrP* and an approximately 20-fold up-
117 regulation of *hprR* and *hprS* in the wild-type strain (Fig. 2A). Using the translational *hiuH-lacZ* and
118 *msrP-lacZ* reporter fusions, we found a 15-fold (*hiuH*) and 110-fold (*msrP*) up-regulation after HOCl
119 stress (Fig. 2B). Collectively, these results indicate that the induction of the *hiuH-msrPQ* operon by
120 HOCl was dependent on the presence of a functional HprSR system. Our results also reveal that HOCl
121 is a better signal than H₂O₂ for HprSR activation. The concentration of H₂O₂ required to activate
122 HprSR led to a survival rate of 33% whereas the concentration of HOCl required led to a survival rate
123 of 92% (Fig 2C).

124 To overcome the lethality, different sub-lethal concentrations of H₂O₂ (0.5 to 3 mM) were tested for
125 *msrP-lacZ* expression. The H₂O₂ was added at OD≈0.2 and the β-galactosidase activities were
126 measured over a period of 3 hours. For all the sub-lethal concentrations tested, no induction of the
127 *msrP-lacZ* fusion was observed (Fig. 3A) compared to the unstressed sample, whereas for the positive
128 control, the *ahpC-lacZ* fusion, which responds to H₂O₂ via activation of OxyR, shows a 2.5-fold
129 induction after 30 min of exposure to 0.5 and 1 mM H₂O₂ (Fig. 3B). These results challenge the
130 relevance of the *in vivo* activation of HprSR by H₂O₂ and raise the question of the real signal detected
131 by this system. Therefore, we decided to investigate the response of HprSR to different type of
132 oxidants *in vivo*. We treated wild-type *E. coli* cells with various reactive oxygen, nitrogen and chlorine
133 species at different sub-lethal concentrations. No significant expression of *msrP* was observed in
134 response to diamide (a thiol-specific oxidant), paraquat (1,1'-dimethyl-4,4'-bipyridinium, a
135 superoxide generator) or nitric oxide (NO) (Fig. 3C, 3E and 3G). The *ahpC-lacZ*, *soxS-lacZ* and the
136 *hmpA-lacZ* fusions, which respond to diamide, paraquat and NO respectively, were used as positive
137 controls (Fig. 3D, 3F and 3H). HprSR is activated by HOCl and *N*-Chlorotaurine (*N*-ChT), a weakly
138 membrane-diffusible but long-lived oxidant produced by the reaction between HOCl and the amino
139 acid taurine (Fig. 4A). Moreover, to overcome the problem of HOCl hyper-reactivity against LB

140 compounds, we performed a time course experiment in minimal media (M9) at a lower HOCl
141 concentration (135 μ M). A significant *msrP* induction was observed from 10 minutes after stress (Fig.
142 4B). These data show that the activation of HprSR is specific to HOCl and related RCS.

143

144 ***Positions Met72 and Met153 are important for HprS activity.***

145 Our next goal was to investigate the molecular mechanism by which HprS senses HOCl. HOCl has
146 been shown to modify proteins via the oxidation of sulfur-containing compounds, such as cysteine
147 and methionine¹⁷. Both residues have the highest degree of reactivity towards HOCl. A previous
148 study had already investigated the implication of cysteine residues in HprS activation, and it had
149 pointed out the residue Cys165, located in the trans-membrane domain, as being important¹⁶. We
150 decided to investigate the involvement of Met residues in HprS function. Analysis of the HprS
151 sequence revealed the presence of 6 Met residues among which 4 (positions 72, 73, 100 and 153) are
152 located in the periplasmic loop, the first segment of HprS that comes into contact with HOCl (Fig. 5A).
153 To assess the role of each periplasmic Met residue, site directed mutagenesis with single Met-to-Ala
154 and single Met-to-Gln substitutions was carried out.

155 The variant containing Met-to-Ala should inform us of the physicochemical constraints at this
156 position, and the variant containing Gln, a mimetic of Met-O¹⁸, should show the effect of oxidizing
157 each Met on HprS activity. To test the effect of these substitutions, an *hprS*-less host carrying the
158 *msrP-lacZ* fusion (strain *CH186*) was complemented with plasmids expressing mutated variants of
159 *hprS*, as well as the plasmid carrying the WT gene. All the HprS variant proteins exhibit a similar level
160 of production (from 1 to 2.6 fold) as revealed by western-blot analysis (Fig. 5B). Next, the strains
161 were grown and treated with HOCl (4 mM) for 1 hour. The *msrP-lacZ* activity and the production of
162 MsrP were monitored. As already reported, a *hprS*-null mutant has a higher basal expression of *msrP*-
163 *lacZ* compared to the strain expressing the WT gene, and it cannot respond to HOCl¹³ (Fig. 5C, Fig.
164 5D). These phenotypes can be complemented with a plasmid *hprS*^{WT} allele. First, we found low levels

165 of MsrP protein and a decrease in β -galactosidase activity of the *msrP-lacZ* fusion in the absence of
166 HOCl; then, we observed that the presence of HOCl led to a high steady-state level of MsrP and an
167 increase in β -galactosidase activity from 20 to 230 Miller Units (Fig. 5C). We then assayed *msrP*
168 expression in the strain *CH186* complemented with different *hprS* mutants with or without HOCl. The
169 mutation of the Met 100 residue into either Ala or Gln had no effect on HprS activity. These mutants
170 show similar levels of β -galactosidase activity and MsrP production compared to HprS^{WT} in the
171 absence and presence of HOCl (Fig. 5C, Fig. 5D). The mutation of the Met73 residue into Ala had no
172 effect on HprS activity either (Fig. 5C). However, when the Met73 residue was mutated into a Met-O
173 mimicking residue (Gln) we observed a higher basal expression of *msrP* compared to HprS^{WT} in the
174 absence of HOCl (around 20 and 280 Miller Units with HprS^{WT} and HprS^{M73Q} respectively). When HOCl
175 was added, we observed an increase in *msrP* expression and β -galactosidase activity compared to
176 those observed with the WT allele (350 Miller Units for HprS^{WT} and 700 for HprS^{M73Q}) (Fig. 5D).
177 Mutating residues Met72 or Met153 into either Ala or Gln resulted in an unexpected observation:
178 these mutations shift the sensor to a “locked-on” state. Strains expressing these variants show a high
179 level of MsrP and increased expression of *msrP-lacZ* even in the absence of HOCl (around 1,100 and
180 8,500 Miller Units with HprS^{M72A} and HprS^{M153A} and 3500 and 4900 Miller Units with HprS^{M72Q} and
181 HprS^{M153Q} respectively). The β -galactosidase activity is not affected by the addition of HOCl and is
182 maintained at high levels (around 1,600 and 5,800 Miller Units with HprS^{M72A} and HprS^{M153A} and 2800
183 and 3600 with HprS^{M72Q} and HprS^{M153Q} respectively) (Fig. 5C, Fig 5D). Taken together, these
184 observations suggest that Met72 and Met153 are important for HprS activity.

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

191 production were observed compared to HprS^{WT} in the absence and presence of HOCl, showing that
192 they are not essential for HprS activity (Fig. 6B). When we combined mutation M73A to M72A, we
193 observed a decrease in the basal expression of *msrP* compared to that observed with HprS^{M72A} (1175
194 and 459 Miller Units with HprS^{M72A} and HprS^{M72-73A} respectively). The addition of HOCl increased the
195 expression of *msrP* in the presence of HprS^{M72-73A} whereas no increase in *msrP* expression was
196 observed with HprS^{M72A} after the addition of HOCl (Fig. 6B). HprS^{M72-100A} shows the same phenotype
197 as the variant HprS^{M72A}: the same high basal level of *msrP* expression in the absence of HOCl (around
198 1180 and 1300 Miller Units with HprS^{M72A} and HprS^{M72-100A} respectively). The addition of HOCl did not
199 affect the expression of *msrP* and it was maintained approximately at the same level in both mutants
200 (around 1600 and 1900 Miller Units with HprS^{M72A} and HprS^{M72-100A} respectively). These results show
201 that M72A is a dominant mutation compared to M100A (Fig. 6B). Mutations M72A or M73A seem to
202 be recessive compared to mutation M153A: the variants HprS^{M72-153A} and HprS^{M73-153A} show very high
203 levels of *msrP-lacZ* expression that are comparable to those observed with the HprS^{M153A} variant
204 (around 8550, 5550 and 6450 Miller Units with HprS^{M153A}, HprS^{M72-153A} and HprS^{M73-153A} respectively).
205 The addition of HOCl does not affect the expression of *msrP* (around 5800, 5100 and 4760 Miller
206 Units with HprS^{M153A}, HprS^{M72-153A} and HprS^{M73-153A} respectively) (Fig. 6B). To assess the importance of
207 residue Met153 in HprS activity, we constructed an HprS mutant with Met72, Met73 and Met100
208 substituted by Ala. In this mutant, only Met153 is available to react with HOCl within the periplasmic
209 loop. The mutant was not able to complement an *hprS* deletion in the strain *CH186*: the basal level of
210 *msrP-lacZ* is around 608 Miller Units in the absence of HOCl. When HOCl is added, *msrP-lacZ*
211 expression increases to around 1000 Miller Units. Nevertheless, when we compared the fold-
212 induction between HprS^{M72-73-100A} and HprS^{WT} in response to HOCl, we found a 30-fold increase of
213 *msrP-lacZ* in an HprS^{WT} background and only a two-fold increase in an HprS^{M72-73-100A} background. This
214 observation suggests that the presence of Met153 alone is not sufficient for a fully functional HprS
215 (Fig. 6C). Based on these observations, we hypothesized that all Met residues might be important for
216 HprS activity and that substituting all Met residues by Ala might render HprS blind to the signal.

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

221

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

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

236

237 ***Residues Met72 and Met153 are highly conserved in HprS homologues.***

238 In order to support a functional role for the periplasmic Met residues in HprS, we assessed their
239 conservation in HprS sequences. To do so, we first established the list of HprS homologues from the
240 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 matter¹⁹. 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 H₂O₂¹⁶. 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*

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

270

271 **DISCUSSION**

272 In bacteria, two-component systems (TCS) are widespread signaling pathways allowing cells to sense,
273 respond and adapt to fluctuating environments and challenging conditions. Based on genome
274 sequencing, *E. coli* MG1655 has been predicted to carry a total of 24 "classical" TCS composed of a
275 histidine kinase and a response regulator²⁰; many of them have yet to be characterized. Other well-
276 studied TCS have been shown to regulate bacterial metabolism, nutrient acquisition and stress
277 response to numerous stimuli such as pH, metals and envelope integrity^{21,22}. Therefore, the
278 characterization of *E. coli* TCS is important for developing complete knowledge on bacterial
279 regulatory networks. The aim of this study was to characterize further the TCS HprSR in *E. coli* and we
280 have found that oxidative stress mediated specifically by RCS (HOCl and N-ChT) can activate this
281 system. We have also provided evidence for the molecular mechanism of signal sensing by HprS.

