Transposon-Directed Insertion-Site Sequencing Reveals Glycolysis Gene gpmA as Part of the H2O2 Defense Mechanisms in Escherichia coli

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Abstract: Hydrogen peroxide (H2O2) is a common effector of defense mechanisms against pathogenic infections. However, bacterial factors involved in H2O2 tolerance remain unclear. Here we used transposon-directed insertion-site sequencing (TraDIS), a technique allowing the screening of the whole genome, to identify genes implicated in H2O2 tolerance in Escherichia coli. Our TraDIS analysis identified 10 mutants with fitness defect upon H2O2 exposure, among which previously H2O2-associated genes (oxyR, dps, dksA, rpoS, hfp and polA) and other genes with no known association with H2O2 tolerance in E. coli (corA, rbsR, nhaA and gpmA). This is the first description of the impact of gpmA, a gene involved in glycolysis, on the susceptibility of E. coli to H2O2. Indeed, confirmatory experiments showed that the deletion of gpmA led to a specific hypersensitivity to H2O2 comparable to the deletion of the major H2O2 scavenger gene katG. This hypersensitivity was not due to an alteration of catalase function and was independent of the carbon source or the presence of oxygen. Transcription of gpmA was upregulated under H2O2 exposure, highlighting its role under oxidative stress. In summary, our TraDIS approach identified gpmA as a member of the oxidative stress defense mechanism in E. coli.

Keywords: E. coli; H2O2; TraDIS; Tn-seq; phosphoglycerate mutase; gpmA

1. Introduction

Escherichia coli is a Gram-negative facultative anaerobic bacterium. It is a frequent member of the normal human microbiota but can also be a pathogen causing food poisoning, urinary tract infection and even septic shock [1]. The burden of diarrheal infections by pathogenic strains of E. coli is immense; in 79 low-income countries alone, more than 200 million episodes of childhood diarrhea due to E. coli and Shigella occur each year [2]. In high-income countries, E. coli is the primary cause of blood stream infections, accounting for 27% of the documented bacteremia episodes [3]. The emergence of antibiotic resistance in Gram-negative bacteria is also concerning and a recent study of 203 countries identified E. coli as the leading pathogen for deaths associated with antimicrobial resistance in 2019 [4].

Reactive oxygen species (ROS), and more specifically hydrogen peroxide (H2O2), have a strong impact on bacterial pathogenesis. Millimolar H2O2 can be produced by certain strains of Lactobacillus of the human normal microbiota [5]. H2O2 production prevents the colonization by pathogens of the urinary tract [6]. Similarly, H2O2 is produced by phagocytes during the oxidative burst, a necessary step for the killing of pathogens [7]. The effect of H2O2 on bacteria has been partially studied, but a complete picture of how
H$_2$O$_2$ affects bacteria and the bacterial response has not been elucidated for any bacterial species. Previous studies on H$_2$O$_2$ tolerance, using DNA microarrays and RNA-seq, identified genes regulated under H$_2$O$_2$ exposure [8–10] in E. coli. These studies permitted a better understanding of the regulation of numerous genes and pathways affected by H$_2$O$_2$ exposure. In particular, OxyR, a specific H$_2$O$_2$-responsive transcription factor, and SoxR which senses oxidative stress and nitric oxide, have been identified as playing an important role in resistance to H$_2$O$_2$ [9,11]. OxyR senses hydrogen peroxide through the oxidation of its cysteine residues, which orchestrate a conformational change allowing it to regulate the expression of 38 genes [9,12]. The iron–sulfur cluster of SoxR is oxidized by redox cycling compounds or superoxide, leading to the activation of the transcription factor which regulates the expression of 11 genes, which includes SoxS, another transcription factor that further regulates 34 genes [9,13]. However, transcriptional analyses do not identify genes required for survival in oxidative conditions. Diverse mutagenesis techniques were used to identify the genes involved in E. coli survival under H$_2$O$_2$ exposure, but only a limited number of genes were identified each time [14–16].

The combination of transposon mutagenesis and high-throughput sequencing is a powerful technique that allows interrogation of the whole genome and represents a new standard for global functional genomic studies [17]. Here, we performed transposon-directed insertion-site sequencing (TraDIS) [18] to identify genes implicated in tolerance to exogenous H$_2$O$_2$ exposure. A similar approach was used on Salmonella Typhimurium to identify genes implicated in H$_2$O$_2$ tolerance, deepening the understanding of how the bacteria survive oxidative burst [19,20]. The results of our study highlighted the role of gpmA, which encodes a phosphoglycerate mutase, an enzyme of the glycolysis, under H$_2$O$_2$ exposure. This is the first study identifying gpmA as a factor of H$_2$O$_2$ tolerance in E. coli.

2. Materials and Methods
2.1. Bacterial Strains, Media and Growth Conditions

All bacterial strains and plasmid used in this study are documented in Table 1. E. coli strains were cultured at 37 °C in Luria–Bertani (LB) (Becton & Dickinson, Basel, Switzerland) broth or on Luria–Bertani Agar (Becton & Dickinson). H$_2$O$_2$ 35% w/w (Acros Organics, VWR Life Science, Nyon, Switzerland) was added at the indicated final concentration. LB was supplemented with 0.4% glucose, 0.4% glycerol, 0.4% sodium acetate, 0.4% sodium citrate, 50 mM sodium nitrate (Sigma-Aldrich, St. Louis, MI, USA, Merck and Cie, Schaffhausen, Switzerland) where indicated. Minimal medium M9 plates, constituted by M9 salts (VWR Life Science), 0.1 mM CaCl$_2$ (Sigma-Aldrich), 0.2 mM MgSO$_4$ (Sigma-Aldrich), 1.5% (w/v) agar (Carl Roth, Arlesheim, Switzerland), were used when indicated. Antibiotics were used when indicated at the following concentrations: ampicillin 100 µg/mL (10044, Sigma-Aldrich), kanamycin 50 µg/mL (PanReac AppliChem, VWR, Switzerland). For anaerobic assays, bacteria were grown in deoxygenated LB with the corresponding antibiotic and every step was performed under anaerobic condition (Coy Laboratory Products, Labgene scientific SA, Châtel-Saint-Denis, Switzerland). Overnight cultures and agar plates were grown overnight at 40 °C in anaerobic chamber.

