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Methionine oxidation under anaerobic conditions in *Escherichia coli*.

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11 ABSTRACT

12 Repairing oxidative-targeted macromolecules is a central mechanism necessary for living
13 organisms to adapt to oxidative stress. Reactive oxygen and chlorine species preferentially
14 oxidize sulfur-containing amino acids in proteins. Among these amino acids, methionine
15 can be converted into methionine sulfoxide. This post-translational oxidation can be
16 reversed by methionine sulfoxide reductases, Msr enzymes. In Gram-negative bacteria, the
17 antioxidant MsrPQ system is involved in the repair of periplasmic oxidized-proteins.
18 Surprisingly, in this study we observed in *Escherichia coli* that *msrPQ* was highly expressed
19 in the absence of oxygen. We have demonstrated that the anaerobic induction of *msrPQ*
20 was due to chlorate (ClO_3^-) contamination of the Casamino Acids. Molecular investigation
21 led us to determine that the reduction of chlorate to the toxic oxidizing agent chlorite
22 (ClO_2^-) by the three nitrate reductases (NarA, NarZ and Nap) led to methionine oxidation of
23 periplasmic proteins. In response to this stress the *E. coli* HprSR two-component system
24 was activated, leading to over-production of MsrPQ. This study therefore supports the idea
25 that methionine oxidation in proteins is part of chlorate toxicity, and that MsrPQ can be
26 considered as an anti-chlorate/chlorite defense system in bacteria. Finally, this study
27 challenges the traditional view on the absence of Met-oxidation during anaerobiosis.

28

29 INTRODUCTION

30 Bacteria must effectively counter a myriad of stresses to survive (Fang *et al.*, 2016),
31 oxidative stress being one of the most challenging (Imlay, 2015). Under aerobic conditions
32 oxygen is used as the terminal electron acceptor for aerobic respiration, during which
33 reactive oxygen species (ROS) are produced endogenously through the reaction between
34 O₂ and univalent electron donors, such as metals, flavin and quinol (Imlay, 2013). In
35 addition to ROS, a wide variety of reactive nitrogen species (RNS) and reactive chlorine
36 species (RCS) act as powerful oxidants (Winterbourn and Kettle, 2013). A huge number of
37 these types of oxidants are released in order to combat pathogens during the host immune
38 oxidative-burst response. ROS, RNS and RCS are toxic and damage biological
39 macromolecules (nucleic acids, proteins, lipids), ultimately becoming life threatening
40 (Imlay, 2015).

41 Living organisms have developed two strategies to protect themselves from oxidants: the
42 first one, pre-oxidation, consists in eliminating harmful oxidants and relies on ROS
43 scavenging enzymes such as superoxide dismutases (SOD) to remove toxic O₂⁻ and
44 catalases/peroxidases to prevent H₂O₂ accumulation (Imlay, 2013); the second, post-
45 oxidation, involves the repair of oxidative-targeted macromolecules (Vaughan, 1997;
46 Merdanovic *et al.*, 2011; Aussel and Ezraty, 2021).

47 Several amino acids can be targeted by oxidants leading to post-translational modifications
48 and protein misfolding (Schramm *et al.*, 2020). Under oxidative stress, sulfur-containing
49 methionine residues are particularly sensitive to oxidation leading to the formation of
50 methionine sulfoxide (Met-O) (Kim *et al.*, 2014; Ezraty *et al.*, 2017). Oxidation of Met into
51 Met-O is reversible because of specific enzymes found in most living organisms: the
52 methionine sulfoxide reductases (Msr) (Ezraty *et al.*, 2005; Boschi-Muller and Branlant,

2014). The main representatives of this oxidized-protein repair family are MsrA and MsrB, which are complementary and ubiquitous enzymes present in all domains of life from humans to bacteria (Ezraty *et al.*, 2005). In prokaryotic cells, MsrA and MsrB are generally located in the cytoplasm with some rare exceptions: for example the extracytoplasmic MsrAB of *Neisseria* or *Streptococcus* species (Skaar *et al.*, 2002; Lowther *et al.*, 2002; Saleh *et al.*, 2013).

A recently characterized member from *Escherichia coli* is MsrP, an enzyme responsible for protein-MetO reduction in the periplasm of most Gram-negative bacteria (Gennaris *et al.*, 2015). MsrP is a molybdoenzyme containing a molybdenum cofactor of the molydopterin type (Mo-MPT), characteristic of the sulfite oxidase family (Loschi *et al.*, 2004). This reductase works with the haem-binding membrane-bound partner MsrQ, which transmits reducing power (Gennaris *et al.*, 2015). We have previously shown that the cistron *hiuH* - encoding a transthyretin-like periplasmic protein (Lee *et al.*, 2005)- and *msrPQ* genes belong to the same operon (El Hajj *et al.*, 2022). The *hiuH-msrPQ* operon is induced by RCS in a two-component system HprSR-dependent manner (El Hajj *et al.*, 2022).

An anaerobic lifestyle is not considered to produce ROS. However, many obligate anaerobes actually have ROS scavenging enzymes (superoxide dismutases or reductases; catalases/ peroxidases) and oxidative-damage repair systems (Sheng *et al.*, 2014; Imlay, 2019). Msr genes (*msrA* and *msrB*) are also present in anaerobic organisms (including *Clostridium acetobutylicum* or *perfringens* and *Chlorobium tepidum*) (Delaye *et al.*, 2007). The presence of antioxidant mechanisms in anaerobes has been suggested to be required following transient exposure to oxygen (Fu *et al.*, 2015).

In this study, we highlight the importance of the antioxidant enzyme MsrP in the absence of oxygen for the facultative anaerobic bacterium *E. coli*. We observed periplasmic protein-bound Met-O appearance during anaerobiosis following chlorate reduction to chlorite by

nitrate reductases. Mechanistically we have shown that MsrPQ production via HprSR during chlorite stress is essential for *E. coli*. Finally, our results challenge the traditional view that Met-oxidation is absent during anaerobiosis.

RESULTS

msrP expression is induced under anaerobic conditions in the presence of Casamino Acids

As reported in previous studies, we found that reactive chlorine species (HOCl and *N*-Chlorotaurine) induce the expression of the *hiuH-msrPQ* operon in *E. coli* (El Hajj *et al.*, 2022). These results corroborate the idea that the oxidized-protein repair system is necessary during oxidative stress. During this study, an unexpected observation was made: this operon is also highly up-regulated under anaerobic conditions in the absence of any apparent exogenous oxidative stress. This initial unpredicted observation suggested that an antioxidant system might be needed in the absence of oxygen and was the subject of subsequent studies. A more precise characterization of this effect led us to observe that the induction of *hiuH-msrPQ* was present in both minimal and rich medium, but only in the presence of Casamino Acids (CASA) (Fig. 1A). Indeed, by using a strain carrying the chromosomal *hiuH-lacZ* translational fusion, we showed that under anaerobic conditions the activity of the fusion increased about 20-fold in the presence of CASA in both media (Fig. 1A), whereas under aerobic conditions the expression of the fusion was not affected by the presence of CASA. This effect was confirmed on analysing MsrP protein levels by western blot (Fig. 1B). These observations led us to hypothesize that the HiuH and/or MsrPQ system might be important for cell growth under anaerobic conditions in the presence of CASA. To test this hypothesis, we plated the $\Delta hiuH-msrPQ$, *hiuH*⁻, $\Delta msrP$ and

ΔmsrQ mutant strains onto LB-agar plates supplemented with CASA under anaerobic conditions and observed that disruption of the *hiuH-msrPQ* operon led to a defect in colony morphology (Fig. 1C). Moreover, we observed that disruption of the single *hiuH* gene had no effect on colony morphology, whereas deletions of *msrP* or *msrQ* showed a severe phenotype (Fig. 1C). Indeed, we observed that the *ΔhiuH-msrPQ*, *ΔmsrP* and *ΔmsrQ* mutant colonies tended to be smaller than wild type colonies. The shape was readily distinguishable, as the colonies of the mutant strains were cauliflower- or donut-shaped. This morphological defect was not dependent on the density of the colonies on the plate (Fig. S1) as previously described (Warne *et al.*, 1990). Overproduction of MsrP using the plasmid pMsrP complemented only the *ΔmsrP* mutant, whereas overproduction of MsrP and MsrQ using the plasmid pMsrPQ was able to complement the mutant strains *ΔhiuH-msrPQ*, *ΔmsrP* and *ΔmsrQ* (Fig. 1D). These results support the conclusion that MsrPQ plays an important role in bacterial growth under anaerobic conditions in the presence of CASA.

FNR has an indirect effect on the regulation of *msrPQ* under anaerobic conditions in the presence of CASA.

We established that *msrPQ* was up-regulated under anaerobic conditions in the presence of CASA and that the absence of the MsrPQ system rendered *E. coli* highly sensitive to these conditions. However, it remained unclear why an antioxidant system appeared essential in the absence of oxygen. An understanding of the regulatory pathway could clarify the situation and therefore we initially investigated whether FNR, the master transcriptional regulator of anaerobiosis (Spiro, 1994; Kiley and Beinert, 1998), was involved. We examined *hiuH-lacZ* expression in a *Δfnr* strain: induction of the *hiuH-lacZ* fusion under

anaerobic conditions with CASA depended on the presence of FNR since no up-regulation was observed in a Δfnr mutant (Fig. 2A). Furthermore, no accumulation of MsrP was observed following immunoblotting of the Δfnr mutant under anaerobic conditions with CASA (Fig. 2B). Interestingly, deletion of *fnr* was able to suppress the colony morphology phenotype of the $\Delta msrP$ strain. The colonies of the $\Delta msrP \Delta fnr$ strain were smooth and circular compared to the rough colonies of the wild-type strain (Fig. 2C). These results suggest that FNR participates in *msrPQ* regulation. One prediction of our model is that FNR directly binds and regulates the *hiuH-msrPQ* operon. To test this, we performed an *in silico* analysis of the *hiuH-msrPQ* promoter region, using the DNA consensus binding site previously determined (Myers *et al.*, 2013), and identified a putative FNR-binding site (5'-**TTGAAGATAATCAA**-3') between positions -148bp and -162bp relative to the start codon of the *hiuH* gene. We then constructed a deletion mutant of this putative FNR-binding site in the *lacZ* fusion and showed that the deletion of this sequence had no impact on the induction of the *hiuH-lacZ* fusion under anaerobic conditions with CASA (Fig. 2A). These results favoured an indirect role for FNR in *msrPQ* regulation in *E. coli*.