282 Until now, HprSR was poorly characterized and initial evidence for the HprSR-dependent regulon was
283 provided by SELEX methodology¹⁵. Four HprR binding sites were identified in the whole genome: one
284 between the two operons *hprRS* and *hiuH-msrPQ*; one within the *cyoABCDE* operon encoding
285 cytochrome oxidase; one near the *qorA* gene encoding a quinone oxido-reductase; and one between
286 the *cusRS* and the *cusCFBA* operons, encoding the copper responding TCS and the copper efflux
287 pump, respectively¹⁵. However, this approach cannot exclude the possibility that some targeted
288 genes could be regulated only by HprR and not by HprS via a cross-talk between TCS. Indeed, such a
289 cross-talk has to be taken into account as HprR was shown to be phosphorylated by CusS, the

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

292 As a first step towards characterizing HprSR, we tried to identify the signals sensed by the HK HprS.
293 Previous studies reported contradictory results: it has been shown that HprSR, in response to H₂O₂
294 stress, increases the mRNA level of its target gene *hiuH*, suggesting that HprS senses H₂O₂ ¹⁵. Another
295 study showed that HprSR (formerly YedVW) induced the expression of the gene encoding the
296 periplasmic methionine sulfoxide reductase system (MsrPQ) in response to HOCl and not to H₂O₂ ¹³.
297 This regulation was seen at both transcriptional and translational levels, by using an *msrP* reporter
298 fusion and by assessing the MsrP protein levels ¹³. These contradictory results prompted us to re-
299 investigate the signals sensed by HprS. In this study, we assessed both mRNA and protein levels of
300 HprSR target genes. The strength of this study is the simultaneous comparison of the effect of HOCl
301 and H₂O₂ on HprSR and the evaluation of the expression of target genes both at the transcriptional
302 and translational level. Our results show that HOCl is a more physiological signal for HprSR compared
303 to H₂O₂, and that the previously described activation of HprSR by H₂O₂ might be irrelevant and non-
304 physiological as only a slight increase in mRNA levels is detected. We also tested other ROS/RCS that
305 might be sensed by HprS by treating cells with different compounds known to generate oxidative
306 stress. Among all the compounds tested, we only detected an activation of HprSR in response to
307 HOCl and *N*-ChT. However, as HOCl reacts rapidly with different compounds of the culture media,
308 especially with LB, producing chloramines and other less toxic chlorine species ²³, we suggest that
309 HprS is a specific RCS sensor in *E. coli*.

310 Until now, two different names have been attributed to this TCS: HprSR for hydrogen peroxide
311 sensor ¹⁶ and regulator and MsrVW ²⁴ for its association with MsrPQ. We think that neither name
312 might be suitable for this TCS in light of the present study: firstly, we have shown that H₂O₂ is not an
313 adequate signal and secondly, the Msr denomination is misleading and should be reserved for
314 proteins that have methionine sulfoxide reductase enzymatic activity or that are essential for Msr

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

317 As a second step towards fully characterising HprSR, we investigated the molecular mechanism
318 underlying HOCl detection by HprS. It has been shown that HOCl activates RCS-specific transcription
319 factors through post-translational modification of Cys or Met residues. Indeed, HOCl activates RclR by
320 a thiol-disulfide switch mechanism and NemR by the formation of a Cys-Lys sulfenamide bond, while
321 HOCl oxidizes three Met residues in HypT, leading to its activation^{9,8,10}. Interestingly, HprS contains
322 four Cys and six Met residues: two Cys residues (Cys165 and Cys172) are located in the second
323 transmembrane domain and the other two (Cys333 and Cys338) are in the cytoplasmic domain. In
324 the case of the Met residues, four of them are located in the periplasmic loop of HprS (Met72,
325 Met73, Met100 and Met153), and the other two are in the cytoplasmic domain (Met225 and
326 Met352). The involvement of Cys residues in the HprS sensing mechanism has already been
327 investigated and the transmembrane Cys165 residue has been identified as being important for H₂O₂
328 detection¹⁶. Met residues are also highly sensitive to HOCl and because HK usually use their
329 periplasmic loop to sense extracellular signals²⁵: we therefore focused our study on the 4 Met
330 residues located in the periplasmic domain. We conducted a systematic Met-to-Ala and Met-to-Gln
331 substitution of each Met residue. We also generated HprS mutants containing different combinations
332 of two, three or four Met-to-Ala substitutions. Replacing Met with Ala prevents the sulfur atom
333 reacting with HOCl and substituting Met with Gln mimics Met-O residues²⁶. We observed that
334 substituting Met72 or Met153 with Ala or Gln had drastic consequences on HprS, leading to
335 constitutive expression of *msrP*. Substituting Met73 with a Gln residue led to a higher basal activity
336 compared to HprS^{WT} but this variant was not blind to the signal. Mutations of residue Met100
337 (M100A and M100Q) were neutral and had no effect on HprS. The analysis of HprS double mutants
338 show that M72A and M153A are dominant compared to M73A and M100A. We also showed that
339 residues M100 and M73 are not essential for HprS activation by HOCl. When analyzing the mutant
340 HprS^{M72-73-100A}, it was clear that residue M153A alone cannot activate HprS to its full potential in

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

348 In addition to the mutational approach discussed earlier, we assayed the effect of MsrP on HprS
349 activation. If the activation of HprS is linked to the oxidation of Met residues, then MsrP could
350 participate in switching HprS off by re-reducing the Met-O residues. Our results show that the level
351 of *msrP-lacZ* expression is dependent on the level of MsrP production. The HOCl-dependent
352 induction of *msrP-lacZ* is regulated by HprS and so this observation is evidence for a central role of
353 the Met redox state in HprS activity. Finally, we reinvestigated the role of the transmembrane Cys165
354 residue using the HprS^{M72Q} constitutive variant. First, we confirmed that an HprS^{C165A} variant is
355 affected in its activity in agreement with a previous study¹⁶. However, the localization and the lack of
356 conservation of Cys165 led us to postulate that this position (165) rather than the Cys residue itself
357 (as it is not conserved) could be important for signal transduction to the catalytic domain and not for
358 signal sensing. The fact that C165A has been found to be dominant over the M72Q mutation, even in
359 the absence of the signal, supports this view. Moreover, the absence of HprS activation by diamide, a
360 thiol-specific oxidant, led us to speculate that the weak HprS activation by H₂O₂ could be caused by
361 Met rather Cys165 oxidation.

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

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

367 If Met residues are involved in HprS activation, this could explain why H₂O₂ cannot activate HprS
368 properly: in terms of kinetics, the oxidizing reactivity of Met residues with H₂O₂ is much lower than
369 with HOCl ($2 \times 10^2 \text{ M}^{-1}\cdot\text{s}^{-1}$ for H₂O₂ versus $3.4 \times 10^7 \text{ M}^{-1}\cdot\text{s}^{-1}$ for HOCl)¹⁷ making the reaction much
370 slower and requiring higher concentrations of H₂O₂ that might not be physiological. However, we
371 have no direct proof of *in vivo* Met oxidation in HprS, therefore additional studies will be required to
372 consolidate our proposed model. Also, our data do not exclude the possibility that HprS might be
373 activated indirectly by RCS through a Met-oxidation process of a third periplasmic component which
374 could interact with HprS. Our model needs to be consolidated in the future by using a biochemical
375 approach to assess the different post-translational modifications occurring in HprS during RCS stress
376 *in vivo*.

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

381

382

383

384 **MATERIAL AND METHODS**

385 ***Strains and microbial techniques***

386 The strains used in this study are listed in table 1. To construct strain SH198 (*hprR* knock-out strain),
387 the *hprR* mutant allele from the Keio collection²⁷ was transferred to the *E. coli* MG1655 strain (wild-
388 type) using a standard P1 transduction procedure²⁸. Kanamycin resistant strains were selected and
389 verified by PCR. To excise the kanamycin cassette, we used the pCP20 plasmid^{27,29}. The *hiuH*-
390 *lacZ* fusion in strain CH184 was constructed using the protocol described by Mandin and
391 Gottesman³⁰. First, the promoter region of *hiuH* located between nucleotides -274 and the ATG
392 initiation triplet was amplified using the primers *lacI*-*hiuH*-For and *lacZ*-*hiuH*-Rev. Using mini lambda
393 recombination, the PCR product was recombined in the chromosome of an *E. coli* strain carrying a
394 P_{BAD}-*cat*-*SacB* cassette inserted in front of the gene *lacZ* (strain PM1205). Recombinants which had
395 lost the *cat*-*sacB* cassette were selected and verified by PCR: these clones carried the translational
396 fusion *hiuH*-*lacZ*. Strain LL1700 containing a CmR cassette was constructed to transfer the *hiuH*-*lacZ*
397 fusion into an *E. coli* MG1655 (Wild-type) background. To do so, we inserted a resistance cassette
398 close to the fusion enabling its transduction: a *Cat* cassette was amplified using primers 5del and
399 3del. The resulting product carried on its 5' end, a 40 bp homologous sequence to *lacI* and on its 3'
400 end, a 41 bp sequence homologous to the *hiuH* promoter (located between the nucleotide -133 and -
401 92, before the *HprR* boxes). The amplicon was then electroporated into the strain CH184 carrying the
402 pKD46 plasmid. *Cat* resistant clones were selected and tested by PCR. The *lacI*-*cat*-*hiuH*-*lacZ*
403 construct was then transferred into MG1655 cells using a standard P1 transduction procedure.
404 Chloramphenicol resistant clones were selected and verified by PCR. Strain SH100 was constructed as
405 follows: (i) a TetR-Kan sequence composed of the promoter TetR and the kanamycin cassette was
406 amplified using the plasmid pBbS2K as the template and the primers YedW_TetR_F and
407 Kan_Boxyedx_R. The resulting PCR product shared a 48 bp sequence homologous to *hprR* and a 41
408 bp sequence homologous to the intergenic region between *hprR* and *hiuH* located upstream of the

409 HprR boxes. (ii) After purification, the PCR product was transformed by electroporation into *E. coli*
410 BW25113 cells (WT) carrying the plasmid pKD46²⁹. Kan resistant clones were selected and verified by
411 PCR. (iii) This construction was then transferred into LL1700 cells expressing *hiuH-lacZ* fusion using
412 standard P1 transduction procedures. Selected clones were verified by PCR.

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

415

416 ***Plasmid constructions***

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

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

441

442 ***Operonic organization analysis***

443 *E. coli* cells (MG1655) were grown to OD₆₀₀ ≈ 2 in the presence of 2 mM HOCl added at the beginning
444 of the culture. Total bacterial RNA was extracted with a Maxwell® 16 LEV miRNA Tissue Kit (Promega)
445 according to the manufacturer's instructions. Contaminating chromosomal DNA was digested using
446 Turbo DNase (Invitrogen). RNA quality was assessed using a Tape station system (Agilent) then
447 quantified at 260 nm using a NanoDrop 1000 (Thermo Fisher scientific). cDNA was synthesized with 1
448 µg of total RNA, 0.5 µg random primers (Promega) and GoScript™ reverse transcriptase
449 (Promega). To analyze the *hiuH-msrP-msrQ* operon structure, two sets of primers, 5QTyedX-
450 3QTmsrP and 5QTmsrP-3QTmsrQ, were used to amplify the possible transcripts. As a negative
451 control, RNA samples were amplified using the same sets of primers in order to verify the absence of
452 chromosomal contamination. As a positive control, chromosomal DNA was amplified using EconoTaq
453 DNA polymerase (Promega). Results were visualized on a 1 % agarose gel prepared with TBE (Tris-
454 Borate-EDTA) and stained with ethidium bromide.

455

456 ***qRT-PCR analysis***

457 Total RNA was extracted from wild type *E. coli* (MG1655) cells and *hprS* mutants (SH198) either
458 cultured to OD₆₀₀≈ 0.6 and subjected to 6 mM H₂O₂ for 30 min or cultured to OD₆₀₀≈ 1 and stressed
459 with 4 mM HOCl for 1 h. mRNA levels of *hprS*, *hprR*, *msrP* and *msrQ* were quantified by qRT-PCR
460 analysis using the incorporation of EvaGreen (Bio-rad) in a CFX96 Real Time system (Bio-Rad)
461 according to the manufacturer's instructions. For these reactions, primers 5QT-3QT yedX, 5QT-3QT
462 *msrP*, 5QT-3QT yedV, and 5QT-3QT yedW were used. For accurate evaluation of gene expression,
463 RNA were quantified and normalized to the 16S rRNA housekeeping gene. qRT-PCR technical
464 triplicates for each condition were carried out. All biological repeats were selected and reported.