Table 1. Bacterial strains and plasmid used in this study.

| Name     | Genotype                      | Source or Reference |
|----------|-------------------------------|---------------------|
| BW25113  | F-, Δ(arD-arB)567, ΔlacZ4787(::rrnB-3), rph-1, Δ(rhaD-rhaB)568, hsdR514 | CGSC 1 [21]         |
| MG1655   | F-, Δ(arD-arB)567, ΔlacZ4787(::rrnB-3), rph-1, Δ(rhaD-rhaB)568, hsdR514 | CGSC 1               |
| BEFB02   | MG1655, ΔoxyR::Cm'             | [22]                |
| JW3914   | BW25113, ΔkatG::kan            | [21]                |
| ΔkatG    | MG1655, ΔkatG::kan             | This study          |
| JW0738   | BW25113, ΔgpmA::kan            | [21]                |
The table continues from before:

Table 1. Cont.

| Name             | Genotype            | Source or Reference |
|------------------|---------------------|---------------------|
| \(\Delta gpmA\)  | MG1655, \(\Delta gpmA::\text{kan}\) | This study          |
| JW4130           | BW25113, \(\Delta hfq::\text{kan}\) | [21]                |
| \(\Delta hfq\)   | MG1655, \(\Delta hfq::\text{kan}\) | This study          |
| JW0797           | BW25113, \(\Delta dps::\text{kan}\) | [21]                |
| \(\Delta hfq\)   | MG1655, \(\Delta dps::\text{kan}\) | This study          |
| JW3789           | BW25113, \(\Delta corA::\text{kan}\) | [21]                |
| \(\Delta hfq\)   | MG1655, \(\Delta corA::\text{kan}\) | This study          |
| JW5437           | BW25113, \(\Delta rpoS::\text{kan}\) | [21]                |
| \(\Delta rpoS\)  | MG1655, \(\Delta rpoS::\text{kan}\) | This study          |
| JW3732           | BW25113, \(\Delta rbsR::\text{kan}\) | [21]                |
| \(\Delta rbsR\)  | MG1655, \(\Delta rbsR::\text{kan}\) | This study          |
| JW0141           | BW25113, \(\Delta dksA::\text{kan}\) | [21]                |
| \(\Delta dksA\)  | MG1655, \(\Delta dksA::\text{kan}\) | This study          |
| JW0018           | BW25113, \(\Delta nhaA::\text{kan}\) | [21]                |
| \(\Delta nhaA\)  | MG1655, \(\Delta nhaA::\text{kan}\) | This study          |
| JW3587           | BW25113, \(\Delta gpmM::\text{kan}\) | [21]                |
| \(\Delta gpmM\)  | MG1655, \(\Delta gpmM::\text{kan}\) | This study          |
| pWSK29           | AmpR                | [23]                |

1 E. coli Genetic Stock Center.

The TraDIS screen was performed using a library of transposon mutants previously generated in *E. coli* strain BW25113 [18]. The *E. coli* strain MG1655 is referred in this paper as the wild-type (WT). The strain BEFB02 with oxyR deletion was a kind gift from B. Ezraty. Other gene deletions were obtained from the Keio collection [21] and transduced in a MG1655 background by P1 transduction.

2.2. TraDIS

The TraDIS library was thawed and diluted in 50 mL of LB broth to reach an OD\(_{595}\) of 0.02 (approximately \(8 \times 10^8\) CFU). H\(_2\)O\(_2\) was added to H\(_2\)O\(_2\)-treated samples to reach a concentration of 2.5 mM whereas pure medium was added in untreated controls. The experiment was performed in duplicates. Bacteria were grown at 37 °C in aerating conditions (250 mL flask, shaking 250 rpm) until an OD\(_{595}\) = 1.

Bacteria were collected and the DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer’s instructions. Samples were prepared for sequencing as described previously [18]. Briefly, genomic DNA was fragmented by ultrasonication, fragments were end-repaired using the NEBNext Ultra I kit (New England Biolabs, Notting Hill, Australia) and transposon fragments enriched by PCR using primers specific for the transposon and adapter. Samples were quantified by qPCR using the NEBNext Library Quant Kit for Illumina kit (New England Biolabs) according to the manufacturer’s instructions and sequenced using an Illumina MiSeq with 150-cycle v3 cartridges.

The TraDIS data were analyzed using Bio::TraDIS pipeline [24] with the following parameters: 50 reads per gene as minimal threshold and 5% trim at each side of gene to avoid the consideration of meaningless transposon insertions that can occur within gene extremities. Sequencing reads were mapped to the *E. coli* BW25113 reference genome (accession: CP009273.1) downloaded from NCBI. Sequencing reads are available at the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB56340 (https://www.ebi.ac.uk/ena/browser/view/PRJEB56340, accessed on 13 October 2022). Processed data are available for viewing at our online browser: https://tradis-vault.qfab.org/.
2.3. P1 Transduction

Strains from the Keio library were grown with 50 mg/mL kanamycin. The deletions of genes of interest from the corresponding Keio library mutant were transduced in *E. coli* MG1655 using phage P1 as previously described [25]. P1 phage was a kind donation from G. Panis (University of Geneva). The deleted mutants were verified using PCR with appropriate gene-specific primers (Supplementary Table S1).