Nitrate reductases are required for the regulation of *msrPQ* under anaerobic conditions with CASA.

Molybdenum cofactor-dependent anaerobic respiration DMSO reductase family system genes are FNR-regulated (Myers *et al.*, 2013). Deletion of the *mobAB* operon required for the synthesis of the bis-Mo-MGD cofactor used by these systems, prevent accumulation of MsrP under anaerobic conditions with CASA and suppressed the colony morphology phenotype of the $\Delta msrP$ mutant strain, the colonies appeared smoother and less

147 segmented than wild-type colonies (Fig. 2B and C). This observation suggested that one or
148 more molybdenum cofactor-dependent anaerobic respiration systems are involved in this
149 phenotype. Indeed, the addition of alternative electron acceptors such as nitrate and TMAO
150 during anaerobic growth abrogated the effect of CASA on the induction of the *hiuH-lacZ*
151 fusion, while adding DMSO had no effect (Fig. 3A and B). Nitrate, and to a lesser extent
152 TMAO, chemically suppressed the $\Delta msrP$ colony morphology phenotype, while DMSO had
153 no impact (Fig. 3C). To perform anaerobic respiration using nitrate or TMAO as alternative
154 electron acceptors, *E. coli* relies on two cytoplasmic and one periplasmic nitrate reductase
155 (NR) (NarGHI, NarZYV and NapABC) (Fig. 4A) or TorAC respectively (Stewart, 1988;
156 Magalon and Mendel, 2015). Deletion of one NR had no effect on the induction of *msrPQ*
157 during anaerobiosis in the presence of CASA. However, the use of the $\Delta narG \Delta narZ \Delta napA$
158 mutant strain abrogated the up-regulation of *msrPQ*, suggesting that NR systems are
159 required for the induction of *msrPQ* during anaerobiosis in the presence of CASA (Fig. 4B).
160 Furthermore, no accumulation of MsrP was observed in the $\Delta narG \Delta narZ \Delta napA$ mutant
161 strain, whereas deleting either *narG*, *narZ*, *napA* had no effect on MsrP accumulation (Fig.
162 4C). We confirmed the involvement of the three NRs by deleting the two genes encoding
163 cytoplasmic nitrate uptake proteins (NarU and NarK) (Clegg *et al.*, 2002), and by showing
164 that the $\Delta narU \Delta narK \Delta napA$ mutant strain abrogated the up regulation of *msrPQ* (Fig. 4B).
165 Given that TMAO could partially suppress the $\Delta msrP$ colony morphology phenotype on
166 CASA, we conducted an experiment using the $\Delta torA$ mutant strain. Deletion of *torA* had no
167 effect on MsrP accumulation and did not suppress the colony morphology phenotype of the
168 $\Delta msrP$ (Fig. 4C and D). Surprisingly, deletion of *narG* alone was able to suppress the colony
169 morphology phenotype of the $\Delta msrP$ strain. The $\Delta msrP \Delta narG$ colonies were larger and
170 more opaque than wild-type colonies (Fig. 4D). These results suggested that the

requirement for MsrPQ during anaerobiosis in the presence of CASA depends on NR activities.

Reduction of chlorate to chlorite by nitrate reductases induces *msrPQ*.

Given the multiple lines of evidence suggesting a link between MsrPQ and NRs during anaerobiosis in the presence of CASA and in the absence of nitrate, we investigated whether a component of the CASA could be used as a substrate for NRs. The 0.4 % CASA solution was analysed by an external laboratory and a chlorate (ClO_3^-) contamination of around 1.5 mg/L was detected. Chlorate, as an analogue of nitrate, is a substrate for NRs which reduce chlorate to the toxic chlorite (ClO_2^-) (Glaser and DeMoss, 1971; Alefounder and Ferguson, 1980; Giordano *et al.*, 1984). By analysing the expression of the *hiuH-lacZ* fusion and the western blot, we confirmed that the addition of chlorate to the LB medium under anaerobic conditions led to an increase in the expression of MsrP (Fig. 5A and B). Moreover, the ΔmsrP strain showed a donut-shaped colony morphology phenotype on LB plates containing chlorate (Fig. 5C), as observed with CASA. These results suggested that the effects observed with CASA were due to the presence of chlorate in the CASA solution. To confirm that the chlorate toxicity of the ΔmsrP strain comes from chlorate reduction to chlorite by NRs, we attempted to suppress the phenotype of the ΔmsrP mutant by overproducing chlorite dismutase (Cld) from *Dechloromonas aromatica* (Goblirsch *et al.*, 2010). The Cld enzyme catalyses the decomposition of chlorite into molecular oxygen and chloride ions. Fig. 6A shows that Cld substantially suppressed the ΔmsrP mutant phenotype. Next, we examined the effect of chlorite on the expression of the *hiuH-lacZ* fusion. We observed that ClO_2^- induced the expression of the fusion in a dose-dependent

manner (Fig. 6B). Cld co-expression abrogated *hiuH-lacZ* up-regulation during ClO_2^- stress (Fig. 6C and D). Overall, these results confirm that chlorite production via chlorate reduction by NRs led to the ΔmsrP colony morphology phenotype and induction of *msrPQ* expression.

HprSR two-component system detects chlorite stress and induces *msrPQ*.

We have previously demonstrated that HOCl induces the expression of *msrPQ* in an HprSR-dependent manner (El Hajj *et al.*, 2022). HprSR is a two-component system in which HprS and HprR are the sensor and the regulator respectively. We thus investigated the role of HprSR in chlorite detection and performed immunodetection of MsrP from WT, ΔhprS and ΔhprR strains cultivated anaerobically in the presence of chlorate. We observed an accumulation of MsrP in the WT strain (Fig. 7), but no over-production of MsrP in the ΔhprS and ΔhprR mutants (Fig. 7). These results indicate that the induction of the *hiuH-msrPQ* operon by chlorate is dependent on the presence of a functional HprSR system and suggest that HprS is able to sense chlorite production in the cell.

Chlorite-damaged periplasmic proteins

Having established that the periplasmic oxidized-protein repair system, MsrPQ, was over-produced during chlorite stress, we sought to determine the effect of chlorite stress on the methionine-redox state of periplasmic proteins by following the primary periplasmic chaperone SurA, a specific substrate of MsrP in *E. coli* (Gennaris *et al.*, 2015). Using western blots, we observed that SurA produced in a ΔmsrP mutant exposed to chlorite treatment migrated more slowly than SurA produced in a WT strain or in a ΔmsrP mutant

that had not been exposed to chlorite (Fig. 8). This mobility-shift seen on SDS-PAGE was evidence for methionine oxidation in SurA. These data suggest that chlorite was able to oxidize Met residues *in vivo*. The findings presented so far suggest that periplasmic proteins are oxidized by chlorite and that MsrP is important in the repair of chlorite-induced protein damage.

Chlorate stress did not induce the expression of cytoplasmic methionine sulfoxide reductases

We also examined whether chlorate stress induced the expression of the cytoplasmic oxidized-protein repair genes, *msrA* and *msrB*. The strains carrying the chromosomal reporter fusions *pmsrA-lacZ* and *pmsrB-lacZ* were cultured in the absence or in the presence of chlorate under anaerobic conditions. No induction of *pmsrA-lacZ* and *pmsrB-lacZ* fusions was observed during chlorate stress under anaerobic conditions (Fig. 9A). Moreover, no colony morphology phenotype was observed using the $\Delta msrA \Delta msrB$ mutant (Fig. 9B). Even the deletion of the *gshA* gene, that leads to decreased intracellular glutathione levels (glutathione being putative quencher of cytoplasmic chlorite), had no effect on colony morphology of the $\Delta msrA \Delta msrB$ mutant (i.e absence of donut/cauliflower-shaped colonies) on LB-CASA under anaerobic conditions (Fig. S2). We conclude that cytoplasmic methionine sulfoxide reductases are not up-regulated and that their absence does not lead to morphological colony defects under chlorate stress.

DISCUSSION

Chlorate toxicity in bacteria has been studied for many decades, especially in relation to anaerobic respiratory systems (Stewart, 1988). This study provides new insight into the molecular mechanism of chlorate toxicity and has characterized, for the first time, protection mechanisms against this stress in *E. coli*. We demonstrated that reduction of chlorate to toxic chlorite leads to periplasmic protein oxidation. In response to this stress, the *E. coli* HprSR two-component system is activated leading to the over-production of the periplasmic oxidized-protein repair system, MsrPQ.

A word of caution on chlorate contaminated Casamino Acids

A fortuitous observation led us to discover that the Casamino Acids were contaminated with chlorate, which represented the starting point for this study. This contamination does not seem to be anecdotal since another global transcriptomic analysis study reported an induction of *hiuH*, *yedY* and *yedZ* gene expression (as *msrP* and *msrQ* were previously named) under anaerobic conditions (Covert *et al.*, 2004). CASA are a product of hydrochloric acid hydrolysis of casein of animal origin and a dedicated study will be necessary to investigate the presence of chlorate depending on the commercial origin of the CASA. We would like to alert the wider population of CASA users to the possibility of chlorite production during anaerobic experiments.