465

466 ***HOCl induction assays***

467 *E. coli* cells expressing *msrP-lacZ* or *hiuH-lacZ* (CH183, CH184 or CH186 carrying pYedV or pYedV
468 derived plasmids) were grown aerobically in LB medium at 37 °C. At OD₆₀₀≈ 1, the culture was divided
469 into two 15 mL falcon tubes each containing 5 mL of this culture: 4 mM HOCl (NaOCl, 10-15% AC
470 Acros Organics) was added to one of these tubes. Cultures were then incubated at 37 °C. To assess
471 the activity of HprS mutants, β-galactosidase activity was measured after 1 h of incubation. To follow
472 the kinetics of *msrP-lacZ* or *hiuH-lacZ* expression in response to HOCl, β-galactosidase activity was
473 measured at different time points. The kinetics of *msrP-lacZ* expression in response to HOCl were
474 also evaluated in minimal medium (M9) supplied with 0.2 % glucose and 0.2 % CASA. For this, strain
475 CH183 expressing the *msrP-lacZ* fusion was cultured to OD₆₀₀≈ 0.2. The culture was then divided into
476 two 50 mL falcon tube each containing 5 mL of the culture. To one of the tubes, 135 μM of HOCl
477 were added and the β-galactosidase activity was measured at different time points. For each
478 protocol, the reaction mixture was prepared as follows: 200 μL of bacteria were added to 800 μL of
479 β-galactosidase buffer. β-galactosidase was measured as previously described by Miller *et al.*³¹.

480

481 ***H₂O₂ induction assays***

482 To test the induction of *msrP-lacZ* or *hiuH-lacZ* (CH183 and CH184) in response to H₂O₂, two different
483 protocols were tested: first, as described by Urano and his collaborators¹⁶, *E. coli* cells were cultured
484 aerobically in LB medium at 37 °C. At OD₆₀₀≈ 0.6, cultures were split into two 50 mL falcon tubes each
485 containing 5 mL of the culture; 6 mM of H₂O₂ was added to one of them and after 30 min of
486 incubation at 37°C, β-galactosidase activity was measured. For the second protocol, *E. coli* cells were
487 aerobically cultured in LB medium at 37°C. At OD₆₀₀≈ 0.2, the culture was divided into 5 mL
488 aliquots in 50mL falcon tubes. Different sub-lethal concentrations of H₂O₂ were added for each (500
489 μM, 1 mM, 2 mM and 3 mM of H₂O₂). 200 μL of culture were harvested at different time points and
490 β-galactosidase assays were conducted. As a positive control for this protocol, strain BE1001 was
491 used to follow the expression of *ahpC-lacZ* under the tested conditions.

492

493 ***Bacteria survival assay in vitro.***

494 To test the effect of H₂O₂ and HOCl on cell viability, overnight cultures of *E. coli* (MG1655) were
495 diluted in LB medium to OD₆₀₀≈ 0.4. Cells were then subjected to 6 mM H₂O₂ and 4 mM HOCl using
496 the protocols described earlier. Cultures were serially-diluted in PBS. 5 μL of each dilution were
497 spotted onto LB agar plates; after 18h at 37 °C, CFU were counted.

498

499 ***Chlorotaurine synthesis***

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

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

505

506 ***Chlorotaurine induction assays***

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

510

511 ***NO induction assays***

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

518

519 ***Superoxide radical induction assay***

520 To test the induction of *msrP-lacZ* by the superoxide radical, CH183 cells were grown in LB medium at
521 37 °C. At $\text{OD}_{600} \approx 0.2$, the culture was split into 5 mL aliquots in 50 mL falcon tubes. Each culture was
522 treated with a different concentration of paraquat (300 μM , 500 μM and 1 mM) followed by
523 incubation at 37 °C. 200 μL of culture were harvested at different time points and β -galactosidase
524 activities were measured. The BE1000 strain carrying *soxS-lacZ* was used as a positive control.

525

526 ***Diamide induction assay***

527 To test the induction of *msrP-lacZ* by diamide, CH183 cells were grown in LB medium at 37 °C. At
528 $OD_{600} \approx 0.2$, the culture was split into 5 mL aliquots in 50 mL falcon tubes. Each culture was treated
529 with a different concentration of diamide (0.2, 0.3 and 0.4 mM diamide) followed by incubation at 37
530 °C. 200 μ L of culture were harvested at different time points and β -galactosidase activities were
531 measured. The BE1001 strain carrying *ahpC-lacZ* was used as a positive control.

532

533 ***Immunoblot analysis of MsrP expression***

534 To assess MsrP induction by HOCl, overnight cultures of the CH186 strain expressing different
535 plasmid alleles of *hprS* were diluted to $OD_{600} \approx 0.04$ in LB medium. Cells were grown aerobically to
536 $OD_{600} \approx 1$. The culture was divided into two 15 mL falcon tubes each containing 5 mL of the culture; 4
537 mM HOCl was added to one tube. Cultures were then incubated at 37 °C for 1 h. Samples were
538 diluted in sample buffer (Laemmli: 2 % SDS, 10 % glycerol, 60 mM Tris-HCl, pH 7.4, 0.01 %
539 bromophenol blue). The amount of protein loaded onto the gel was standardized for each culture
540 based on their A_{600} values. Samples were then heated for 10 min at 95 °C and loaded onto an SDS-
541 PAGE gel for immunoblot analysis. Immunoblot analysis was performed according to standard
542 procedures: the primary antibody is a guinea pig anti-MsrP antibody (provided by Jean-François
543 Collet Lab, De Duve Institute, Belgium). The secondary antibody is anti-guinea pig IgG conjugated to
544 horseradish peroxidase (HRP) (Promega). An ImageQuant LAS4000 camera (GE Healthcare Life
545 Sciences) was used for image Chemiluminescence.

546

547 ***Immunoblot analysis of HprS production***

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

556

557 ***Analysis of the effect of MsrP on hiuH expression***

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

562

563 **Table 1- Strains used in this study.**

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

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

566

567

568 **Table 2- Plasmids used in this study.**

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

571

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 <i>et al</i> , 2015 ¹³
pAG195	pTAC-MAT-Tag-2-NdeI MsrP-MsrQ	Gennaris <i>et al</i> , 2015 ¹³
pBbS2K	pBbS2K-RFP $\Delta RFP1$, selfligated	Germain <i>et al</i> , 2019 ³⁴
pHprS	pBBR-MCS1- <i>XhoI</i> -HprS ^{WT}	This study
pHprSM72A	pBBR-MCS1- <i>XhoI</i> -HprS ^{M72A}	This study
pHprSM73A	pBBR-MCS1- <i>XhoI</i> -HprS ^{M73A}	This study
pHprSM100A	pBBR-MCS1- <i>XhoI</i> -HprS ^{M100A}	This study
pHprSM153A	pBBR-MCS1- <i>XhoI</i> -HprS ^{M153A}	This study
pHprSM72Q	pBBR-MCS1- <i>XhoI</i> -HprS ^{M72Q}	This study
pHprSM73Q	pBBR-MCS1- <i>XhoI</i> -HprS ^{M73Q}	This study
pHprSM100Q	pBBR-MCS1- <i>XhoI</i> -HprS ^{M100Q}	This study
pHprSM153Q	pBBR-MCS1- <i>XhoI</i> -HprS ^{M153Q}	This study
pHprSC165A	pBBR-MCS1- <i>XhoI</i> -HprS ^{C165A}	This study
pHprSM72Q-C165A	pBBR-MCS1- <i>XhoI</i> -HprS ^{M72Q-C165A}	This study
pHprSM72-73A	pBBR-MCS1- <i>XhoI</i> -HprS ^{M72A-M73A}	This study
pHprSM72-100A	pBBR-MCS1- <i>XhoI</i> -HprS ^{M72A-M100A}	This study
pHprSM72-153A	pBBR-MCS1- <i>XhoI</i> -HprS ^{M72A-M153A}	This study
pHprSM73-100A	pBBR-MCS1- <i>XhoI</i> -HprS ^{M73A-M100A}	This study
pHprSM73-153A	pBBR-MCS1- <i>XhoI</i> -HprS ^{M73A-M153A}	This study
pHprSM72-73-100A	pBBR-MCS1- <i>XhoI</i> -HprS ^{M72A-M73A-M100A}	This study
pHprSM72-73-100-153A	pBBR-MCS1- <i>XhoI</i> -HprS ^{M72A-M73A-M100A-M153A}	This study

572

573

574

575 **Table 3- Primers used in this study.**

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

578

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	CCGAAAAGCTTTAATTTATGGCGTACTGAAGCCCTATG
YedV_M72A_F	CCTGTGTACTTTAACCGGGCGATGGATGTTAGTCAGGATATC
YedV_M72A_R	GATATCCTGACTAACATCCATCGCCGGTTAAAGTACACAGG
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
YedV_M72Q_R	ATC TTG ATT ATT CAT GGT GAT 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	TACCCATTCTGGGTCCTTTGATTATCTTCAATAAGTAGAATCTTCATATGT ATATCTCCTTCTTAAAAGATCTTTTGAATTCCTTTCTCTATCACTGATAGG
Kan_Boxyedx_R	GGCTGTTTCTATAACATATGATTTATGGCATATT ATTTTCATCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATGC
lacI-HiuH-for	CGAAGCGGCATGCATTTACGTTGACACCA TCGAATGGCGCATCAATGCATAATCATCCTTC

lacZ-HiuH-rev TAACGCCAGGGTTTTCCAGTCACGACGTT
GTAAAACGACCATGTTTATATCCTTGTCATG

579

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588

589

590 **FIGURE LEGENDS**

591

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

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

602

603

604 **Figure 2- Influence of H₂O₂ and HOCl on the expression of the *hiuH-msrPQ* and *hprRS* operons.**

605 **A)** Fold-expression level of *hprS*, *hprR*, *hiuH* and *msrP* transcripts under H₂O₂ and HOCl stresses in
606 MG1655 (WT) and SH198 ($\Delta hprR$) cells. Both strains were cultured in LB and subjected to either 6
607 mM H₂O₂ for 30 min, as described by Urano and his collaborators¹⁵, or to 4 mM HOCl for 1 hour. For
608 each condition, quantitative real-time PCR (RT-qPCR) was performed to amplify *hprS*, *hprR*, *hiuH* and
609 *msrP* cDNA. Values are expressed as means \pm SEM of three independent experiments. ND: not
610 determined. **B)** Evaluation of *msrP* and *hiuH* expression under HOCl or H₂O₂ stress. The expression of
611 *msrP-lacZ* and *hiuH-lacZ* fusions, in strains CH183 and CH184 respectively, were used as a proxy for
612 *hiuH* and *msrP* expression. Strains CH183 and CH184 were cultured in LB and subjected to H₂O₂ or
613 HOCl stress, followed by β -galactosidase assays. Results are expressed as means \pm SD, n=3. **C)** Effect
614 of 6 mM H₂O₂ and 4 mM HOCl on the viability of WT *E. coli* cells. *E. coli* MG1655 (wild-type) strain
615 was grown in LB medium, each culture was subjected to either 6 mM H₂O₂ for 30 min¹⁵ or to 4 mM
616 HOCl for 1h. Cultures were then serially diluted in PBS and spotted onto LB agar plates. After 18 h of
617 incubation, CFU (colony forming unit) were counted and compared to untreated cells. Values shown
618 represent the ratio between the CFU of treated cells and that of untreated cells at a dilution of 1x10⁷.
619 ⁷. The results are expressed as means \pm SD of three independent experiments.