2.4. *H*₂*O*₂ Susceptibility Assessed by Disk Diffusion Assay

To assess the susceptibility to *H*₂*O*₂ and other oxidants, we used disk diffusion assay as previously described [10]. Briefly, an overnight culture of bacteria was diluted in LB to McFarland 0.5 using a Densimat (bioMérieux, Marcy-l’Ètoile, France) and LB agar plates were inoculated using a sterile cotton swab. Sterile cellulose disks (5 mm diameter) were placed on the plate and 10 µL of 1 M *H*₂*O*₂ diluted in sterile water was added to the center of the disk. Other oxidants were used at the following concentrations: methylhydroquinone (Sigma-Aldrich) MHQ 0.5 M in water; methyl viologen dichloride hydrate, also called paraquat, (Sigma-Aldrich) PQ 1 M in water; diamide (Sigma-Aldrich) DI 0.2 M in water; menadione (Sigma-Aldrich) K3 360 mM in DMSO; cumene hydroperoxide (Sigma-Aldrich) CHP 0.25 M in DMSO; sodium hypochlorite (Sigma-Aldrich) NaOCl 5%; ciprofloxacin (Sigma-Aldrich) CIP 0.5 µg/µL in water; ampicillin AMP 1 µg/µL in water.

Plates were incubated at 37 °C for 18 h and the diameter of inhibition was measured in mm. The area of inhibition was calculated as: \[\text{area of inhibition} = \frac{\text{diameter of inhibition}^2}{4} \times \pi\]. To compare the effect of different oxidants, data were normalized as following: \[\frac{\text{area of inhibition of the interested mutant}}{\text{area of inhibition of the WT}}\] × 100.

2.5. *H*₂*O*₂ Survival Assay

For survival assay, the susceptibility of *E. coli* to *H*₂*O*₂ was tested in liquid medium. Briefly, overnight cultures were diluted to 2 × 10⁷ CFU/mL in 10 mL LB. 1 mL of *H*₂*O*₂ diluted in LB was added to the bacterial suspension to reach a final concentration of 2.5 mM. The corresponding control received 1 mL LB without *H*₂*O*₂. Bacteria were grown at 37 °C, 180 rpm. At indicated time points, 20 µL of each sample were serially diluted in LB by 10-fold dilutions. 10 µL of each dilution were spotted on LB agar supplemented with 100 U/mL of bovine liver catalase (Sigma-Aldrich). Plates were incubated overnight at 37 °C. Percent survival was calculated as: \[\frac{\text{CFU from H}_2\text{O}_2-treated sample}}{\text{CFU from untreated sample}}\] × 100.

2.6. Expression Levels Assessed by qRT-PCR

Overnight cultures were diluted in 10 mL of LB to OD₅₉₅ 0.02. These fresh cultures were grown at 37 °C, 180 rpm for 2 h to reach exponential phase. Bacterial suspension was divided in 2 mL samples, and 200 µL of *H*₂*O*₂ diluted in LB was added to reach the final concentrations indicated in the figures. The same volume of LB was added in the corresponding control conditions. Samples were incubated at 37 °C for 10 min. Subsequently, 1 mL was stabilized with 2 mL RNAprotect Bacteria Reagent (Qiagen, Hombrechtikon, Switzerland). RNA was purified using RNeasy Plus Mini Kit (Qiagen) according to the manufacturer instructions with on-column DNA digestion by RNase-Free DNase Set (Qiagen).

Quantitative PCR (qRT-PCR) was performed on RNA samples as previously described [10]. Briefly, the cDNA was produced by reverse-transcribing 500 ng of total RNA using a mix of random hexamers and oligo d(T) primers and Primescript reverse transcriptase enzyme (Takara Bio, Saint Germain-en-Laye, France). The efficiency of each pair of primers was tested with four serial dilutions of cDNA. Oligonucleotides are indicated in Table 2. PCR reactions (10 µL volume) contained 1:20 diluted cDNA, 2 × Power SYBR Green Master Mix (Thermo Fisher, Fisher Scientific AG, Reinach, Switzerland), and 300 nM of forward and reverse primers. PCRs were performed on a SDS 7900 HT instrument (Thermo Fisher) with the following parameters: 50 °C for 2 min, 95 °C for 10 min, and
45 cycles of 95 °C for 15 s, 60 °C for 1 min. Each reaction was performed in three replicates on 384-well plate. Raw Ct values obtained with SDS 2.2 (Thermo Fisher) were imported into Excel and normalization factors were calculated using the GeNorm method as described by Vandesompele et al. [26]. The absence of residual genomic DNA in RNA samples was verified by performing PCR reactions without RTase with the primer pair gyrB_N. Significance was assessed by one-way ANOVA with ad hoc Tukey’s multiple comparisons test.

Table 2. Primers used in this study.