New insight into chlorate reduction in *E. coli*

The ability of nitrate reductases to reduce chlorate was discovered in the sixties through the isolation of chlorate-resistant (Chl^r) mutants and subsequent genetic studies (Glaser and DeMoss, 1971; Alefounder and Ferguson, 1980; Giordano *et al.*, 1984). Retrospectively, the analysis of this plethora of mutants has made it possible to identify all the steps from

gene expression to enzyme maturation involved in the activity of nitrate reductases. The current model for chlorate reduction in *E. coli* is that chlorate is a substrate for the cytoplasmic nitrate reductase A (NarA), but not for the second cytoplasmic reductase (NarZ) or the periplasmic one (Nap). Moreover, it has been proposed that chlorate transport into *E. coli* occurs independently of the nitrate transporters NarK and NarU (Clegg *et al.*, 2002), however, these conclusions were made based on studies using high chlorate concentrations (1 to 20 mM). Here, for the first time, we highlight an *E. coli* chlorate phenotype at low chlorate concentrations (10 to 200 μ M), which allowed reinvestigation of the chlorate reduction model. Our results showed that the three nitrate reductases were able to reduce chlorate, as the concomitant deletion of the three nitrate reductases was necessary to avoid chlorate induction of *msrP*. Moreover, given that the $\Delta narU \Delta narK \Delta napA$ mutant phenocopy the $\Delta narG \Delta narZ \Delta napA$ mutant strain, we propose that the nitrate transporters NarK and NarU are involved in chlorate import.

New insight into chlorate/chlorite toxicity

While chlorite production is known to be toxic to bacteria, the molecular mechanism behind the toxicity of this oxidizing agent was unknown. Previous *in vitro* studies showed that chlorite was able to oxidize protein-bound methionine (Melnik *et al.*, 2015). In this study, we show that chlorite production by the anaerobic respiratory complex NRs damages periplasmic proteins, such as the primary chaperone SurA. Moreover, we show that chlorite activates the HprRS two-component system, whose activation has been proposed to be mediated through a methionine redox switch located in the periplasmic loop of the sensor (El Hajj *et al.*, 2022). Chlorite-HprRS activation leads to expression of the periplasmic methionine sulfoxide reductase *msrPQ*. Together, these elements suggest that protein methionine oxidation contributes to the molecular mechanisms of chlorate

290 toxicity. While the expression of cytoplasmic methionine sulfoxide reductases (*msrA* and
291 *msrB*) is not induced by chlorate treatment and that there is no pronounced morphological
292 defect in the $\Delta msrA \Delta msrB$ double mutant, the possibility that cytoplasmic proteins are
293 damaged by chlorite cannot be rule out, especially since NarA and NarZ have a
294 cytoplasmic-facing orientation, implying that chlorite is produced in the cytoplasm.
295 However, the fact that a strain lacking the periplasmic nitrate reductase, NapA, is still able
296 to activate *msrPQ* expression implies that cytoplasmic chlorite is, at least partially,
297 transferred to the periplasm, most likely driven by the membrane potential and the
298 nitrate/nitrite antiporter. Under high chlorate content, the rate of chlorite production
299 could be higher than the rate of periplasmic transfer, leading to oxidation of cytoplasmic
300 proteins and therefore MsrA/B could be important for chlorate resistance.

302 **MsrPQ appears as an anti-chlorite defense system**

303 The up-regulation of *msrPQ* during chlorate stress in *E. coli* is reminiscent of the response
304 found in the dissimilatory (per)chlorate-reducing bacteria (DPRB) which use (per)chlorate
305 as an electron acceptor (Wang and Coates, 2017). In the canonical perchlorate (ClO_4^-)
306 reduction pathway, perchlorate reductase (PcrAB) reduces ClO_4^- to chlorate (ClO_3^-) and
307 then to chlorite (ClO_2^-) in the periplasm. Chlorite dismutase (Cld) detoxifies chlorite by
308 generating chloride (Cl^-) and oxygen (O_2), and then a terminal oxygen reductase reduces
309 oxygen to water. In addition to the periplasmic anti-chlorite enzyme Cld, it has been shown
310 in the DPRB *Azospira suillum* that the *msrQP-mrpX* operon is induced during chlorite stress
311 via a sigma/anti-sigma factor system (SigF/NrsF). MrpX is a methionine-rich periplasmic
312 protein suggested to be an MsrP-dependent recyclable 'sponge' for oxidants (Melnyk *et al.*,
313 2015). Moreover, chlorate reduction in *Shewanella algae* ACDC has been shown to induce
314 the expression of *msrPQ* (Clark *et al.*, 2014). In this study, the *huiH-msrPQ* operon in *E. coli*

is not shown to be regulated by FNR, in accordance with previous studies which have not reported *msrPQ* as direct FNR target genes (Myers *et al.*, 2013). Interestingly, in *Shewanella oneidensis* the *msrPQ* operon has been suggested to be under the direct control of the FNR homolog, EtrA (Zupok *et al.*, 2019), but to our knowledge this has not been experimentally demonstrated. Therefore, it appears that different regulatory pathways (HprRS, SigF/NrsF, FNR) are involved in adaptive regulation to chlorite, leading to the production of MsrPQ, which can be considered as a necessary and efficient anti-chlorite defense system for bacteria. The conservation of *msrPQ* (*yedYZ*) genes throughout Gram-negative bacteria (Gennaris *et al.*, 2015) and more precisely in chlorate-respiring bacteria (Barnum *et al.*, 2018) illustrates the importance of having a periplasmic methionine sulfoxide reductase system to overcome chlorite production.

Methionine oxidation under anaerobic conditions

This study reports for the first time a molecular explanation for the importance of methionine sulfoxide reductases under physiological anaerobic conditions. This finding is reminiscent of the study placing MsrA/B at the centre of the *Bacillus cereus* antioxidant system under anaerobic fermentative conditions, however the molecular source of methionine oxidation was not demonstrated (Duport *et al.*, 2021). Therefore, our study shows that methionine redox control is not a phenomenon that is solely linked to a reaction with reactive oxygen species.

Finally, these results become relevant if we assume that the natural life cycle of *E. coli* takes place in the gastrointestinal tract and, most of its existence is spent under anaerobic conditions. Chlorate contamination of drinking water could lead to the presence of chlorate

in the human gut and therefore put MsrPQ at the forefront of adaptive responses in bacteria.

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The authors declare no conflict of interest.

352 EXPERIMENTAL PROCEDURES

353 Chemical reagents and antibiotics

354 All chemicals used were purchased from Sigma, Euromedex, or Fisher Scientific. CASA used
355 was purchased from Gibco™ Ref: #223050; batch n° 6266538. Sodium chlorate used was
356 purchased from Acros Organics Ref: #223222500. Antibiotics were used at the following
357 concentrations: Ampicillin (50 µg/ml) and kanamycin (25 µg/ml). Arabinose was used at
358 0.02%. Nitrate (40 mM), and trimethyl amine oxide (TMAO) (40 mM) were used as
359 electron acceptors.

360 Strains and microbial techniques

361 The strains used in this study are listed in Table 1. The *fnr::Kan^R*, *narG::Kan^R*, *narZ::Kan^R*,
362 *napA::Kan^R*, *narU::Kan^R*, *narK::Kan^R* and *torA::Kan^R* mutations from the BW25113 strain
363 (Keio library) (Baba *et al.*, 2006) were transduced into the MG1655 wild-type strain by P1
364 transduction standard procedure and checked by PCR. Strains were cured using pCP20
365 plasmid for successive transductions in order to obtain double and triple mutants. Primer
366 sequences used in this study are listed in Supplementary Table 2.

367 The *ΔhiuH-msrPQ* scarless mutant (strain LL996) was generated by lambda red
368 recombination adapted from Blank *et al.* (Blank *et al.*, 2011). Briefly, the DNA fragment was
369 amplified by PCR using primers 5-yedX-scarless and 3-wanner-msrQ (see Table 2) with
370 plasmid pWRG100 as a template. The resulting PCR product was electroporated into
371 BW25113 cells carrying pKD46 plasmid. After 1 h of expression, the cells were plated on
372 LB containing 25 µg/mL chloramphenicol. Selected colonies contained a *ΔhiuH-msrPQ sce-*
373 *I::cm* cassette which has been transduced in MG1655 cells harboring the pWRG99 plasmid.
374 The chloramphenicol cassette was then removed by counter selection using lambda red
375 recombination to insert a PCR product complementary to the flanking regions of the *sce-*
376 *I::cm* cassette on the chromosome. DsDNA is prepared from two complementary 96 mer
377 oligonucleotides generated with primers 5-scarlessyedXPQ and 3-scarlessyedXPQ. DsDNA
378 was then electroporated into MG1655 *ΔhiuH-msrPQ sce-I::cm* expressing lambda
379 recombinase from pWRG99, and after 1 hour expression serially diluted and plated on LB
380 containing 100 µg/mL ampicillin and 1 µg/mL anhydrotetracycline. pWRG99 also encoded
381 for the meganuclease I-Sce-I under the control of an anhydrotetracycline (aTc) inducible

promoter. The proper integration of the $\Delta hiuH$ -*msrPQ* was confirmed by diagnostic PCR and sequenced.

The *hiuH* 3stop mutant (strain LL1913) was performed using the same method as described above. Briefly, a $\Delta yedX$ *sce-I::cm* mutant was obtained by PCR using primers 5-yedX-scarless and 3-yedX-scarless with plasmid pWRG100 as a template. The chloramphenicol cassette was then removed by counter selection using lambda red recombination to insert a *hiuH* 3stop PCR product amplified by PCR using primers *yedX*_ATG_TAAx3_F and *yedX*_ATG_TAAx3_R.

The Δ FNR-putative-box::*cat*-*hiuH*-*lacZ* mutation was constructed in a one-step inactivation by using lambda red recombination. A *cat* gene cassette was inserted into the putative FNR box upstream *hiuH* gene. A DNA fragment containing the *cat* gene flanked with 5' and 3' regions bordering the *E. coli* putative FNR box contained in the *hiuH*-*lacZ* construction was amplified by PCR using pKD3 as a template and oligonucleotides 5del-wanner-*yedW* and 3del-wanner-*yedW*. PM1205 *hiuH*-*lacZ* strain, carrying the pKD46 plasmid, was transformed by electroporation with the amplified fragment and Cm^r colonies were selected.