620

621 **Figure 3- Effect of different types of oxidative stress on HprSR activation.** The induction of the *msrP-*
622 *lacZ* fusion in strain CH183 was used as a proxy for HprSR activation. **A-B)** Effect of H₂O₂ on *msrP-*
623 *lacZ* and *ahpC-lacZ* expression. Strain CH183 was subjected to sub-lethal concentrations of H₂O₂,
624 ranging from 0.5 to 3 mM, and *msrP-lacZ* expression was followed at different time points. Strain
625 BE1001 carrying the *ahpC-lacZ* fusion, known to respond to H₂O₂, was used as a positive control.
626 Results are expressed as means \pm SD (n=5). **C-D)** Effect of diamide on *msrP-lacZ* and *ahpC-lacZ*
627 expression. Strain CH183 was subjected to sub-lethal concentrations of diamide (0.2, 0.3 and 0.4
628 mM), and *msrP-lacZ* expression was followed at different time points. Strain BE1001 carrying the
629 *ahpC-lacZ* fusion, known to respond to diamide, was used as a positive control. Results are expressed

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

639

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

648

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

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

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

668

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

670 **A)** Immunoblot analysis showing the expression of different plasmid *hprS* alleles (WT and double Ala-
671 to Met mutations) in CH186 cells. CH186 cells carrying the empty plasmid pBBRMCS1 or expressing a
672 variant of *hprS* (WT or mutants) were cultured overnight at 37 °C in LB medium in the presence of 1
673 mM IPTG. Immunoblot analysis was carried out using rabbit anti-HprS as a primary antibody. The
674 abundance of each HprS variant was quantified and the relative HprS amount was calculated and
675 normalized to HprS^{WT}. **B)** Effect of combining two Met-to-Ala mutations on HprS activity. The
676 expression of *msrP* is used as a proxy for HprSR activity. MsrP production is assessed by immunoblot
677 analysis using an anti-MsrP antibody and by measuring *msrP-lacZ* expression. Both analyses were
678 carried out on CH186 cells carrying either the empty plasmid pBBRMCS1 (negative control),
679 expressing wild-type *hprS* (positive control) or *hprS* mutants: M72-73A, M72-100A, M72-153A, M73-
680 100A, M73-153A. *msrP* expression was analyzed in each strain after treatment with HOCl. Values of
681 the β-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 β -galactosidase assays are expressed as mean \pm 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 Δ msrP cat-hiuH-lacZ*) were cultured
691 in M9 minimal medium. 135 μ M of HOCl was added to each strain at OD₆₀₀=0.2, and *hiuH-lacZ*
692 expression was followed for 4 hours. Values of the β -galactosidase assays are expressed as means \pm
693 SD (n=4). An asterisk indicates a statistically significant difference between the β -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 μ M HOCl was added to each strain at OD₆₀₀=0.2 and *hiuH-lacZ*
697 expression was followed for 4 hours. Values of the β -galactosidase assays are expressed as means \pm
698 SD (n=3). An asterisk indicates a statistically significant difference between β -galactosidase activity of
699 different strains. *P \leq 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

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

714

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

728

729

730 **REFERENCES:**

- 731 1. Winterbourn, C. C., Hampton, M. B., Livesey, J. H. & Kettle, A. J. Modeling the Reactions of
732 Superoxide and Myeloperoxidase in the Neutrophil Phagosome. *J. Biol. Chem.* **281**, 39860–39869
733 (2006).
- 734 2. Hawkins, C. L. Hypochlorous acid-mediated modification of proteins and its consequences.
735 *Essays Biochem.* **64**, 75–86 (2019).
- 736 3. da Cruz Nizer, W. S., Inkovskiy, V. & Overhage, J. Surviving Reactive Chlorine Stress: Responses of
737 Gram-Negative Bacteria to Hypochlorous Acid. *Microorganisms* **8**, (2020).
- 738 4. Winterbourn, C. C. Comparative reactivities of various biological compounds with
739 myeloperoxidase-hydrogen peroxide-chloride, and similarity of the oxidant to hypochlorite.
740 *Biochim. Biophys. Acta* **840**, 204–210 (1985).
- 741 5. Deborde, M. & von Gunten, U. Reactions of chlorine with inorganic and organic compounds
742 during water treatment-Kinetics and mechanisms: a critical review. *Water Res.* **42**, 13–51 (2008).
- 743 6. Winterbourn, C. C. Reconciling the chemistry and biology of reactive oxygen species. *Nat. Chem.*
744 *Biol.* **4**, 278–286 (2008).
- 745 7. Gray, M. J., Wholey, W.-Y. & Jakob, U. Bacterial responses to reactive chlorine species. *Annu.*
746 *Rev. Microbiol.* **67**, 141–160 (2013).
- 747 8. Drazic, A. *et al.* Methionine oxidation activates a transcription factor in response to oxidative
748 stress. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 9493–9498 (2013).
- 749 9. Parker, B. W., Schwessinger, E. A., Jakob, U. & Gray, M. J. The RclR protein is a reactive chlorine-
750 specific transcription factor in Escherichia coli. *J. Biol. Chem.* **288**, 32574–32584 (2013).
- 751 10. Gray, M. J., Wholey, W.-Y., Parker, B. W., Kim, M. & Jakob, U. NemR is a bleach-sensing
752 transcription factor. *J. Biol. Chem.* **288**, 13789–13798 (2013).
- 753 11. Ezraty, B., Gennaris, A., Barras, F. & Collet, J.-F. Oxidative stress, protein damage and repair in
754 bacteria. *Nat. Rev. Microbiol.* **15**, 385–396 (2017).

- 755 12. Whitworth, D. E. & Cock, P. J. A. Evolution of prokaryotic two-component systems: insights from
756 comparative genomics. *Amino Acids* **37**, 459–466 (2009).
- 757 13. Gennaris, A. *et al.* Repairing oxidized proteins in the bacterial envelope using respiratory chain
758 electrons. *Nature* **528**, 409–412 (2015).
- 759 14. Lee, Y. *et al.* Transthyretin-related proteins function to facilitate the hydrolysis of 5-
760 hydroxyisourate, the end product of the uricase reaction. *FEBS Lett.* **579**, 4769–4774 (2005).
- 761 15. Urano, H., Umezawa, Y., Yamamoto, K., Ishihama, A. & Ogasawara, H. Cooperative regulation of
762 the common target genes between H₂O₂-sensing YedVW and Cu²⁺-sensing CusSR in *Escherichia*
763 *coli*. *Microbiol. Read. Engl.* **161**, 729–738 (2015).
- 764 16. Urano, H. *et al.* Cross-regulation between two common ancestral response regulators, HprR and
765 CusR, in *Escherichia coli*. *Microbiol. Read. Engl.* **163**, 243–252 (2017).
- 766 17. Varatnitskaya, M., Degrossoli, A. & Leichert, L. I. Redox regulation in host-pathogen interactions:
767 thiol switches and beyond. *Biol. Chem.* **402**, 299–316 (2021).
- 768 18. Bigelow, D. J. & Squier, T. C. Thioredoxin-dependent redox regulation of cellular signaling and
769 stress response through reversible oxidation of methionines. *Mol. Biosyst.* **7**, 2101–2109 (2011).
- 770 19. Keene, William. C. *et al.* Composite global emissions of reactive chlorine from anthropogenic and
771 natural sources: Reactive Chlorine Emissions Inventory. *J. Geophys. Res. Atmospheres* **104**, 8429–
772 8440 (1999).
- 773 20. Mizuno, T. Compilation of all genes encoding two-component phosphotransfer signal
774 transducers in the genome of *Escherichia coli*. *DNA Res. Int. J. Rapid Publ. Rep. Genes Genomes* **4**,
775 161–168 (1997).
- 776 21. Yamamoto, K. *et al.* Functional Characterization in Vitro of All Two-component Signal
777 Transduction Systems from *Escherichia coli*. *J. Biol. Chem.* **280**, 1448–1456 (2005).
- 778 22. Laub, M. T. The Role of Two-Component Signal Transduction Systems in Bacterial Stress
779 Responses. in *Bacterial Stress Responses* 45–58 (John Wiley & Sons, Ltd, 2010).
780 doi:10.1128/9781555816841.ch4.

- 781 23. Ashby, L. V., Springer, R., Hampton, M. B., Kettle, A. J. & Winterbourn, C. C. Evaluating the
782 bactericidal action of hypochlorous acid in culture media. *Free Radic. Biol. Med.* **159**, 119–124
783 (2020).
- 784 24. Danchin, A. Coping with inevitable accidents in metabolism. *Microb. Biotechnol.* **10**, 57–72
785 (2016).
- 786 25. Buschiazzo, A. & Trajtenberg, F. Two-Component Sensing and Regulation: How Do Histidine
787 Kinases Talk with Response Regulators at the Molecular Level? *Annu. Rev. Microbiol.* **73**, 507–
788 528 (2019).
- 789 26. Vogt, W. Oxidation of methionyl residues in proteins: tools, targets, and reversal. *Free Radic.*
790 *Biol. Med.* **18**, 93–105 (1995).
- 791 27. Baba, T. *et al.* Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the
792 Keio collection. *Mol. Syst. Biol.* **2**, 2006.0008 (2006).
- 793 28. Bremer, E., Silhavy, T. J., Weisemann, J. M. & Weinstock, G. M. Lambda placMu: a transposable
794 derivative of bacteriophage lambda for creating lacZ protein fusions in a single step. *J. Bacteriol.*
795 **158**, 1084–1093 (1984).
- 796 29. Datsenko, K. A. & Wanner, B. L. One-step inactivation of chromosomal genes in Escherichia coli
797 K-12 using PCR products. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 6640–6645 (2000).
- 798 30. Mandin, P. & Gottesman, S. A genetic approach for finding small RNAs regulators of genes of
799 interest identifies RybC as regulating the DpiA/DpiB two-component system. *Mol. Microbiol.* **72**,
800 551–565 (2009).
- 801 31. Miller, J. H. *A short course in bacterial genetics: a laboratory manual and handbook for*
802 *Escherichia coli and related bacteria.* (Cold Spring Harbor Laboratory Press, 1992).
- 803 32. Ezraty, B., Henry, C., Hérissé, M., Denamur, E. & Barras, F. Commercial Lysogeny Broth culture
804 media and oxidative stress: a cautious tale. *Free Radic. Biol. Med.* **74**, 245–251 (2014).