| Name          | Sequence                       | Gene Accession ID | Efficiency (RT-qPCR Primers) | Reference |
|---------------|--------------------------------|-------------------|------------------------------|-----------|
| RT-qPCR primers                         |                                |                  |                              |           |
| gyrB_N_qPCR_F   | GTCCTGAAAGGGCTGGATG            | EG10424           | 1.89 (89.37%)                | [27]      |
| gyrB_N_qPCR_R   | CGAATACCATGTGGTG-CAGA          |                   |                              |           |
| gyrB_V_qPCR_F   | GAAATTCTCTCTCCCAGACCA          | EG10424           | 1.83 (82.56%)                | [27]      |
| gyrB_V_qPCR_R   | GCAGTTCGTTCATCCTGCTGT         |                   |                              |           |
| katG_qPCR_F     | GGGCCGCACCTGTTTATCCTC         | EG10511           | 1.92 (92.09%)                | [10]      |
| katG_qPCR_R     | ATCCGATCCGGTTCCCCAGA          |                   |                              |           |
| gpmA_qPCR_F     | AGCCATGCCTGATCCAGCTTC        | EG11699           | 2.00 (100.45%)               | This study|
| gpmA_qPCR_R     | TTTACCCGTTGTTACGACG           |                   |                              |           |
| hfg_qPCR_F      | CTACTGTGTGGCCGCTTCGC        | EG10438           | 2.01 (101.14%)               | This study|
| hfg_qPCR_R      | TCGGTTTCTCGCTGCTCTG          |                   |                              |           |
| ahpC_qPCR_F     | TGCCACCTCCTGTGTGGACC         | EG11384           | 2.00 (100.23%)               | This study|
| ahpC_qPCR_R     | CGGAGCCAGACTGGTTTCAC         |                   |                              |           |
| katE_qPCR_F     | TCCGAATACGAACTGGGCT          | EG10509           | 2.08 (108.44%)               | This study|
| katE_qPCR_R     | ATTTTGCCGACACGCTGTAAC       |                   |                              |           |
| Cloning primers for gpmA (EG11699)       |                                |                  |                              |           |
| pWSK_gpmA_KpnI_R | GGGGTACCCCGACGTTAATGCTGTTACCTC |                     | This study                   |           |
| pWSK_EcoRI_gpmA_F   | GGAATTCCTACACAGCAGAACACCCGAC |                     | This study                   |           |
| gpmA_His11Ala_F   | CTGGTTCGTTGCAGGCGGAAATGTCAG  |                     | This study                   |           |
| gpmA_His11Ala_R   | CTGACTTTTGCCCGACGAAACCAGAC  |                     | This study                   |           |

2.7. H$_2$O$_2$ Degradation Measurements by Amplex Red

Overnight cultures were diluted in LB to McFarland 1.0 using a Densimat (bioMérieux) and further diluted 10 fold in fresh LB. 10 mL were grown in a Falcon 50 at 37 °C for 2 h to reach exponential phase of growth. Pellets were washed with DPBS (Gibco Thermo Fisher) and resuspended to reach OD$_{595}$ = 0.1 in DPBS. 1 mL of H$_2$O$_2$ diluted in sterile water was added to 10 mL of bacterial suspension for a final concentration of 1 mM of H$_2$O$_2$. At indicated time points, 10 µL were taken from each sample and diluted 1:200 in DPBS; 100 µL of each sample were transferred into a 96-well black plate with clear bottom (Corning). Amplex Red (Thermo Fisher) was used to detect H$_2$O$_2$ according to manufacturer’s instructions. Briefly, 100 µL of Amplex Red mix was added to each well for a final concentration of 27.5 µM Amplex Red and 0.1 U/mL horseradish peroxidase (Sigma-Aldrich). The plate was incubated for 10 min at 37 °C and the fluorescence (excitation 535 nm, detection 595 nm) was read in a Spectramax Paradigm (Molecular Devices, Wokingham, UK). A H$_2$O$_2$ calibration curve was generated by 1:2 serial dilutions of H$_2$O$_2$ in DPBS (from 0.11 mM to 1.07 × 10$^{-4}$ mM) and used to calculate the H$_2$O$_2$ concentration of the samples by linear regression.
2.8. Complementation of gpmA

The *E. coli* MG1655 *gpmA* gene with its native promoter was amplified from genomic DNA using KOD DNA polymerase (Toyobo) and the primers in Table 2 (gene ID Ecocyc database: EG11699). The single amino acid replacement of the 11th histidine by an alanine (*gpmA* His11Ala) was obtained by overlap PCR using primers described in Table 2. The pWSK29 plasmid [23] was a kind gift from M. Roch (Geneva University). The plasmid and the PCR products were digested with the restriction enzymes *EcoRI* and *KpnI* (Thermo Fisher) and were gel-purified using QIAquick gel cleanup kit (Qiagen). T4 ligase (New England Biolabs) was used for the ligations and the ligation products (pWSK29 with either *gpmA* or *gpmA* His11Ala) were transformed in TOPO10 Chemically Competent *E. coli* (C404010, Thermo Fisher). The coding region of the two cloned plasmids was verified by Sanger sequencing. Plasmids were electroporated in either WT or ΔgpmA strains.

The complemented ΔgpmA strains (pWSK29 with either *gpmA* or *gpmA* His11Ala) were compared to the WT and the ΔgpmA strains harboring the pWSK29 (empty) plasmid on LB agar plates containing ampicillin 100 µg/mL.

2.9. Software

Artemis v.18.1.0 for Windows (Wellcome Sanger Institute, Cambridge, UK) was used to visualize the TraDIS data [28]. Graphpad Prism v.9.4.1 for Windows (GraphPad Software, San Diego, CA, USA) was used for data processing, graph plotting and statistical analysis. Inkscape v.1.1.1 for Windows was used for image editing (https://inkscape.org). Representation on metabolic map of previously acquired RNA-seq data was performed using the metabolism tool of Ecocyc [29,30]. All TraDIS data can be visualized at http://tradis-vault.qfab.org/.

3. Results

3.1. TraDIS Was Performed under Sublethal H₂O₂ Exposure

To determine the optimal dose of H₂O₂ to apply for the TraDIS experiment, we tested different concentrations of H₂O₂ on the *E.coli* strain BW25113, the strain used to generate the TraDIS library [18] (Figure 1A). The application of 2.5 mM H₂O₂ increased the lag phase by 70 min, whereas 5 mM or more resulted in complete absence of bacterial growth. The growth rate of bacteria during the exponential phase (between OD 0.2 and 1.6) was identical when treated with 2.5 mM compared to no treatment.