Growth conditions

Cells were grown in Lysogeny Broth (LB) medium or in minimal medium (M9) supplied with 0.2% glucose; for solid medium, 15 g/L of agar was supplemented. In aerobic conditions, cells were grown in flask with a 1/10 volume of the flask capacity at 37 °C with shaking at 150 rpm. In anaerobic conditions, cells were grown in 2 ml tubes full to the brim, and for solid medium by using the GENbox Anaer generator (bioMérieux) in a dedicated chamber.

CASA induction assays

The *hiuH*-*lacZ* -containing strain (CH184) was grown over night at 37 °C under aerobic or anaerobic conditions in M9-glucose minimal medium or LB supplemented or not with CASA (0.4% w/v). Levels of β -galactosidase were measured as previously described (Miller, 1992). Growth under anaerobiosis was achieved by using 2 ml tubes full to the brim.

Colony morphology assays

Strains were spread onto LB-agar plates containing CASA (0.4 % w/v) or NaClO₃ (16 µM). The plates were incubated at 37 °C in anaerobic conditions for 48 hours. Growth under anaerobiosis was achieved by using the GENbox Anaer generator (bioMérieux) in a dedicated chamber. Ampicillin (50 µg/ml) and arabinose (0.02 %) were added to solid media when required. The plates are then scanned using CanonScan 4200F and colony morphology are observed using binocular magnifier Nikon SMZ800N. Colony shape homogeneity was verified, and a representative colony was shown in the figure.

Chlorate detection in CASA solution

CASA solution (0.4 %) was analysed by Flandres-analyses laboratory (59180 Cappelle la Grande) by liquid phase ion chromatography approach according to AFNOR NF EN ISO 10304-1 protocol.

Heterologous expression of the chlorite dismutase enzyme.

Genewiz from Azenta Life Sciences synthesized the pBAD24-clD-6His. The synthetic gene was inserted into the *NcoI/Sall* sites of pBAD24 vector. This chimeric gene encode for a protein composed by the 1-44 amino acids of the TAT signal sequence of MsrP of *E. coli* and the 40-282 amino acids of chlorite dismutase of *Dechloromonas aromatica*. A His6-tag at the *C terminus* of the polypeptide chain was introduced during the synthesis. 0.02 % arabinose was used to induce the expression of *clD* gene. Immunoblot analysis using an anti-His-tag antibody show the production of the non-mature form of Cld, suggesting that the enzyme is not efficiently translocated the periplasm. However, this Cld form is able to attenuate chlorite toxicity. MG1655 or CH184 strains harbouring pCld plasmid was grown aerobically at 37 °C in LB supplemented with ampicillin (50 µg/ml) and arabinose (0.02%). When cells reached an OD_{600nm} of 0.1, NaClO₂ (200 µM) was then added for 3.5 hours, followed by β-galactosidase assays or immunoblot analysis using an anti-His-tag antibody.

Immunoblot analysis of MsrP production.

To assess MsrP production by CASA (0.04%) or chlorate (1, 10, 100 and 200 µM), cells from overnight anaerobically cultures of the CH184, *ΔhprR* and *ΔhprS* strains were harvested. The pellets were suspended in Laemmli buffer (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 its A600 value. Samples were then heated for

10 min at 95 °C and loaded on SDS-PAGE. Immunoblot analysis was performed according to standard procedures: the primary antibody was a guinea pig anti-MsrP antibody (provided by Jean-François Collet Lab, De Duve Institute, Belgium). The secondary antibody was an anti-guinea pig IgG conjugated to horseradish peroxidase (HRP) (Promega). An ImageQuant LAS4000 camera (GE Healthcare Life Sciences) was used for image chemiluminescence.

In vivo repair of oxidized SurA by MsrP.

MG1655 and $\Delta msrP$ strains were grown in LB at 37 °C in aerobic conditions. At OD₆₀₀ of 0.1 cells were treated or not with NaClO₂ (200 µM). Cells were harvested after 2 hours of NaClO₂ addition. The pellets were suspended in Laemmli buffer. The amount of protein loaded onto the gel was standardized for each culture based on its A600 value. Samples were then heated for 10 min at 95 °C and loaded on SDS-PAGE for immunoblot analysis using anti-SurA antibody (provided by Jean-François Collet Lab, De Duve Institute, Belgium).

464 **Table 1. Strains used in this study**

465 This table contains the information regarding the strains used in this study, including
466 strain names, genotypes, description and source.

467

Strain	Genotype and description	Source
MG1655	WT	Laboratory collection
BW25113	<i>lacI^q rrnB_{T14} ΔlacZ_{WJ16} hsdR514 ΔaraBAD_{AH33} ΔrhaBAD_{LD78}</i>	(Datsenko and Wanner, 2000)
PM1205	MG1655 <i>mal::lacI^q ΔaraBAD araC⁺ lacI':P_{BAD}-cat-sacB-lacZ, mini λ tet^R</i>	(Mandin and Gottesman, 2009)
LL996	MG1655 <i>ΔhiuH-msrPQ</i>	This study
LL1913	MG1655 <i>hiuH</i> 3stop mutant	This study
BE107	MG1655 <i>ΔmsrP::Kan^r</i>	(Gennaris <i>et al.</i> , 2015)
CH380	MG1655 <i>ΔmsrP</i>	This study
BE206	MG1655 <i>ΔmsrQ::Kan^r</i>	This study
CH184	PM1205 <i>hiuH-lacZ</i>	(El Hajj <i>et al.</i> , 2022)
LL1103	PM1205 <i>hiuH-lacZ Δfnr::Kan^r</i>	This study
LL1699	PM1205 <i>(lacI-yedW::cat)-hiuH-lacZ</i> (deletion of the putative FNR box)	This study
LL216	MG1655 <i>Δfnr::Kan^r</i>	This study
TP1004	RK4353 <i>ΔmobAB::Kan^r</i>	Received from Tracy Palmer
LL1119	MG1655 <i>ΔmsrP Δfnr::Kan^r</i>	This study
LL1120	MG1655 <i>ΔmsrP ΔmobAB::Kan^r</i>	This study
LL1138	MG1655 <i>ΔnarG::Kan^r</i>	This study
LL1170	LL1138 <i>(lacI-yedW::cat)-hiuH-lacZ</i>	This study
LL1130	MG1655 <i>ΔnarZ::Kan^r</i>	This study
LL1168	CH184 <i>ΔnarZ::Kan^r</i>	This study
LL1129	MG1655 <i>ΔnapA::Kan^r</i>	This study
LL1166	CH184 <i>ΔnapA::Kan^r</i>	This study
LL1144	MG1655 <i>ΔnarG::Kan^r ΔnarZ ΔnapA</i>	This study
LL1171	LL1144 <i>(lacI-yedW::cat)-hiuH-lacZ</i>	This study

LL1169	MG1655 $\Delta narU \Delta narK::Kan^r$	This study
LL1231	LL1169 (<i>lacI-yedW::cat</i>)- <i>hiuH-lacZ</i>	This study
LL1187	MG1655 $\Delta napA \Delta narU \Delta narK::Kan^r$	This study
LL1232	LL1187 (<i>lacI-yedW::cat</i>)- <i>hiuH-lacZ</i>	This study
LL1146	MG1655 $\Delta msrP \Delta narZ::Kan^r$	This study
LL1147	MG1655 $\Delta msrP \Delta napA::Kan^r$	This study
LL1148	MG1655 $\Delta msrP \Delta narG::Kan^r$	This study
LL1145	MG1655 $\Delta torA::Kan^r$	This study
LL1153	MG1655 $\Delta msrP \Delta torA::Kan^r$	This study
BE152	MG1655 $\Delta msrA \Delta msrB$	(Henry <i>et al.</i> , 2021)
LL1296	MG1655 $\Delta msrA \Delta msrB \Delta gshA::Kan^r$	This study
JB56	IBPC5321 <i>msrA'</i> - <i>'lacZ</i>	(Bos <i>et al.</i> , 2013)
JB44	IBPC5321 <i>msrB'</i> - <i>'lacZ</i>	(Bos <i>et al.</i> , 2013)
BE255	MG1655 $\Delta hprR::Kan^r$	This study
BE254	MG1655 $\Delta hprS::Kan^r$	This study

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Table 2. Primers used in this study

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

Name	Sequence (5' to 3')
5-yedX-scarless	cgctatcctcaaaactcattcacatgacaaggatataaaccgccttacgccccgcctgc
3-wanner-msrQ	acatccggcagggataacgcgtactttgtcggcaatatgaagcatatgaatatcctcctta
5-scarlessyedXPQ	gcttttcgctatcctcaaaactcattcacatgacaaggatataaacttcatattgccgacaaagtacgc gttatccctgccggatgtggcgcgag
3-scarlessyedXPQ	ctcgcgccacatccggcagggataacgcgtactttgtcggcaatatgaagttatccttgcctgtga atgagttttgaggatagcgaanaagc
3- yedX-scarless	ccgtaacatctgattcttttaaaattgattcttttcatctagactatattaccctgtt
yedX_ATG_TAAx3_F	cgctatcctcaaaactcattcacatgacaaggatataaacatgtaataataatatttagtactctcggt gcaacg
yedX_ATG_TAAx3_R	ccctaacagtagcagatctgctttacatatttatgcattaactgccacgataggtgaataccc
5del-wanner-yedW	cgaagcggcatgcatttacgttgacaccatcgaatggcgcgtgtaggctggagctgcttc
3del-wanner-yedW	ggctgtttctataacatgatgattatggcatatttttcacatgaatatcctcctta

Table 3. Plasmids used in this study

This table contains the information regarding the plasmids used in this study, including plasmid names, genotypes, description and source.