- 805 33. Vinella, D., Loiseau, L., Choudens, S. O. de, Fontecave, M. & Barras, F. In vivo [Fe-S] cluster
806 acquisition by IscR and NsrR, two stress regulators in Escherichia coli. *Mol. Microbiol.* **87**, 493–
807 508 (2013).
- 808 34. Germain, E. *et al.* YtfK activates the stringent response by triggering the alarmone synthetase
809 SpoT in Escherichia coli. *Nat. Commun.* **10**, 5763 (2019).
- 810
- 811

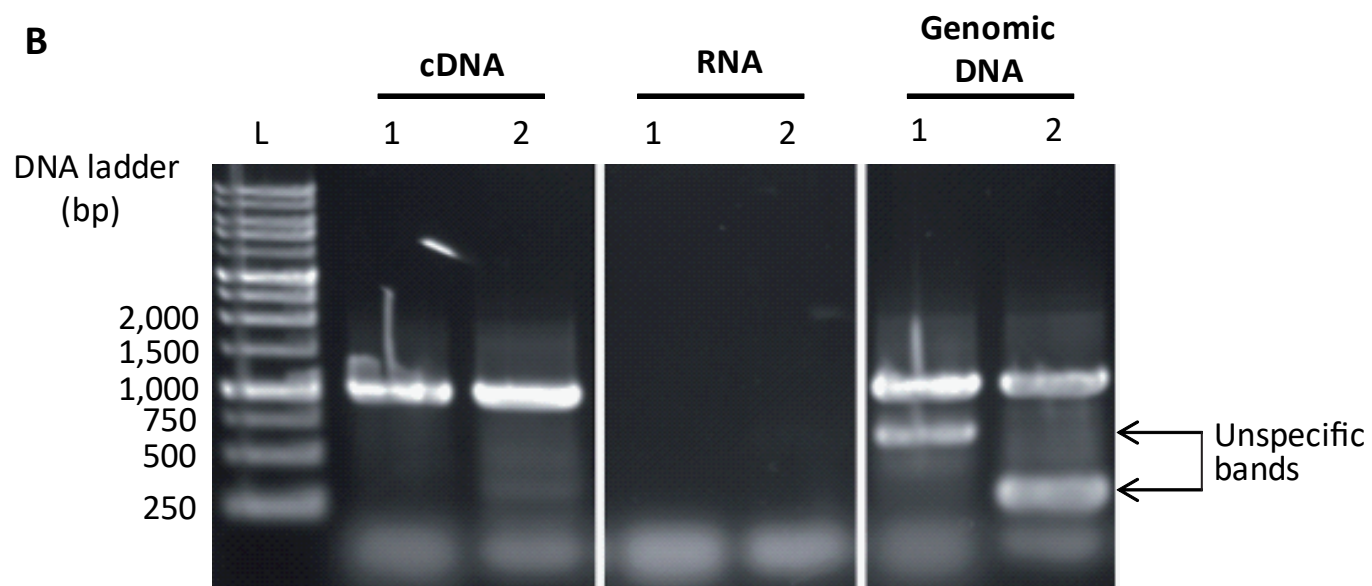
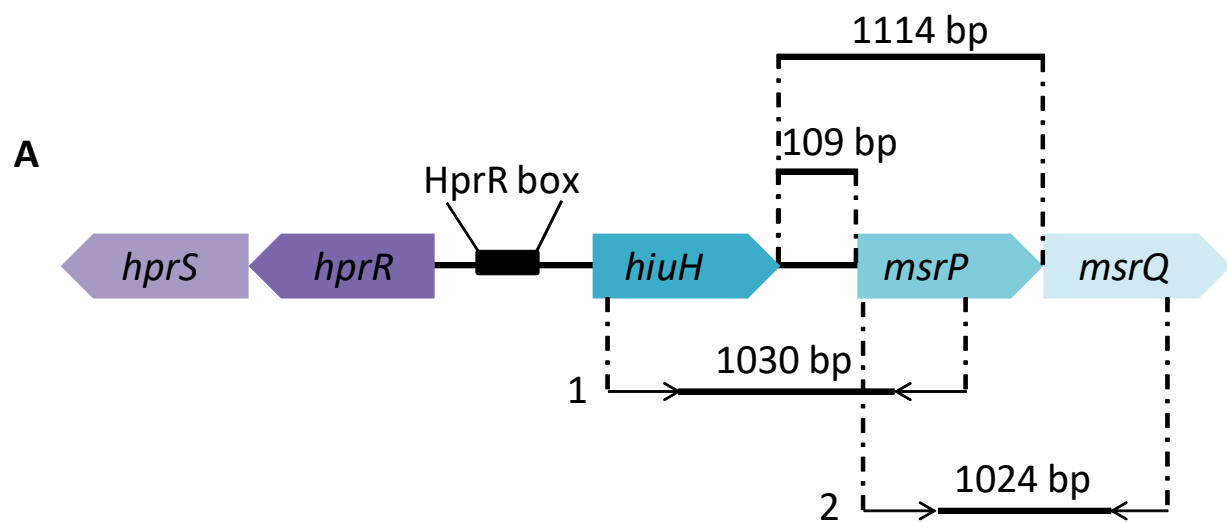


Fig.1

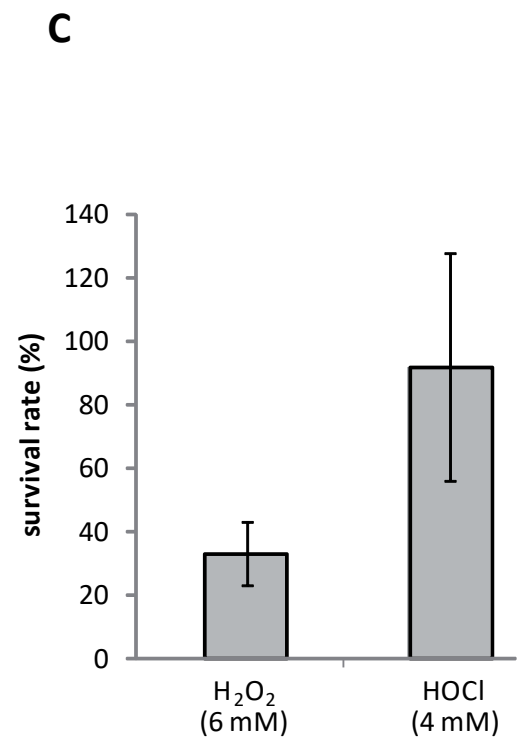
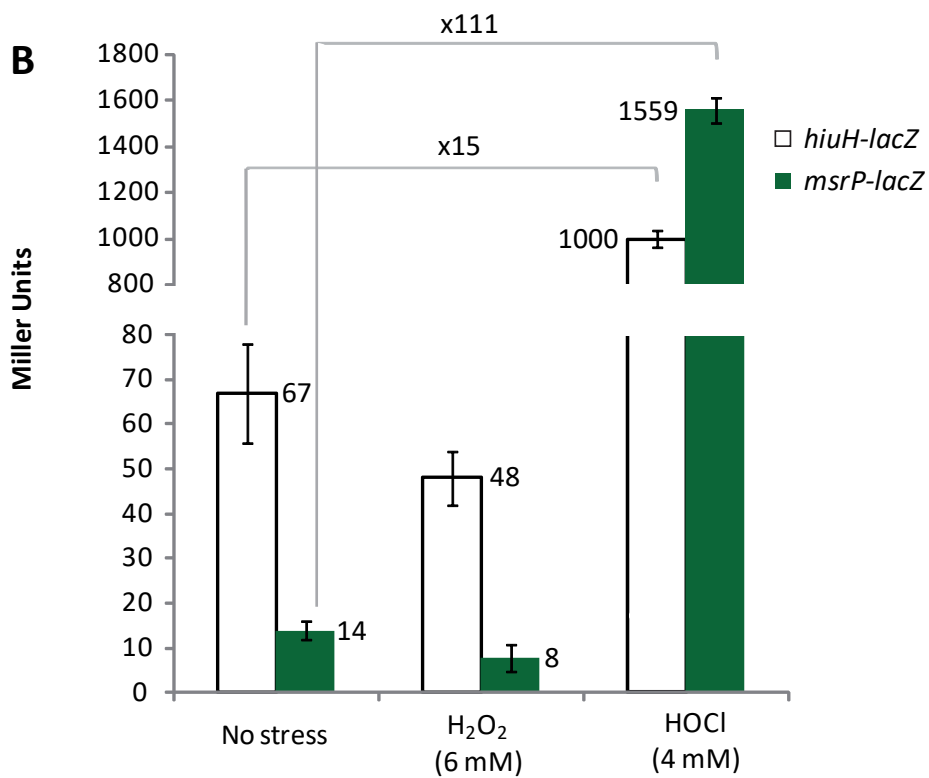
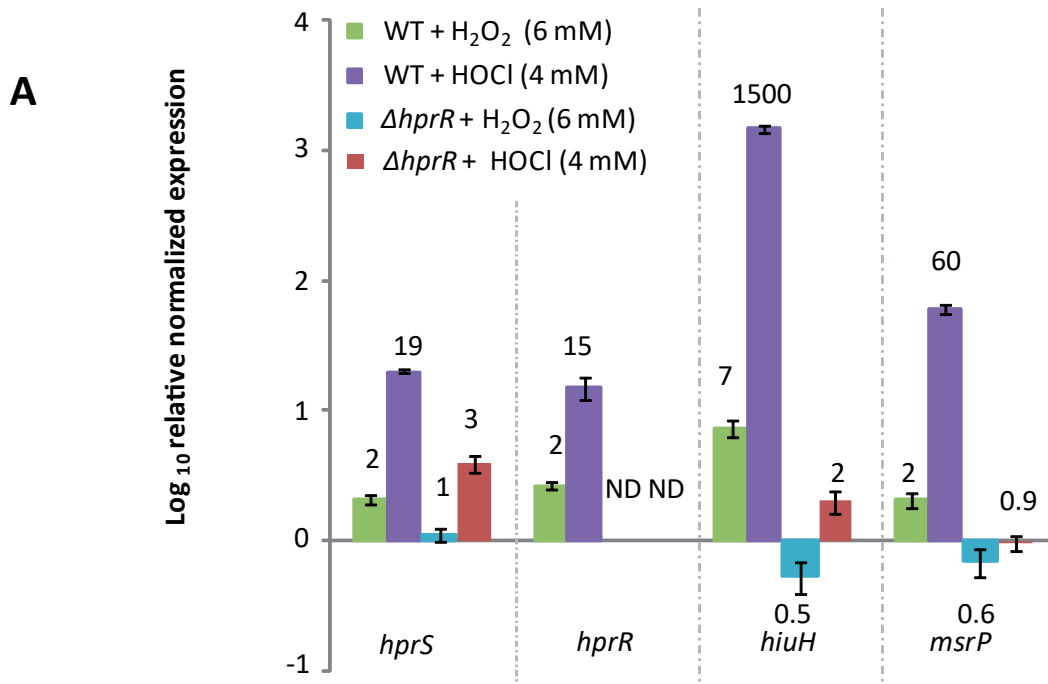