We performed the TraDIS experiment in similar conditions with 2.5 mM H₂O₂. The H₂O₂-treated condition reached OD = 1 approximatively 140 min after untreated controls. We used the genome browser Artemis to observe the insertion site of the transposons (Figure 1B). To analyze the comparative fitness of each gene under both conditions, we performed fitness analysis with the Bio::TraDIS pipeline.

In TraDIS and other Tn-seq techniques, the fitness of each gene deletion is assessed by sequencing. Mutants that are less fit in a given condition will be outcompeted and therefore less abundant, which is approximately measured by insertion frequency. To ensure the relevance of our data, we scrutinized the TraDIS data for an impact on oxyR. The transcription factor OxyR is a well-described H₂O₂ sensor that regulates *E. coli* antioxidant response and deletion of the gene has been shown to increase sensitivity against oxidative stress [31]. As expected, the frequency of insertions was significantly reduced after exposure to H₂O₂ indicating oxyR mutants are less fit than the wild type in the presence of H₂O₂ (Figure 1C). Using the values derived for oxyR as a threshold, we identified nine genes that displayed higher fold-change values suggesting of a role for each of these genes in H₂O₂ tolerance (Table 3). Several genes were already described in the oxidative stress response.
Figure 1. Transposon-directed insertion sequencing (TraDIS) screen of *E. coli* BW25113 under sublethal concentration of H$_2$O$_2$ (A) Bacterial growth of *E. coli* BW25113 over time with 2.5, 5 mM H$_2$O$_2$ and untreated control. The concentration of 2.5 mM was chosen for TraDIS experiment as it displayed a high reproducibility and a delay of approximately 70 min compared to control (mean ± SD, N = 3); (B) Visualization of the TraDIS data using Artemis where each sample is represented by a histogram depicting the localization (X-axis) and the frequency (Y-axis) of transposon insertion sites (in red are antisense insertions, in blue are same sense insertions). Representative examples of essential gene (*murI* in red box), non-essential genes (*btuB*, in green box) and genes with a reduced fitness in the H$_2$O$_2$ condition (*dps* in orange box), (N = 2) (C) Fitness analysis of the TraDIS data, H$_2$O$_2$-treated condition compared to control. Each dot represents a gene, X-axis represents the difference in number of insertions in the H$_2$O$_2$ condition compared to control, Y-axis represents the statistical significance. Nine genes (in pink) displayed a significant and more extreme change than the H$_2$O$_2$-sensor gene *oxyR*. 
Table 3. Genes underrepresented in the H$_2$O$_2$ condition of the TraDIS experiment. The gene function and the fold change compared to control are detailed.

| Gene Name | Function | Log$_2$ FC | q Value |
|-----------|----------|------------|---------|
| corA      | magnesium/nickel/cobalt transporter | $-2.37$ | $2.11 \times 10^{-43}$ |
| dksA      | transcriptional regulator of rRNA transcription, DnaK suppressor protein | $-2.11$ | $1.56 \times 10^{-11}$ |
| dps       | Fe-binding and storage protein; stress-inducible DNA-binding protein | $-3.61$ | $1.37 \times 10^{-42}$ |
| gpmA      | phosphoglyceromutase 1 | $-2.57$ | $4.52 \times 10^{-35}$ |
| hfq       | global sRNA chaperone; HF-I, host factor for RNA phage Q beta replication | $-2.28$ | $5.73 \times 10^{-14}$ |
| nhaA      | sodium-proton antiporter | $-2.50$ | $2.71 \times 10^{-27}$ |
| oxyR      | oxidative and nitrosative stress transcriptional regulator | $-1.88$ | $1.17 \times 10^{-70}$ |
| polA      | fused DNA polymerase 1 5′→3′ polymerase/3′→5′ exonuclease/5′→3′ exonuclease | $-3.25$ | $1.29 \times 10^{-58}$ |
| rboS      | transcriptional repressor of ribose metabolism | $-1.90$ | $3.27 \times 10^{-71}$ |
| rpoS      | RNA polymerase, sigma S (sigma 38) factor | $-2.80$ | $1.11 \times 10^{-32}$ |

No transposon insertion was significantly overrepresented in the H$_2$O$_2$ condition, suggesting that no gene deletion is protective against H$_2$O$_2$ in these conditions. This analysis considered only transposon insertions inside the coding regions of genes. Transposons disrupting promoters or altering the expression of genes such as polar effect were not considered by this analysis.

3.2. H$_2$O$_2$ Susceptibility of Single-Gene Deletion Identified by TraDIS

Single-deletion mutants of genes identified by the TraDIS experiments were tested against H$_2$O$_2$ to evaluate the sensitivity of each mutant. To ensure the absence of undesired mutations, cognate E. coli strain MG1655 mutants were created by P1 phage transduction of the relevant mutations from the Keio collection [21]. The susceptibility to H$_2$O$_2$ of the single-gene deletion mutants was assessed by disk diffusion assay. The mutant deleted for the catalase katG, known as the principal H$_2$O$_2$ scavenger at high concentration [32], was used as positive control. As expected, the deletion of oxyR led to a dramatic increase in the inhibition diameter generated by H$_2$O$_2$ (Figure 2). The deletion of gpmA increased the sensitivity to H$_2$O$_2$ to the same extent as the katG deletion. Similarly, loss of hfq also increased significantly the sensitivity to H$_2$O$_2$. Other genetic deletions did not significantly alter the H$_2$O$_2$ susceptibility in the disk diffusion assay.