Plasmid	Genotype and description	Source
pAG177 (p-empty)	pTAC-MAT-Tag-2-NdeI, Amp ^R	(Gennaris <i>et al.</i> , 2015)
pAG192 (pMsrP)	pTAC-MAT-Tag-2-NdeI MsrP, Amp ^R	(Gennaris <i>et al.</i> , 2015)
pAG195 (pMsrPQ)	pTAC-MAT-Tag-2-NdeI MsrP-MsrQ, Amp ^R	(Gennaris <i>et al.</i> , 2015)
pKD46	λ Red-expression under control of arabinose-inducible promoter, temperature-sensitive, Amp ^R	(Datsenko and Wanner, 2000)
pCP20	<i>FLP⁺</i> , <i>ΔΔcl857⁺</i> , <i>ΔΔP_R</i> Rep ^{ts} , Amp ^R , Cm ^R	(Cherepanov and Wackernagel, 1995)
pWRG99	pKD46 with I-SceI endonuclease under control of tetracycline-inducible promoter (<i>P_{tetA}</i>), temperature-sensitive, orientation 2 (5'→3': I-SceI- <i>tetR</i>), Amp ^R	(Blank <i>et al.</i> , 2011)
pWRG100	pKD3 with I-SceI recognition site, Cm ^R	(Blank <i>et al.</i> , 2011)
pBAD24	PBAD promoter, pACYC184-based, arabinose inducible, Amp ^R	Laboratory collection
<i>pBAD24-cld-6His</i>	pBAD24-NcoI/Sall containing <i>cld</i> gene from <i>Dechloromonas aromatic</i> , Amp ^R	Genewiz/Azenta Life Sciences

LEGENDS

Figure 1. A central role for MsrPQ under anaerobic conditions in the presence of CASA.

A) Evaluation of *hiuH-msrPQ* expression under aerobic and anaerobic conditions. The expression of the *hiuH-lacZ* fusion, in strain CH184, was used as a proxy for *msrPQ* expression. Strain CH184 was cultured overnight at 37°C in M9-Glucose or LB supplemented or not with 0.4 % CASA, under aerobic or anaerobic conditions, followed by β -galactosidase assays. Results are expressed as means $\pm$ SD (n = 3). Asterisks indicate a statistically significant difference between the absence and the presence of CASA, ***P<0.001 (Ordinary one-way ANOVA with Tukey's multiple comparison test) **B)** Immunoblot analysis using an anti-MsrP antibody showing the production of MsrP under anaerobic conditions in the presence of 0.4 % CASA. The image is representative of experiments carried out in triplicate. **C)** Evaluation of colony morphology on LB-CASA agar plates. Wild-type (MG1655), Δ *hiuH-msrPQ* (LL996), *hiuH*⁻ (LL1913), Δ *msrP* (CH380) and Δ *msrQ* (BE206) strains were plated onto LB-agar containing 0.4 % CASA. The plates were incubated at 37 °C under anaerobic conditions for 48 hours. The plates were then scanned (left panel) and observed using a binocular magnifier (right panel). The images are representative of experiments carried out (at least) in triplicate. **D)** Colony morphology of Δ *hiuH-msrPQ* (LL996), Δ *msrP* (CH380) and Δ *msrQ* (BE206) strains carrying either an empty vector, pMsrP or pMsrQ plasmids. The strains were plated onto LB-ampicillin-agar containing 0.4 % CASA. The plates were incubated at 37 °C under anaerobic conditions for 48 hours before being observed using a binocular magnifier. The images are representative of experiments carried out at least three times.

Figure 2. An indirect role for FNR in *msrPQ* expression.

A) Effect of *fnr* deletion on *hiuH-msrPQ* expression. Wild-type and Δ *fnr* strains carrying the *hiuH-lacZ* fusion and the wild-type strain carrying the p^{*}*hiuH-lacZ* fusion (deletion of the putative FNR box) were cultured overnight in LB supplemented with 0.4 % CASA under anaerobic conditions, followed by β -galactosidase assays. Results are expressed as means $\pm$ SD (n = 3). Asterisks indicate a statistically significant difference between mutant and the WT, ***P<0.001 (Ordinary one-way ANOVA with Tukey's multiple comparison test) **B)** Immunoblot analysis using an anti-MsrP antibody, showing the production of MsrP from wild-type, Δ *msrP* (CH380), Δ *fnr* (LL216) and Δ *mobAB* (TP1004) strains cultivated under

anaerobic conditions in the presence of 0.4 % CASA. The image is representative of experiments carried out in triplicate. **C)** Evaluation of colony morphology on LB-CASA agar plates. Wild-type (MG1655), $\Delta msrP$ (CH380), $\Delta msrP \Delta fnr$ (LL1119) and $\Delta msrP \Delta mobAB$ (LL1120) strains were plated onto LB-CASA (0.4 %) agar and incubated at 37 °C under anaerobic conditions for 48 hours before being scanned (upper part) and observed using a binocular magnifier (lower panel). The images are representative of experiments carried out at least three times.

Figure 3. Nitrate chemically suppresses the effect of CASA on MsrP.

A) Effect of the addition of alternative electron acceptors (nitrate, DMSO and TMAO) on *hiuH-msrPQ* expression. Wild-type strain carrying the *hiuH-lacZ* fusion was cultured overnight in LB supplemented with 0.4 % CASA in the presence of nitrate, DMSO or TMAO (40 mM) under anaerobic conditions, followed by β -galactosidase assays. Results are expressed as means $\pm$ SD (n = 3). Asterisks indicate a statistically significant difference between the absence and the presence of electron acceptors, **P<0.01 and ***P<0.001 (Ordinary one-way ANOVA with Tukey's multiple comparison test) **B)** Immunoblot analysis using an anti-MsrP antibody showing the production of MsrP from wild-type cultivated following the procedure described for panel (A). The image is representative of experiments carried out in triplicate. **C)** Evaluation of colony morphology on LB-CASA agar plates supplemented with alternative electron acceptors. The $\Delta msrP$ (CH380), strain was plated onto LB-CASA (0.4 %) agar containing nitrate, DMSO or TMAO (40 mM). Plates were incubated at 37 °C under anaerobic conditions for 48 hours before scanning. The images are representative of experiments carried out at least three times.

Figure 4. The three nitrate reductases are required for the regulation of *msrPQ* under anaerobic conditions with CASA.

A) Schematic representation of the respiratory nitrate reductase systems in *E. coli*. The cytoplasmic nitrate reductases NarA (in blue) and NarZ (in orange) composed of NarGHI and NarZYV subunits respectively, are able to reduce nitrate (NO_3^-) to nitrite (NO_2^-) in the cytoplasm. The periplasmic nitrate reductase NapA (in green) composed of NapABC subunits, reduce NO_3^- in the periplasmic compartment. The nitrate importers NarU (in yellow) and NarK (in purple) move nitrate into the cytoplasm. **B)** Effect of *nar* and *nap* deletions on *hiuH-msrPQ* expression. Wild-type, $\Delta narG$ (LL1170), $\Delta narZ$ (1168), $\Delta napA$

(LL1166), $\Delta narG \Delta narZ \Delta napA$ (LL1171), $\Delta narU \Delta narK$ (LL1231) and $\Delta narU \Delta narK \Delta napA$ (LL1232) strains carrying the *hiuH-lacZ* fusion were cultured overnight in LB supplemented with 0.4 % CASA under anaerobic conditions, followed by β -galactosidase assays. Results are expressed as means $\pm$ SD ($n = 3$). Asterisks indicate a statistically significant difference between mutants and the WT, $***P < 0.001$ and ns, not significant (Ordinary one-way ANOVA with Tukey's multiple comparison test). **C)** Immunoblot analysis using an anti-MsrP antibody showing the production of MsrP from strains indicated below the panel, cultivated following the same procedure as in panel (B). The image is representative of experiments carried out in triplicate. **D)** Evaluation of colony morphology on LB-CASA agar. Wild type (MG16555), $\Delta msrP$ (CH380), $\Delta msrP \Delta narG$ (LL1148), $\Delta msrP \Delta narZ$ (LL1146), $\Delta msrP \Delta napA$ (LL1147) and $\Delta msrP \Delta torA$ (LL1153) strains were plated onto LB-CASA (0.4 %) agar. The plates were incubated at 37 °C under anaerobic conditions for 48 hours before being scanned (upper panel) and observed using a binocular magnifier (lower panel). The images are representative of experiments carried out at least three times.

Figure 5. Chlorate (ClO_3^-) contaminated CASA.

A) Effect of chlorate on *hiuH-msrPQ* expression under anaerobic conditions. The expression of the *hiuH-lacZ* fusion in strain CH184 was used as a proxy for *msrPQ* expression. Strain CH184 was cultured overnight at 37 °C in LB supplemented or not with $NaClO_3$ (10 μM), under anaerobic conditions, followed by β -galactosidase assays. Results are expressed as means $\pm$ SD ($n = 3$). Asterisks indicate a statistically significant difference between the absence and the presence of $NaClO_3$, $***P < 0.001$ (Ordinary one-way ANOVA with Tukey's multiple comparison test). **B)** Immunoblot analysis using an anti-MsrP antibody showing the production of MsrP from the wild-type strain cultivated overnight at 37 °C in LB supplemented with $NaClO_3$ (1 to 100 μM) under anaerobic conditions. The image is representative of experiments carried out in triplicate. **C)** The wild type (MG16555) strain was plated onto LB-agarose supplemented or not with $NaClO_3$ (16 μM). The plates were incubated at 37 °C under anaerobic conditions for 48 hours before being observed using a binocular magnifier. The images are representative of experiments carried out at least three times.

Figure 6. Effect of the heterologous expression of chlorite dismutase.