Fig.2

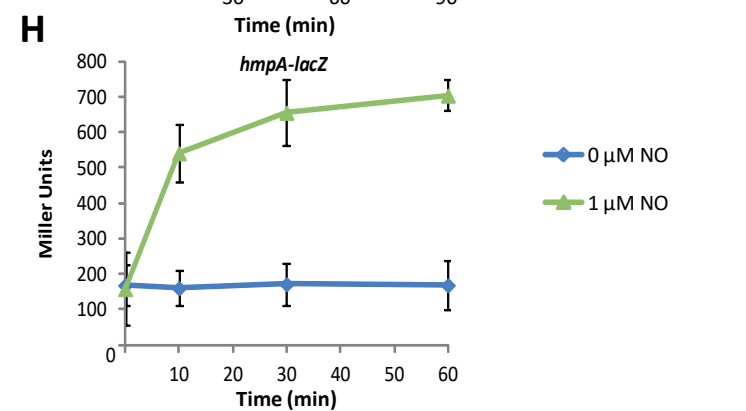
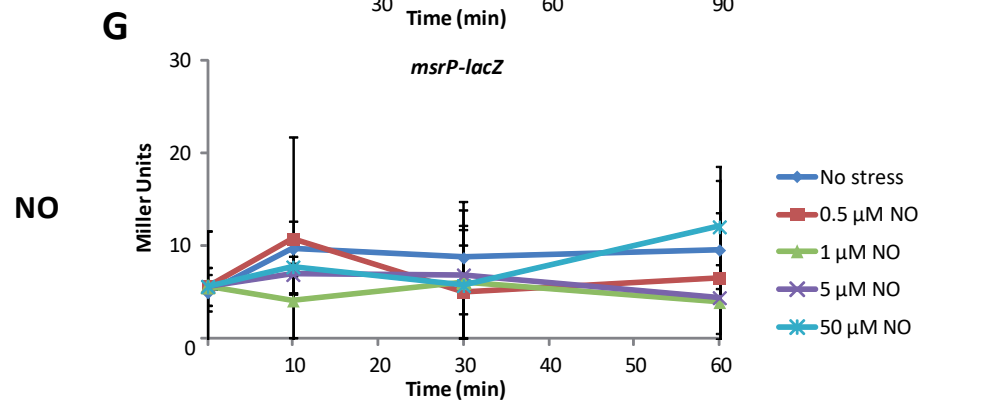
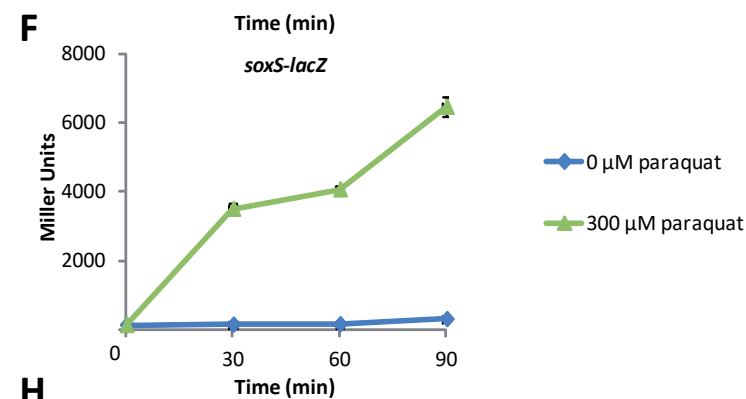
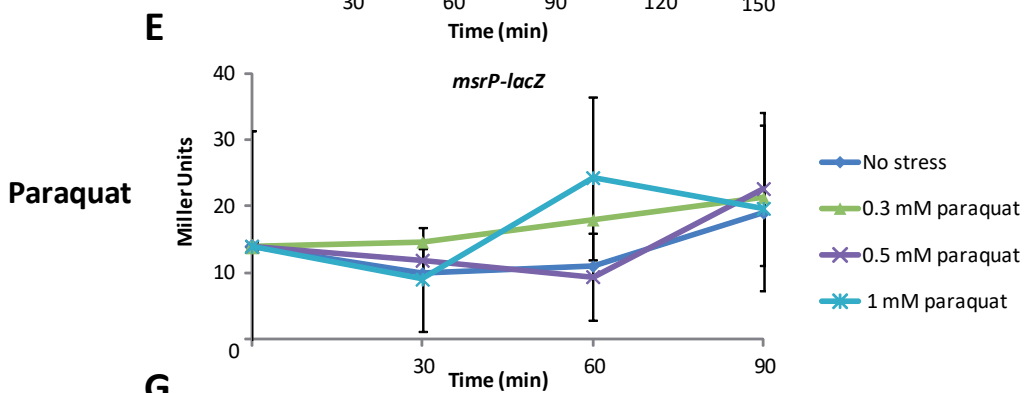
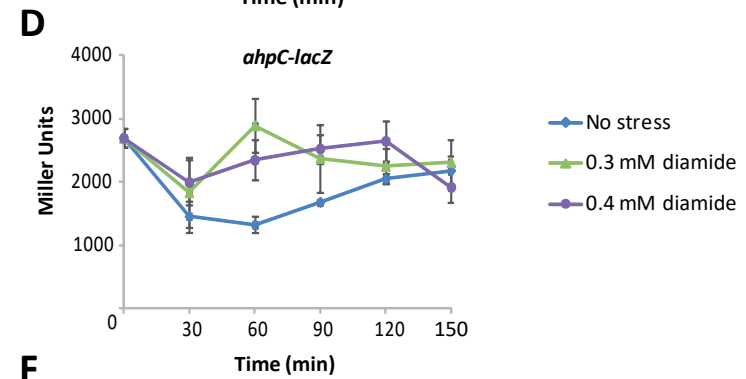
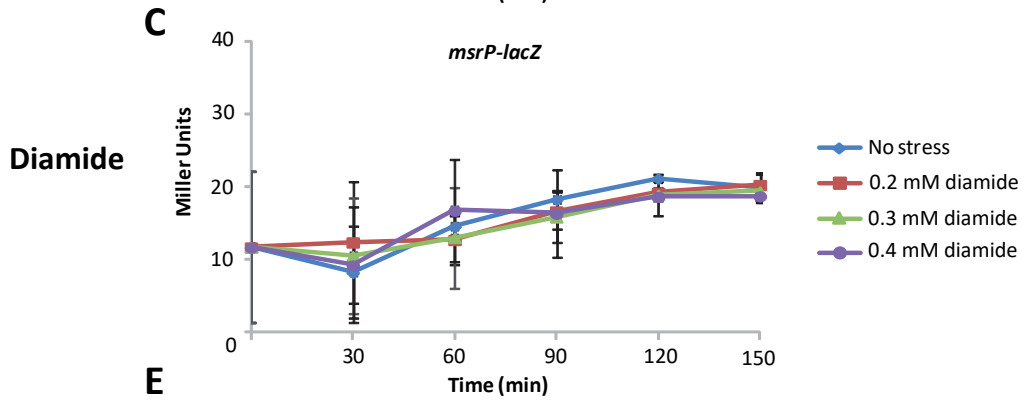
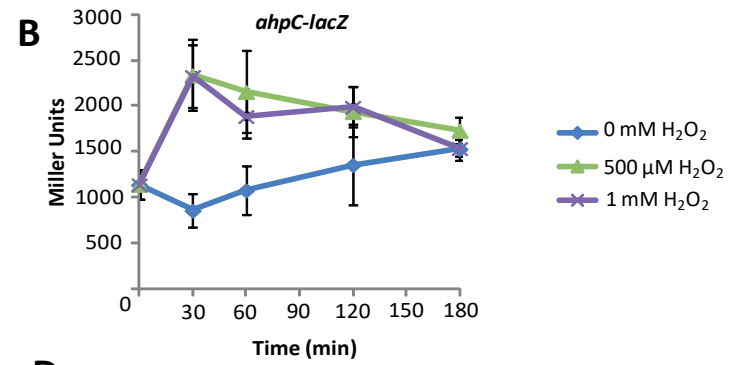
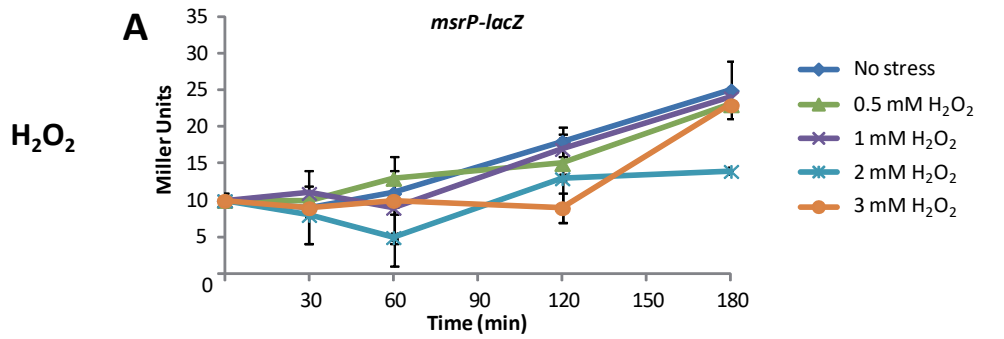