Figure 2. Sensitivity to H$_2$O$_2$ of genes identified by TraDIS. The sensitivity to 10 μL of 1 M H$_2$O$_2$ of each single-gene deletion mutant identified by the TraDIS was assessed by disk diffusion assay. The coding regions of E. coli MG1655 (WT) were replaced by the kanamycin cassette of the corresponding mutant originated from the Keio collection using the phage P1 to ensure the absence of undesired mutation. Data were analyzed by one-way ANOVA with Tukey test for multiple comparison and *, **** correspond to $p < 0.05$ and 0.0001 respectively (mean ±/− SD, $N = 3$).
3.3. ΔgpmA Mutant Was More Sensitive to H$_2$O$_2$ but Not to Other Oxidants

Single-deletion mutants were tested against other oxidants by disk diffusion assay (Figure 3). The deletion of oxyR and hfq led to an increase of the inhibition area of a wide range of oxidants (Figure 3C,E). The deletion of gpmA led to a hypersensitivity to H$_2$O$_2$ but not to other oxidants or antibiotics (Figure 3B,E). This pattern was highly similar to the sensitivity of the ΔkatG mutant used as positive control (Figure 2E).

Figure 3. Sensitivity of the ΔoxyR, ΔgpmA and Δhfq mutants exposed to various oxidants. (A) WT, (B) ΔgpmA, (C) Δhfq. (D) Oxidants applied on each disk (CHP: cumene hydroperoxide, MHQ: methylhydroquinone, H$_2$O$_2$: hydrogen peroxide, CIP: ciprofloxacin, DI: diamide, AMP: ampicillin, K3: menadione, NaOCl: sodium hypochlorite, DMSO: dimethylsulfoxide). (E) Quantification of the area of inhibition normalized to WT for each oxidant. One-way ANOVA with Tukey multiple comparison was performed separately for each oxidant on the area of inhibition of the WT, ΔkatG and the 9 mutants identified by TraDIS (Figure S1). The significance of the difference with the WT is represented on the normalized data by stars (mean ± SD, N = 3). *, **, *** correspond to p < 0.05, 0.01, 0.001, and 0.0001, respectively.
The deletion mutants of the other genes identified by TraDIS were also tested against these oxidants (Supplementary Materials Figure S1). The ΔdksA mutant was more sensitive to methylhydroquinone, cumene peroxide, diamide and ciprofloxacin, and the ΔnhaA mutant was slightly more sensitive to diamide and ciprofloxacin. This suggests that these mutants, despite no increased sensitivity to \( \text{H}_2\text{O}_2 \) in these conditions, were more sensitive to other oxidative stresses. Other mutants did not display significant differences compared to WT.

3.4. *gpmA* Is Upregulated by \( \text{H}_2\text{O}_2 \) Exposure

In a previous study, we performed a RNA-seq analysis of *E. coli* BW25113 after a 10 min exposure to a sublethal concentration (2.5 mM) of \( \text{H}_2\text{O}_2 \) [10]. Among the ten genes identified by TraDIS, only two genes, *dps* and *gpmA*, were significantly dysregulated by \( \text{H}_2\text{O}_2 \) (Figure 4A). In these settings, *gpmA* was upregulated over fourfold. As *gpmA* is part of the glycolysis reaction in *E. coli*, we extracted the transcriptomic data for the glycolysis and the TCA cycle (Supplementary Materials Figure S2). Other enzymes from the glycolysis (pgi, pfkAB, fbaAB, pgk) were also upregulated, suggesting an increased activity of glycolysis following exposure to \( \text{H}_2\text{O}_2 \).

![Figure 4](image)

**Figure 4.** *gpmA* expression is upregulated following exposure to \( \text{H}_2\text{O}_2 \). (A) Differential expression of the 10 genes identified by TraDIS 10 min after exposition to 2.5 mM \( \text{H}_2\text{O}_2 \) compared to no treatment (mean +/- SD, \( N = 4 \)). Data originated from previously performed RNA-seq (deposited on ENA with the accession number: PRJEB51098) [10]. (B, C) Levels of expression of *katG* and *gpmA*, respectively, in the strain MG1655 under increasing concentration of \( \text{H}_2\text{O}_2 \), assessed by qRT-PCR, ** corresponds to \( p < 0.01 \). (SEM +/- SD, \( N = 3 \)).

We confirmed the impact of \( \text{H}_2\text{O}_2 \) on *gpmA* expression in the MG1655 strain used in this study by qRT-PCR. The *gpmA* RNA was upregulated following sublethal exposure of \( \text{H}_2\text{O}_2 \) in a dose-dependent manner (Figure 4C). Induction of *gpmA* expression was less impressive than the catalase *katG*, a known \( \text{H}_2\text{O}_2 \)-responsive gene (Figure 4B).
3.5. Catalase Activity Is Not Involved in the Increased Sensitivity of ΔgpmA to H₂O₂

As the ΔgpmA mutant displayed a similar sensitivity to oxidants compared to the ΔkatG mutant (Figure 3E), we measured catalase expression and activity in the presence of H₂O₂. The ΔgpmA mutant did not exhibit a growth defect compared to the WT in liquid LB (Supplementary Figure S3), so we first assessed the sensitivity of the gpmA mutant to H₂O₂ in liquid LB medium by counting surviving bacteria after H₂O₂ exposure (Figure 5A). Two hours after the addition of 2.5 mM H₂O₂, a 100-fold difference in the number of surviving bacteria in the gpmA and the oxyR mutant compared to the WT was observed (Figure 5B).