A) Chlorite dismutase (Cld) from *Dechloromonas aromatica* was expressed using the pCld plasmid. The $\Delta msrP$ strain carrying the empty vector (pBad24) or pCld was spread onto LB-agar plates supplemented or not with NaClO_3 (16 μM), containing ampicillin and arabinose (0.02 %). The plates were incubated at 37 °C under anaerobic conditions for 48 hours before being observed using a binocular magnifier. The images are representative of experiments carried out at least three times. **B)** Effect of chlorite (ClO_2^-) on *hiuH-msrPQ* expression under aerobic conditions. The expression of the *hiuH-lacZ* fusion in strain CH184 was used as a proxy for *msrPQ* expression. Strain CH184 was cultured at 37°C in LB to $\text{OD}_{600} \approx 1.5$ under aerobic conditions, NaClO_2 (20 or 200 μM) was then added and β -galactosidase assays were conducted at different time points for each condition. **C)** Effect of the production of Cld on *hiuH-lacZ* fusion during chlorite stress. The CH184 strain carrying empty vector (pBad24) or pCld was cultured at 37°C in LB (supplemented with ampicillin and arabinose 0.02 %) to $\text{OD}_{600} \approx 0.1$ under aerobic conditions. NaClO_2 (200 μM) was then added for 3.5 hours, followed by β -galactosidase assays. Results are expressed as means $\pm$ SD (n = 3). Asterisks indicate a statistically significant difference between the absence and the presence of Cld, *** $P < 0.001$ (Ordinary one-way ANOVA with Tukey's multiple comparison test). **D)** Immunoblot analysis using an anti-MsrP antibody, showing the production of MsrP from the strains indicated above the panel, cultivated following the same procedure as described for panel (C).

Figure 7. Chlorate regulates *msrPQ* expression in an HprSR-dependent manner.

Immunoblot analysis using an anti-MsrP antibody showing the production of MsrP from wild-type (MG1655), $\Delta hprR$ (BE255) and $\Delta hprS$ (BE254) strains cultivated overnight at 37 °C in LB supplemented with NaClO_3 (20 μM) under anaerobic conditions. The image is representative of experiments carried out in triplicate.

Figure 8. MsrP repairs the chlorite-oxidized SurA.

Wild type and $\Delta msrP$ cells were grown in LB at 37°C under aerobic conditions. At an OD_{600} of 0.1 cells were treated or not with NaClO_2 (200 μM) for 2 hours and the SurA protein was analysed by immunoblotting. In the $\Delta msrP$ strain, chlorite treatment led to the appearance of the oxidized form of SurA (SurAox) which exhibits a mobility shift on SDS-PAGE resulting from Met-O formation. This blot is representative of three independent experiments.

Figure 9. Cytoplasmic methionine sulfoxide reductases are not induced by chlorate.

A) Effect of chlorate on *msrA* and *msrB* expression under anaerobic conditions. The strains JB56 and JB44, harbouring the *pmsrA-lacZ* and *pmsrB-lacZ* fusions respectively, were cultured overnight at 37°C in LB supplemented or not with NaClO₃ (10 µM), under anaerobic conditions, followed by β-galactosidase assays. The *hiuH-lacZ* fusion (CH184) was used as a control. Results are expressed as means +/- SD (n = 3). Asterisks indicate a statistically significant difference between the absence and the presence of NaClO₃, ***P<0.001 and ns, not significant (Ordinary one-way ANOVA with Tukey's multiple comparison test). **B)** Colony morphology of *ΔmsrP* (CH380) and *ΔmsrA ΔmsrB* (BE152) strains. The strains were plated onto LB-CASA (0.4 %) agar and incubated at 37 °C under anaerobic conditions for 48 hours. The plates were then scanned (upper panel) and observed using a binocular magnifier (lower panel). The images are representative of experiments carried out at least three times.

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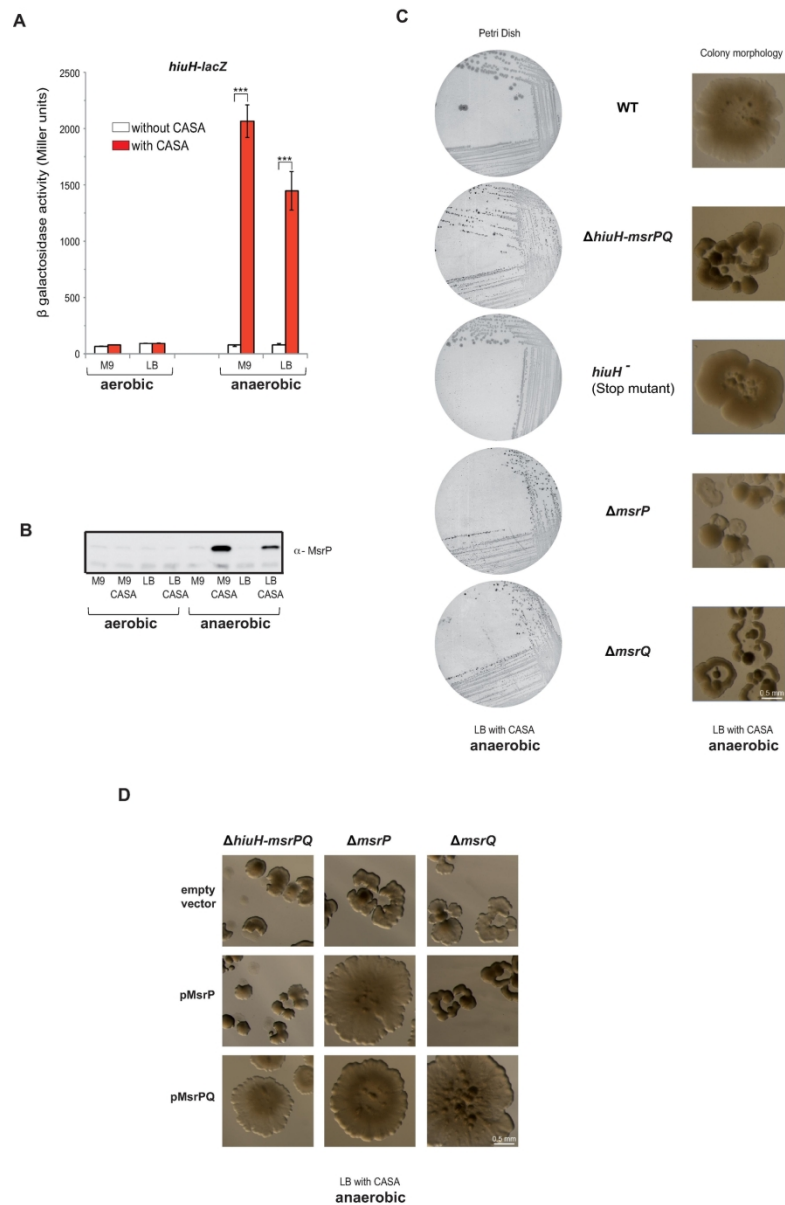


figure 1

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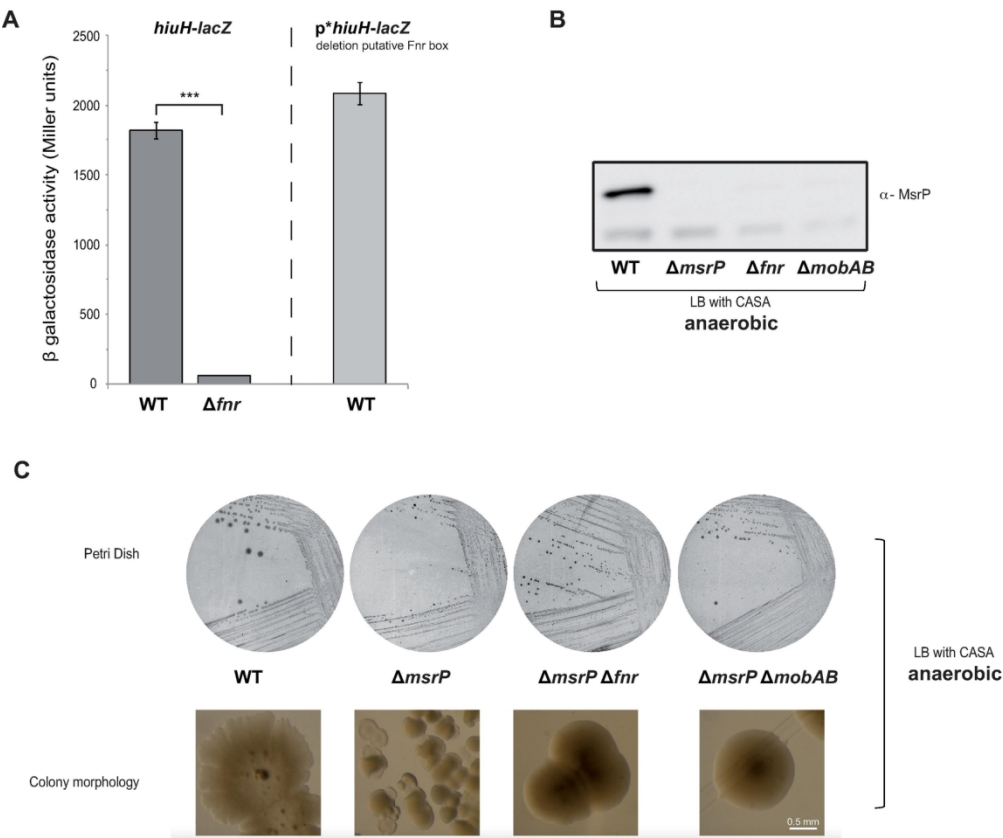