Fig.3

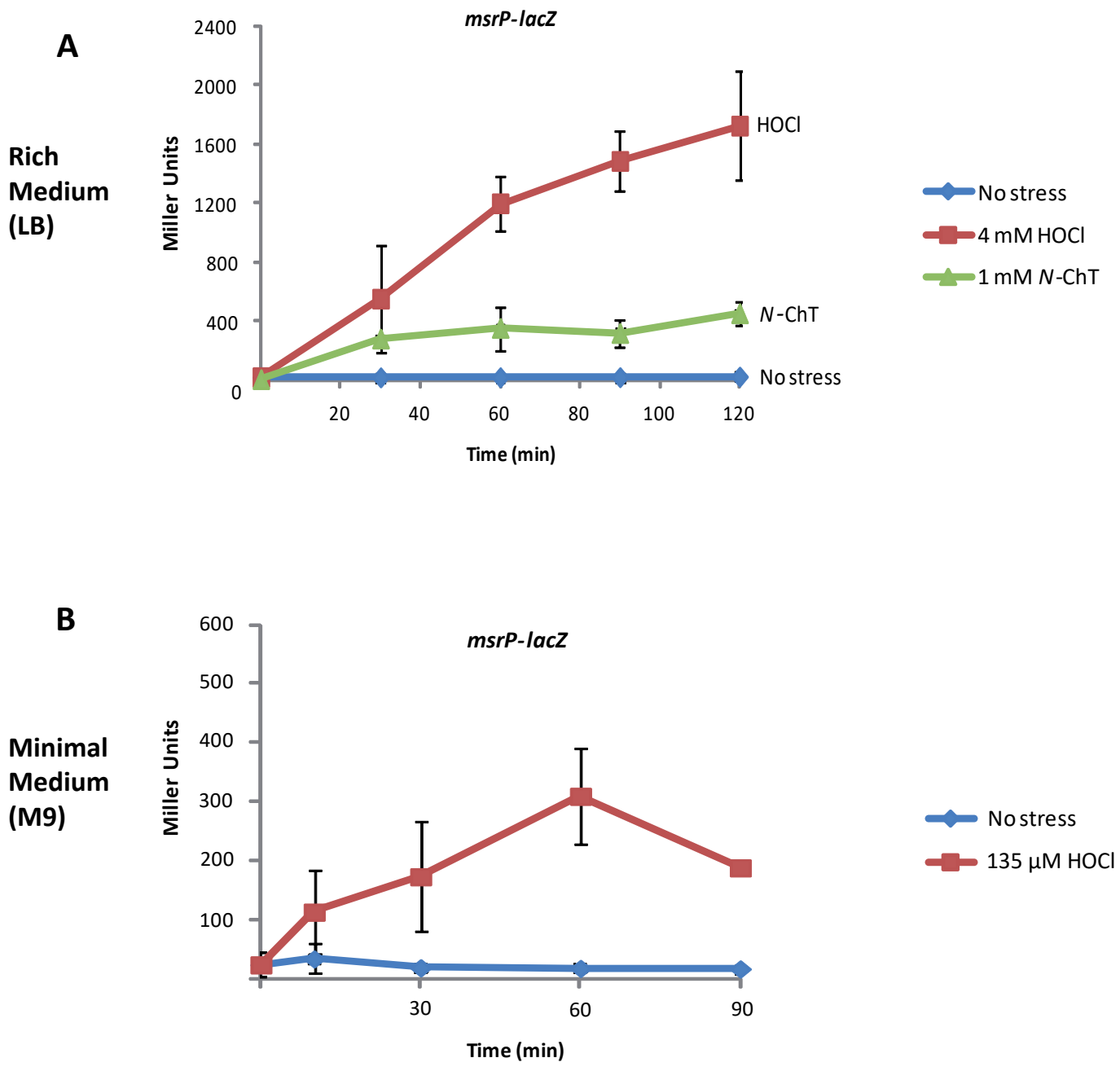


Fig.4

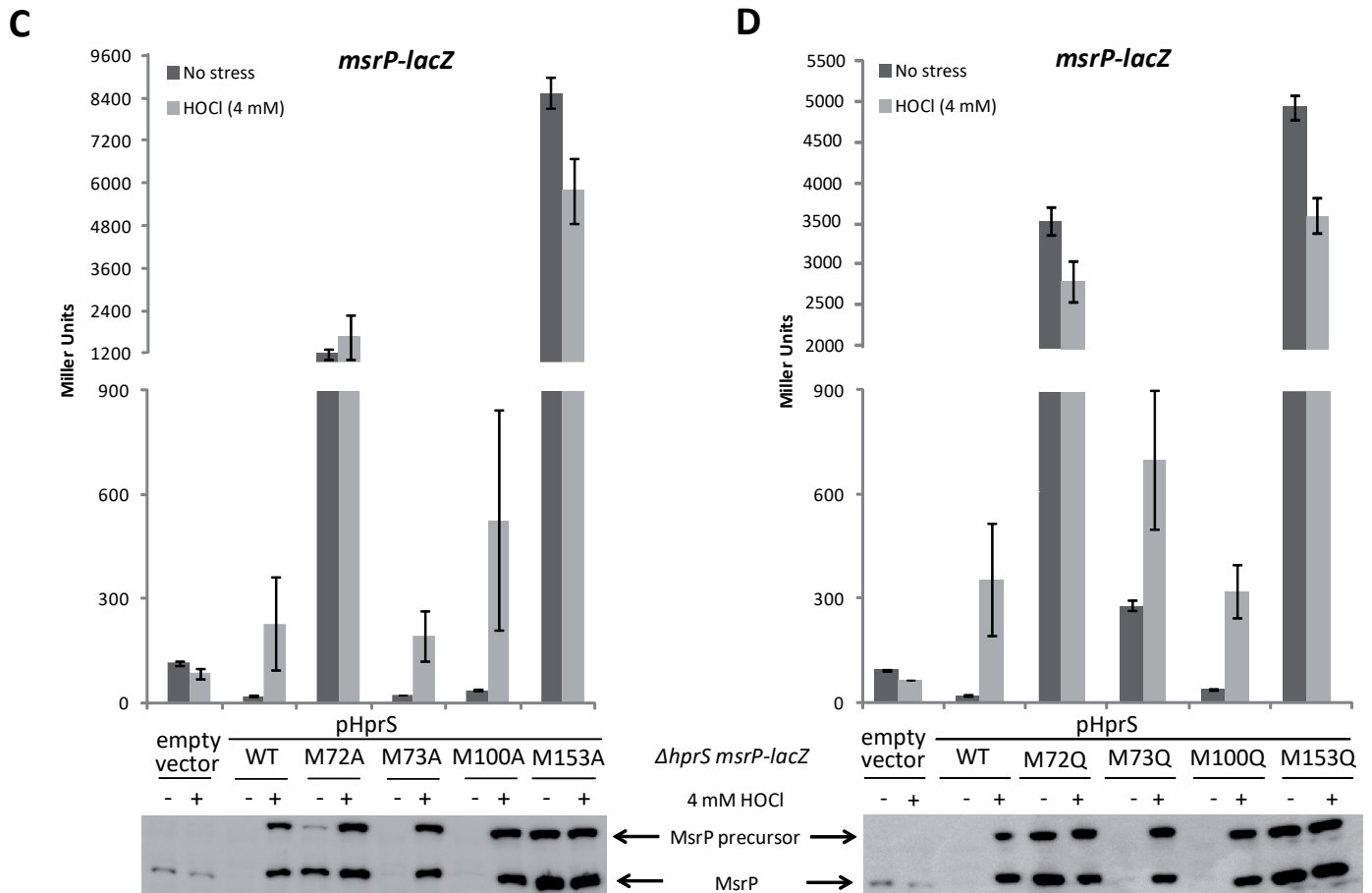
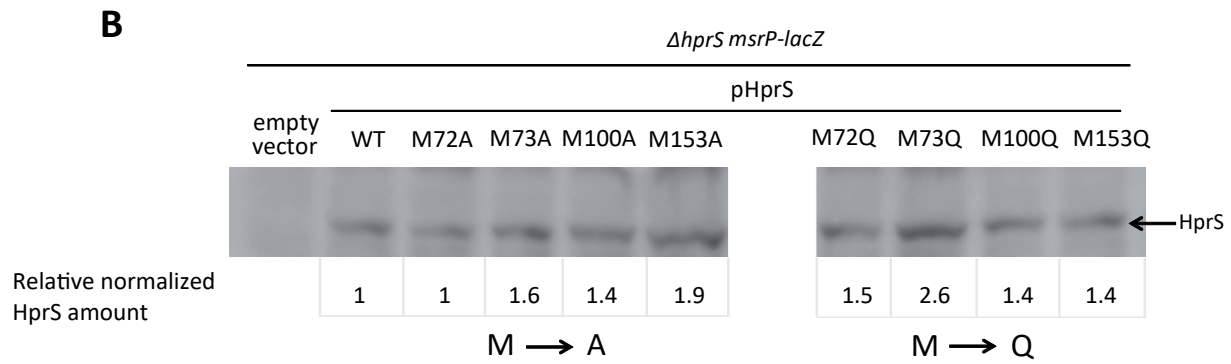
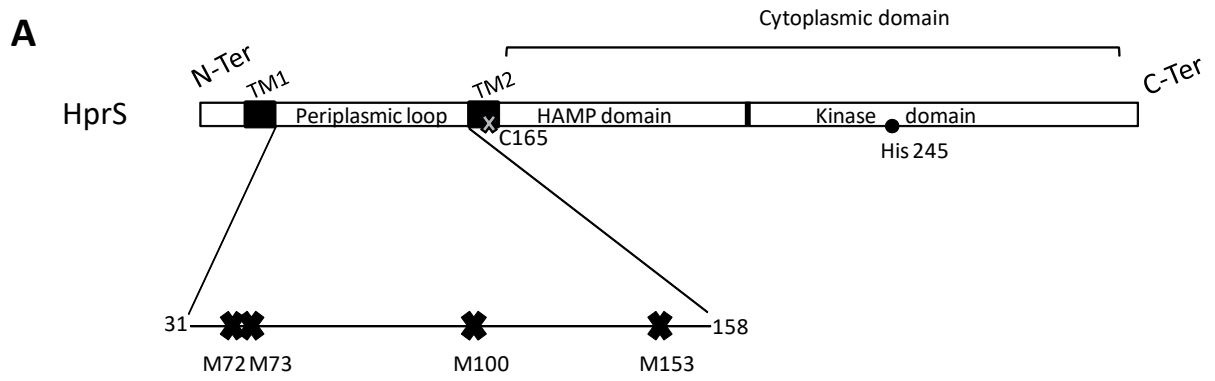