Figure 5. WT and gpmA displayed no difference in catalase expression and activity. (A) Survival of 2 × 10⁷ cells of WT (black), ΔgpmA (blue) and ΔoxyR (orange) over time after an exposure to 2.5 mM H₂O₂ in liquid LB (mean +/− SD, N = 3). (B) Representative image of the survival 2h after H₂O₂ treatment. Each spot represents 10 μL at the given dilution factor. (C) Expression levels of ahpC, katG and katE in WT (black) or ΔgpmA (blue) 10 min after the addition of 2.5 mM H₂O₂ or corresponding control (mean +/− SEM, N = 3). Data were analyzed by Welch T-test, and *, **, *** correspond to p < 0.05, 0.01, 0.001, respectively. (D) Degradation of 1 mM H₂O₂ over time by WT (black), ΔgpmA (blue) and ΔkatG (red) assessed by Amplex Red (mean +/− SD, N = 3).
We measured the expression levels of the three enzymes of *E. coli* that are known to degrade H$_2$O$_2$, the alkyl hydroperoxide reductase encoded by *ahpC*, the catalase/hydroperoxidase HPI encoded by *katG* and the catalase HPII encoded by *katE*. We compared the WT and the Δ*gpmA* strain, in presence or in absence of H$_2$O$_2$ (Figure 5C). There was no significant difference in the expression of the three genes between the WT and the Δ*gpmA* strain. Upregulation of *ahpC* and *katG* was observed after the addition of 2.5 mM of H$_2$O$_2$ in both strains. There was no significant difference in katE expression after H$_2$O$_2$ exposure, which was expected as it is not regulated by OxyR but by RpoS and upregulated during the stationary phase of bacterial growth [33].

To test if the catalase activity was affected by the deletion of *gpmA*, we measured the degradation of 1 mM of H$_2$O$_2$ of the WT and the Δ*gpmA* using the H$_2$O$_2$-sensitive probe Amplex Red. There was no difference in H$_2$O$_2$ degradation between the WT and the Δ*gpmA* strains. The ΔkatG strain, which is defective for the main H$_2$O$_2$ scavenger at high concentration, was unable to degrade H$_2$O$_2$. Altogether, this suggests that the higher sensitivity of the Δ*gpmA* to H$_2$O$_2$ is independent of catalase activity.

### 3.6. Other Carbon Sources Cannot Compensate the H$_2$O$_2$ Hypersensitivity of Δ*gpmA* Mutant

In LB medium, amino acids are the main source of carbon and there is virtually no glucose [34]. We wondered if the supplementation with metabolites entering the central metabolism at different levels could affect the H$_2$O$_2$ sensitivity of the Δ*gpmA* mutant. The addition of alternative carbon source did not significantly modify the H$_2$O$_2$ susceptibility of the WT or the Δ*gpmA* mutant (Figure 6A). We also tested H$_2$O$_2$ sensitivity in M9 minimal media with these metabolites as the only carbon source. There was no difference in the sensitivity of the WT in M9 + glucose compared to LB + glucose. The WT strain was slightly more sensitive in M9 + acetate compared to M9 + glucose. The Δ*gpmA* strain displayed a higher sensitivity in the M9 conditions compared to LB conditions. M9 plates with citrate as the sole source of carbon led to limited growth even after 48 h and were therefore not measurable. Addition of 0.5% pyruvate led to a complete disappearance of the zone of inhibition (data not shown) probably because pyruvate reacts with H$_2$O$_2$ to produce CO$_2$, acetate and water [35].

In *Salmonella Typhimurium*, the Δ*gpmA* mutant was more susceptible to H$_2$O$_2$ than the WT in aerobic conditions, but not in anaerobic conditions, and the addition of the electron acceptor nitrate restored the hypersusceptibility of Δ*gpmA* [20]. We tested the H$_2$O$_2$ susceptibility of *E. coli* WT and Δ*gpmA* in anaerobic conditions. Interestingly, it appeared that the WT was slightly more sensitive to H$_2$O$_2$ in anaerobic conditions than in aerobic conditions suggesting that the exposure to oxygen protect in part against H$_2$O$_2$ damage. However, the Δ*gpmA* mutant did not display any difference in H$_2$O$_2$ sensitivity between anaerobic and aerobic conditions and the difference between the Δ*gpmA* mutant and the WT was maintained in anaerobic conditions. As *E. coli* is also able to use other electron acceptors than oxygen for respiration, we tested the addition of sodium nitrate in anaerobic conditions, but this did not change the area of inhibition induced by H$_2$O$_2$ compared to the anaerobic condition without nitrate (data not shown).

Altogether, we explored a potential impact of factors affecting glycolysis following H$_2$O$_2$ exposure, but we did not observe significant changes in conditions of low oxygen or using different carbon sources.

### 3.7. The Function of *gpmA* Is Necessary for H$_2$O$_2$ Tolerance

The Δ*gpmA* was complemented by native *gpmA* gene including its natural promoter using the low copy plasmid pWSK29. The complemented strain displayed similar H$_2$O$_2$ susceptibility than the WT strain (Figure 7A). A mutation previously described as to be necessary for the function of *gpmA*, namely the substitution of the histidine 11 residue by an alanine [36], resulted in restauration of the hypersensitivity to H$_2$O$_2$. These data suggest that the function of *gpmA* is necessary to reach the WT levels of tolerance against H$_2$O$_2$. 

[33] Reference number

[34] Reference number

[35] Reference number

[36] Reference number
Figure 6. The difference in H$_2$O$_2$ tolerance between WT and ΔgpmA mutant is not affected by the addition of other carbon sources or the absence of oxygen. (A) Area of inhibition assessed by disk diffusion assay of WT and ΔgpmA strain on LB and M9 minimal medium complemented with diverse carbon source. GLU: glucose, GLY: glycerol, ACE: acetate, CIT: citrate (mean $\pm$ SD, N = 3). (B) Area of inhibition of the WT and ΔgpmA strain under aerobic and anaerobic conditions (mean $\pm$ SD, N = 3). Data in (A, B) were analyzed by one-way ANOVA with Tukey test for multiple comparison, and *, ***, **** correspond to $p$ < 0.05, 0.001, 0.0001 respectively.