figure 2

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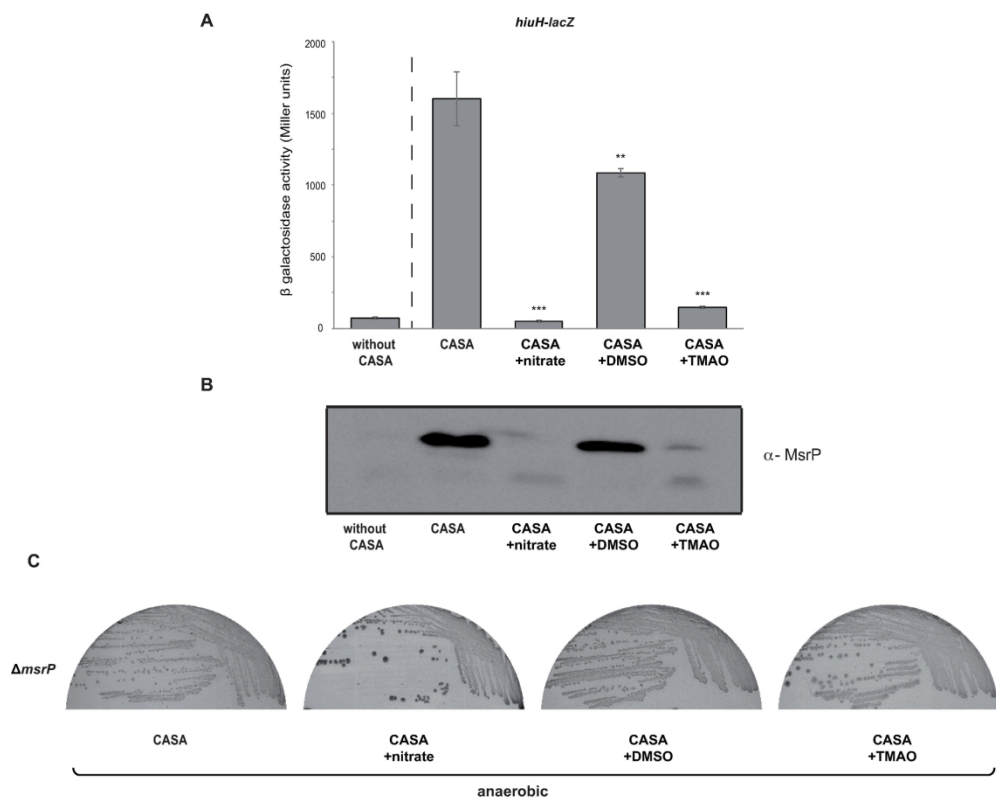


figure 3

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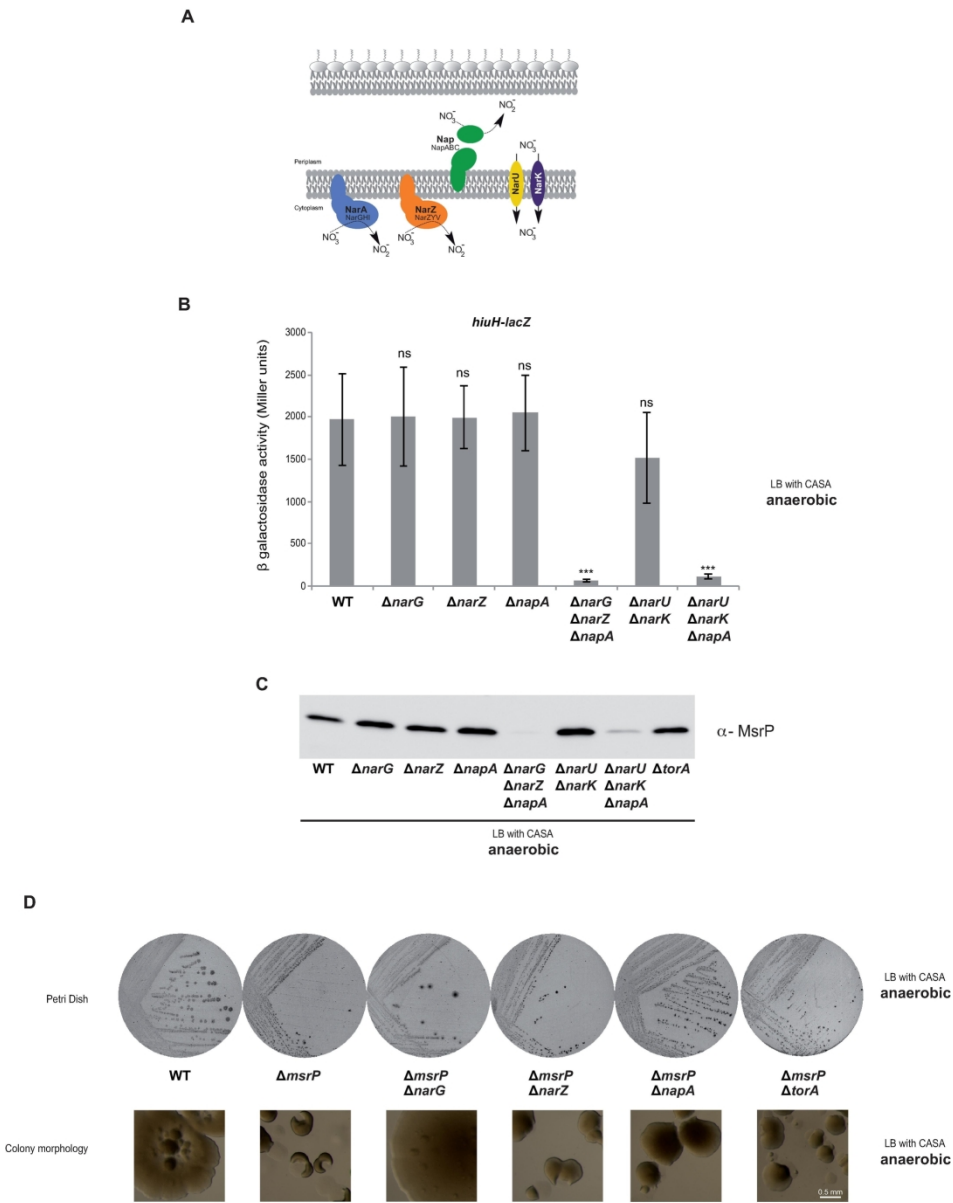


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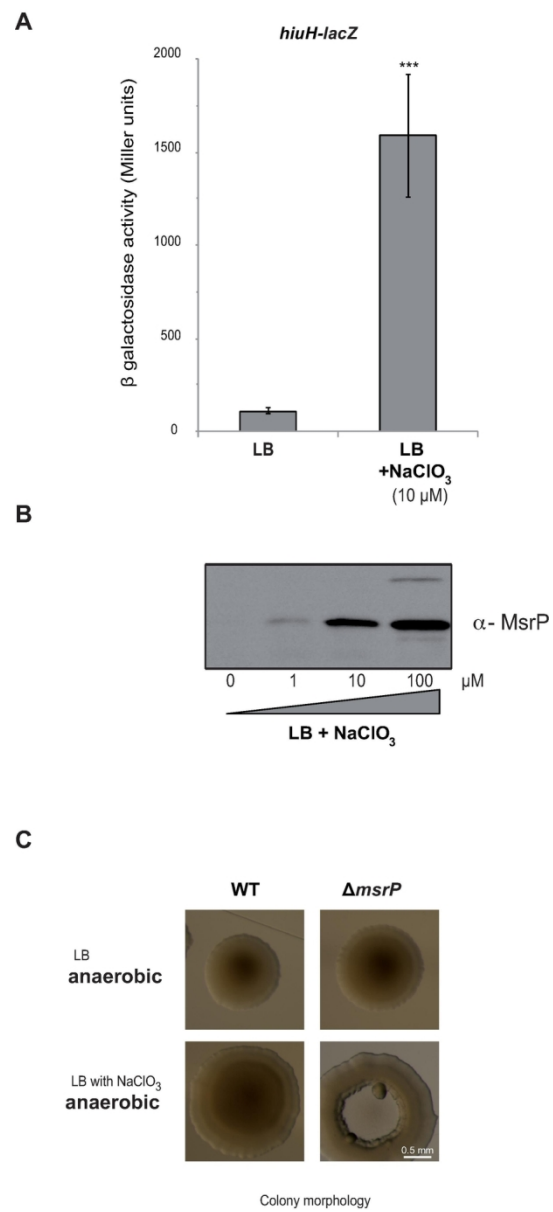


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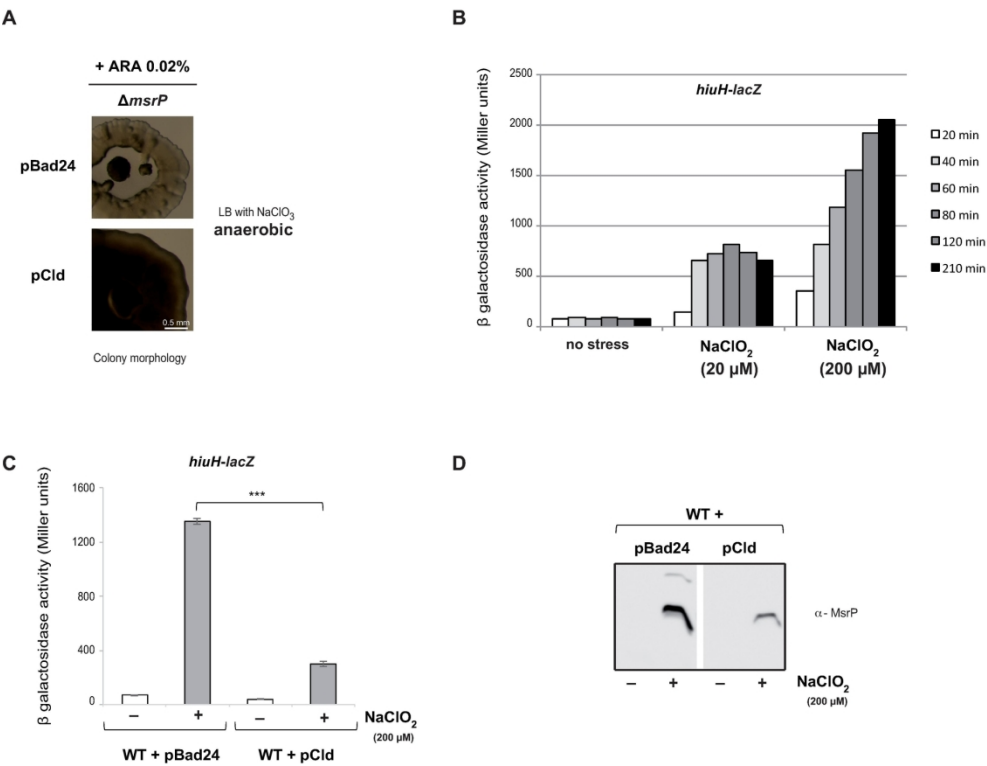