Fig.5

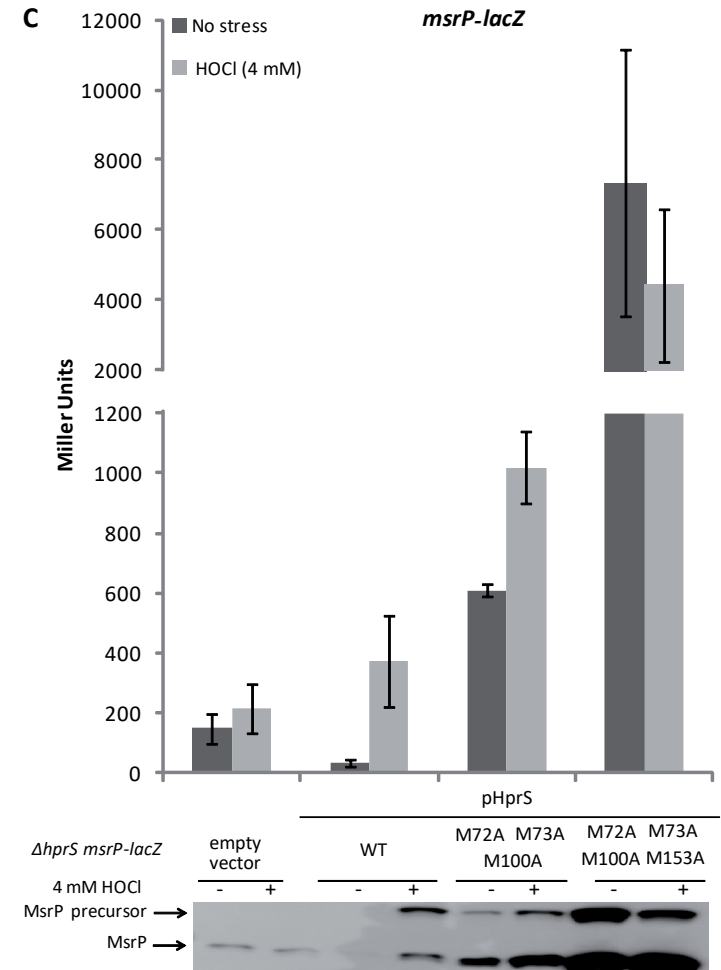
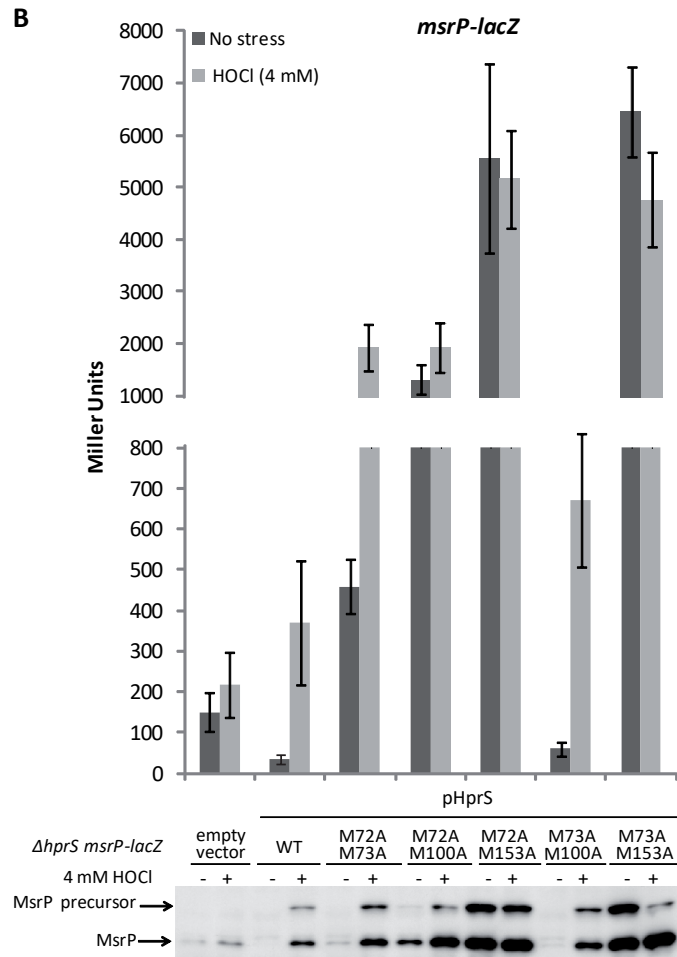
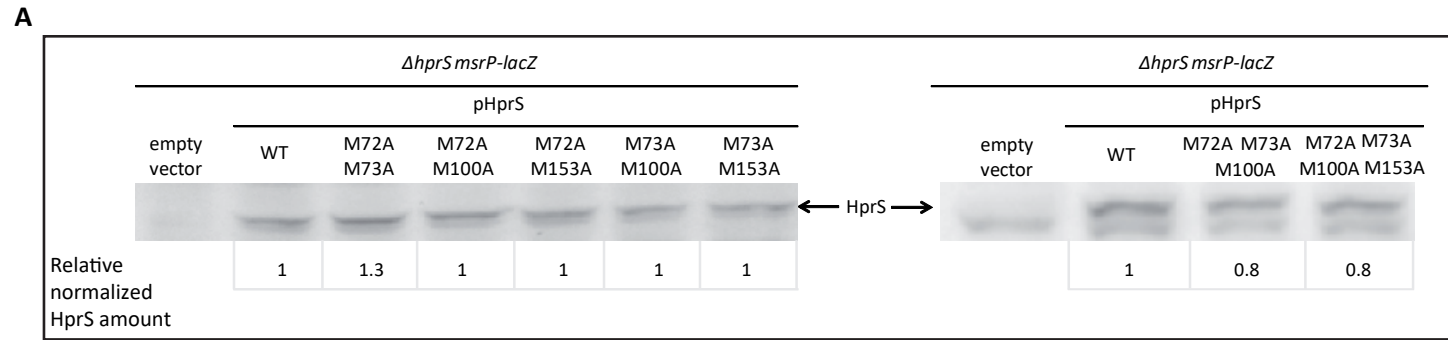
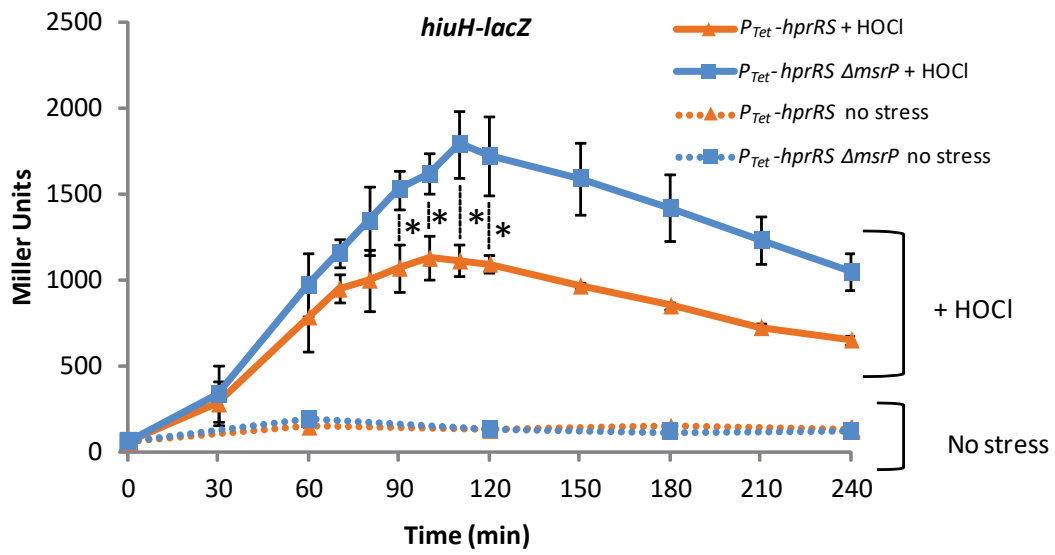
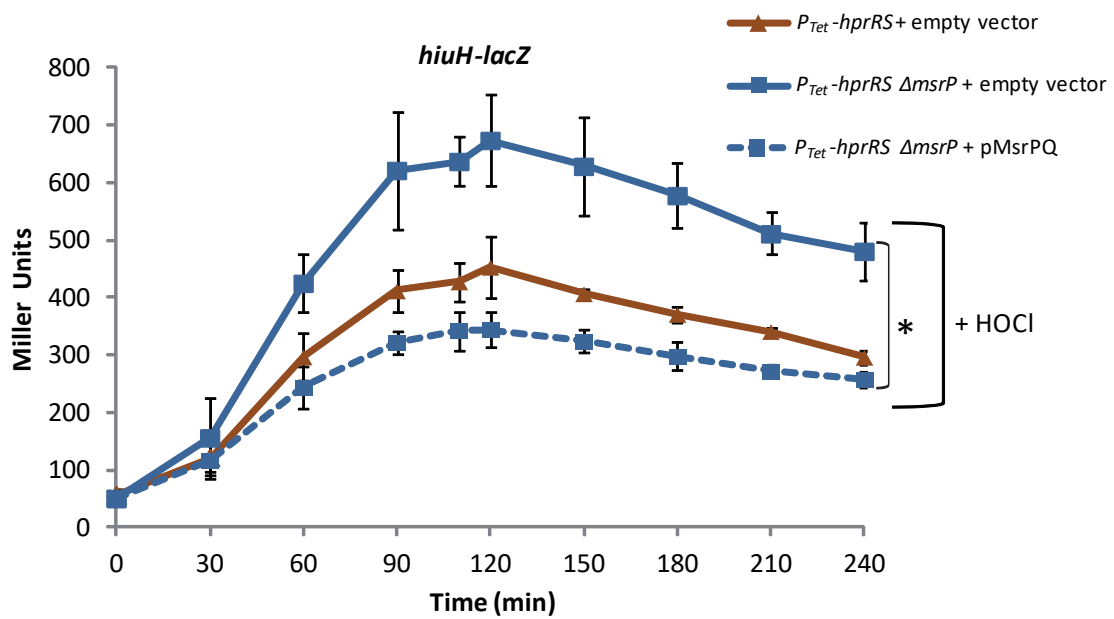
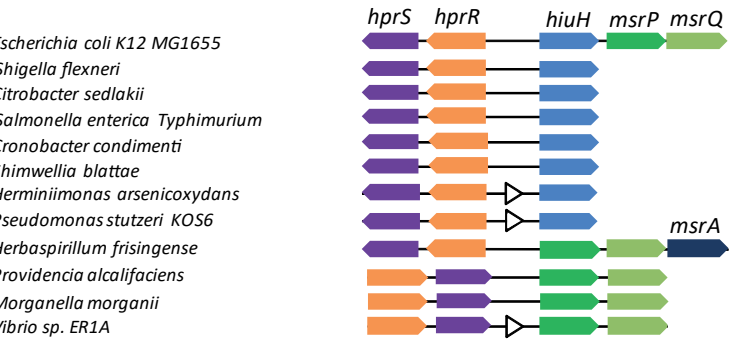


Fig.6

A**B****Fig.7**

A



B

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

<i>Escherichia coli</i> 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
<i>Shigella flexneri</i>	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
<i>Citrobacter sedlakii</i>	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
<i>Salmonella enterica</i> 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
<i>Cronobacter condimenti</i>	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
<i>Shimwellia blattae</i>	V	M	N	R	T	G	V	V	L	P	E	S	F	S	R	-	-	R	A	F	D	A	G	K	
<i>Herminiimonas arsenicoxydans</i>	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
<i>Pseudomonas stutzeri</i> KOS6	E	I	N	P	A	R	L	P	I	P	T	L	P	A	S	A	-	-	-	-	-	-	-	-	-
<i>Herbaspirillum frisingense</i>	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
<i>Providencia alcalifaciens</i>	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
<i>Morganella morganii</i>	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
<i>Vibrio sp. ER1A</i>	V	A	G	D	K	N	V	V	L	N	L	P	F	A	D	Y	-	-	-	-	-	Q	I	D	E

- Met72
- Met73
- Met100
- Met153
- Cys165

<i>Escherichia coli</i> 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
<i>Shigella flexneri</i>	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
<i>Citrobacter sedlakii</i>	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
<i>Salmonella enterica</i> 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
<i>Cronobacter condimenti</i>	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
<i>Shimwellia blattae</i>	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
<i>Herminiimonas arsenicoxydans</i>	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
<i>Pseudomonas stutzeri</i> 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
<i>Herbaspirillum frisingense</i>	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
<i>Providencia alcalifaciens</i>	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
<i>Morganella morganii</i>	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
<i>Vibrio sp. ER1A</i>	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

Fig.8

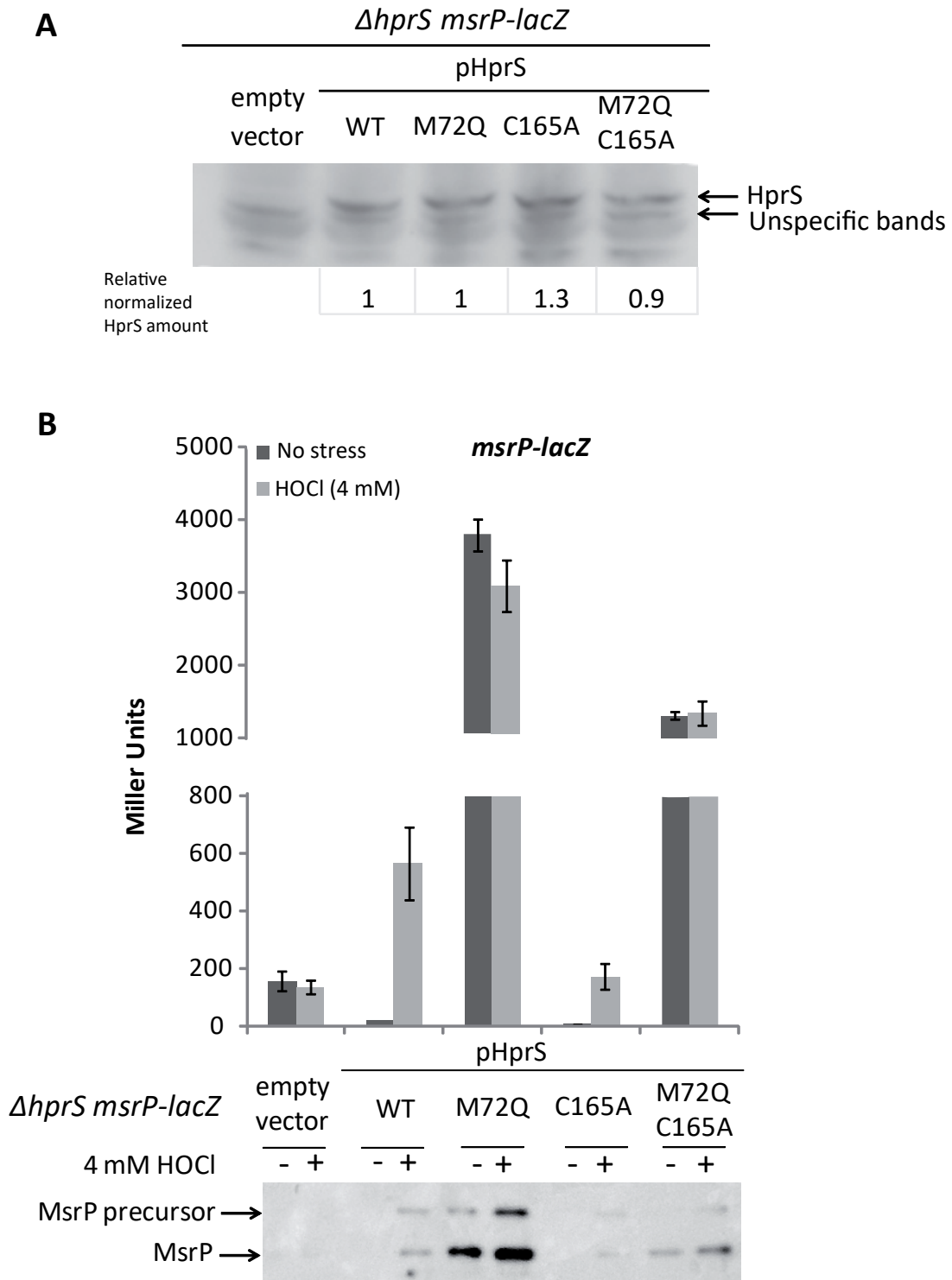


Fig.9