figure 6

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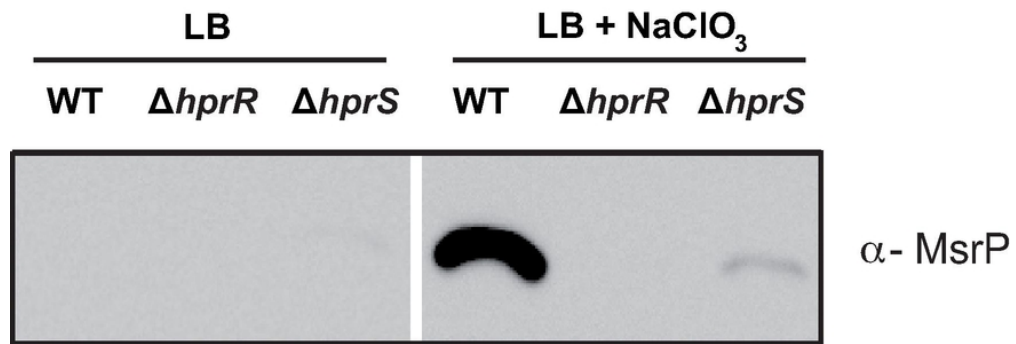


figure 7

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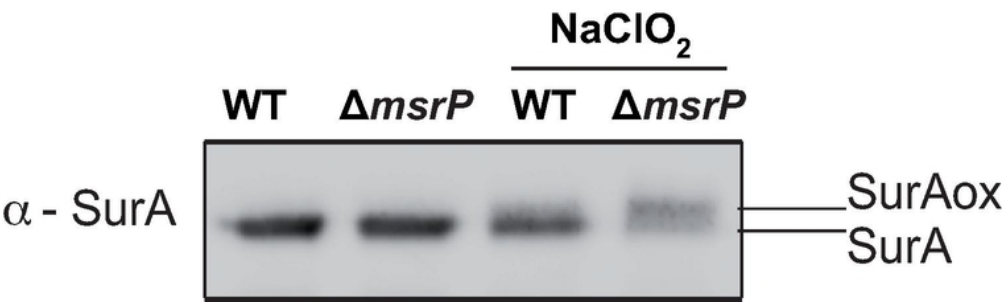


figure 8

64x18mm (300 x 300 DPI)

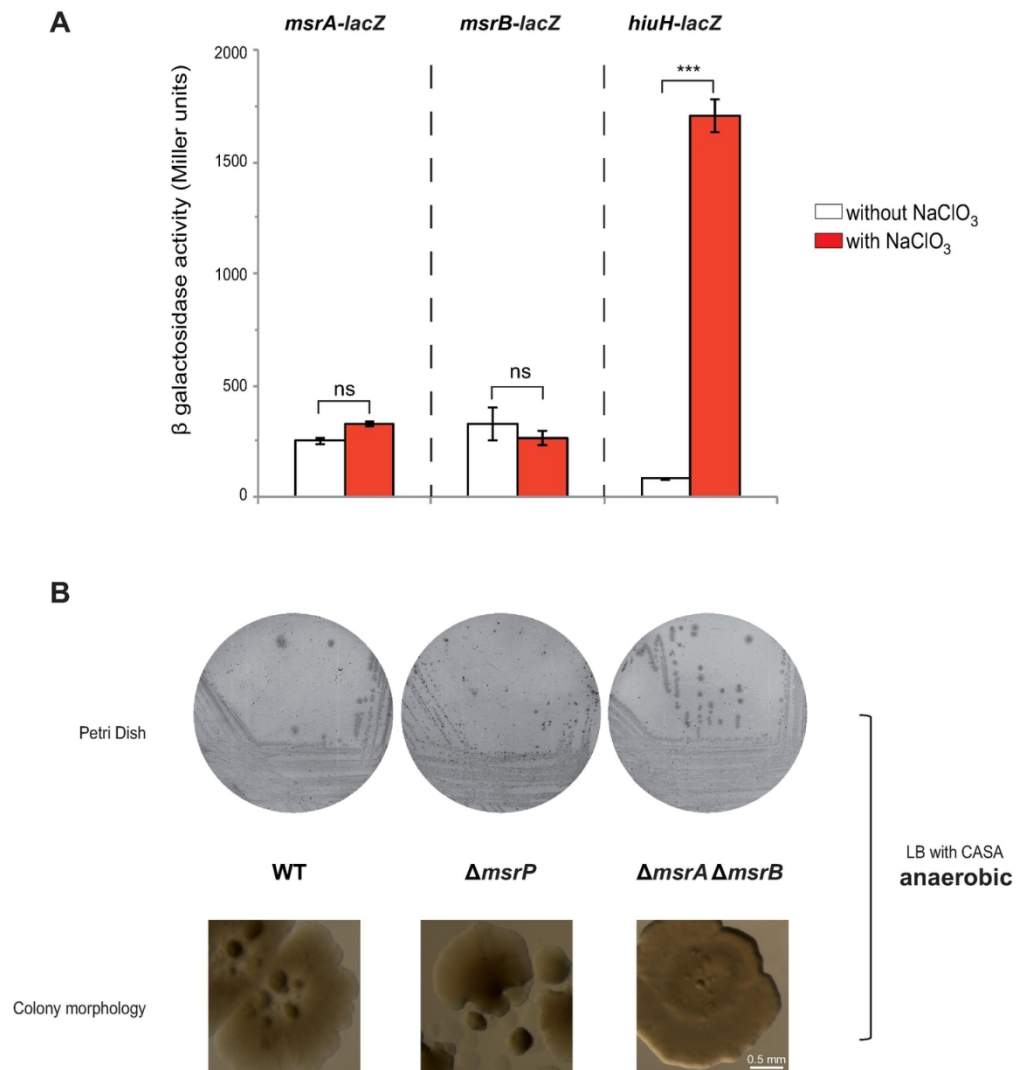
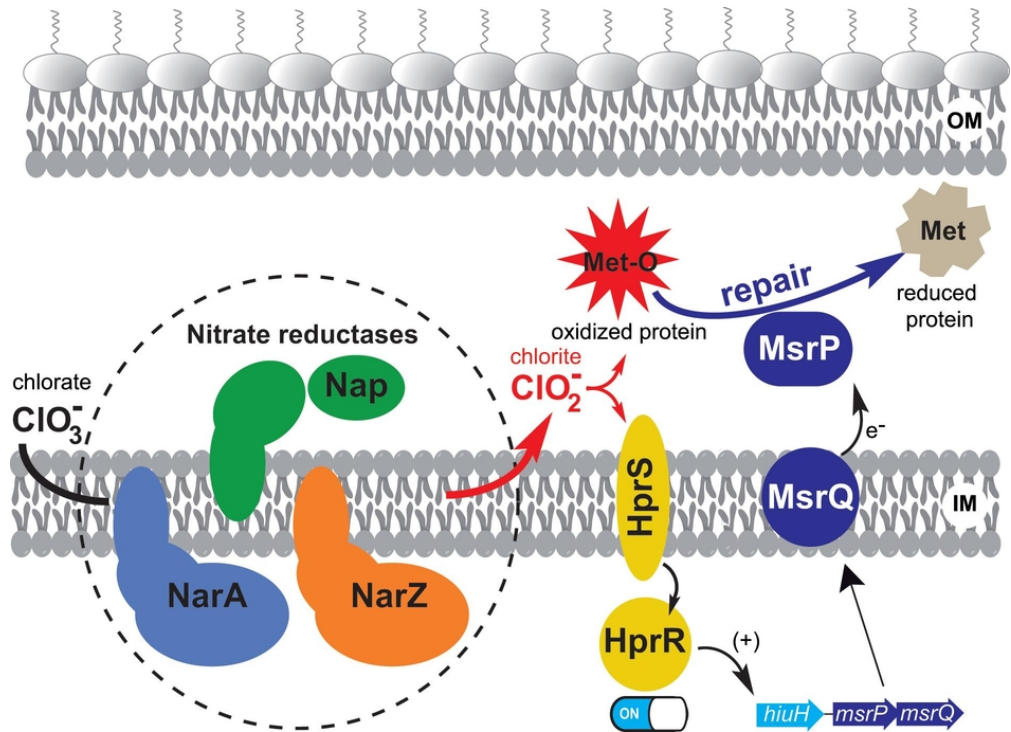


figure 9

121x129mm (300 x 300 DPI)



In anaerobic condition, reduction of chlorate (ClO_3^-) to the toxic oxidizing agent chlorite (ClO_2^-) by the three nitrate reductases (NarA, NarZ and Nap) led to methionine oxidation of periplasmic proteins. In response to this stress the *E. coli* HprSR two-component system was activated, leading to over-production of the periplasmic methionine sulfoxide reductase (MsrPQ) and the repair of the oxidized proteins.

80x57mm (300 x 300 DPI)

Summary graphical abstract :

In anaerobic condition, reduction of chlorate (ClO_3^-) to the toxic oxidizing agent chlorite (ClO_2^-) by the three nitrate reductases (NarA, NarZ and Nap) led to methionine oxidation of periplasmic proteins. In response to this stress the *E. coli* HprSR two-component system was activated, leading to over-production of the periplasmic methionine sulfoxide reductase (MsrPQ) and the repair of the oxidized proteins.

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