E. coli possess a second phosphoglycerate mutase encoded by gpmM, which presents no sequence similarity with gpmA [37]. Contrary to gpmA, the expression level of gpmM was slightly downregulated after the addition of H$_2$O$_2$ in a previous RNA-seq dataset (Figure 7B). This suggests that following exposure to H$_2$O$_2$, gpmA represents the principal form of phosphoglycerate mutase. We tested the ΔgpmM mutant for H$_2$O$_2$ sensitivity. Contrary to gpmA, the deletion of gpmM did not increase the sensitivity to H$_2$O$_2$ (Figure 7C), suggesting a possible alternative function of gpmA in conditions of H$_2$O$_2$ exposure.
The function of gpmA, but not gpmM, is necessary to reach WT level of H₂O₂ tolerance. (A) Sensitivity to H₂O₂ assessed by disk diffusion assay for the WT with the empty plasmid, the ΔgpmA mutant with the empty plasmid, the ΔgpmA mutant with the plasmid encoding the native sequence of gpmA (compl) and the ΔgpmA mutant complemented with the plasmid encoding gpmA with the replacement of the histidine 11 by an alanine (His11Ala). All tests were performed in LB + ampicillin. Data were analyzed by one-way ANOVA with Tukey test for multiple comparison and **** correspond to p < 0.0001 (mean +/− SD, N = 3). (B) Differential expression of gpmA and gpmM 10 min after exposition to 2.5 mM H₂O₂ compared to no treatment (mean +/− SD, N = 4). Data from previously performed RNA-seq (deposited on ENA with the accession number: PRJEB51098) [10]. (C) Sensitivity to H₂O₂ assessed by disk diffusion assay of WT, ΔgpmA and ΔgpmM mutants. Data were analyzed by Welch t-test and *** correspond to p < 0.001 (mean +/− SD, N = 3).

4. Discussion

The production of H₂O₂ by phagocytes from the human immune system and by Lactobacilli species of the normal microbiota are essential for the prevention of colonization from various opportunistic pathogens. Although H₂O₂ effects on bacteria have been studied for years, the mechanisms by which H₂O₂ exerts its antimicrobial activity is still incompletely understood [14,16].

Our TraDIS analysis identified 10 mutants with fitness defect upon H₂O₂ exposure, implicating a role for these genes under H₂O₂-induced oxidative stress. Only three of the ten genes, oxyR, gpmA and hfg, showed a significantly higher susceptibility to H₂O₂ when knocked-out. This could be due to the differences in the settings between the TraDIS experiment and the disk diffusion assay. For example, the DNA-binding protein encoded by dps protects DNA from H₂O₂ damage through iron sequestration and this defense is more important in stationary phase of growth [38]. However stationary phase cultures of each knockout was treated with H₂O₂ in liquid medium, their respective growth was not different compared to the WT, except for the ΔoxyR strain (data not shown). The majority of
genes we identified by TraDIS (oxyR, dps, rpoS, dksA, hsf, polA) have already been reported to respond to oxidative stress in E. coli. The transcription factor OxyR is a well described sensor of H₂O₂, which regulates an extensive and coordinated antioxidant transcriptional response [9,39]. The RNA polymerase subunit RpoS regulates the general stress response and was previously described to be activated by oxidative stress [40], and the deletion of this gene increases sensitivity to H₂O₂ [41]. The RNA polymerase accessory protein DksA senses oxidative stress through its cytochrome residues and participates to the transcriptional response against oxidative stress [42]. Hsf, a RNA-binding protein that affects many cellular processes influences both the small RNA OxyS and the translation of rpoS described above in E. coli [43,44]. The DNA polymerase I encoded by polA is implicated in DNA repair and non-functional PolA increases H₂O₂-sensitivity [45,46]. The polA, rbsR, dps, oxyR, corA, rpoS genes were also identified in a similar experiment performed previously on Salmonella enterica serovar Typhimurium under sublethal H₂O₂ exposure [19]. The dksA and nhaA mutants, despite showing no increase in sensitivity to H₂O₂, were slightly more sensitive to other oxidants than WT using disk diffusion assay. Other validation experiments, such as competition assay with WT under H₂O₂ stress, might better reflect the TraDIS experimental conditions.

On the other hand, several genes previously identified in the literature as necessary for H₂O₂ tolerance were not identified by this TraDIS experiment. For example, xthA, whose deletion mutant is more sensitive to H₂O₂ [47], displayed a decreased fitness in H₂O₂ condition but did not reach the threshold of significance. An explanation could lie in the fact that unlike antibiotics, H₂O₂ is rapidly degraded by bacteria. The duration of the exposure to H₂O₂ performed for the TraDIS may have been insufficient to identify all genes implicated in H₂O₂ tolerance. Secondly, the stress was applied against pooled mutants in liquid where other mutants could provide cross-protection for susceptible mutants. For example, the catalase KatG, which is known to protect against H₂O₂, was not identified by TraDIS, probably because of this phenomenon. This was also the case in a previous study that used Tn-seq with H₂O₂ in Salmonella Typhimurium, where none of the catalase genes were identified [19]. Thus, our TraDIS data only identified those mutants that showed fitness defects despite cross-protection and inherent H₂O₂ degradation.

The TraDIS experiment also identified genes that, to our knowledge, were not previously associated with E. coli H₂O₂ sensitivity. The magnesium ion transporter encoded by corA had been shown to be more sensitive to lactoperoxidase–thiocyanate stress but not to H₂O₂ [48]. rbsR controls the transcription of the operon involved in ribose catabolism and transport and the salvage pathway of purine nucleotide synthesis [49]. corA and rbsR were also identified in a similar Tn-seq experiment using H₂O₂ on Salmonella enterica serovar Typhimurium [19]. The Na⁺:H⁺ antiporter nhaA is implicated in other stress responses against sodium ion, pH homeostasis and in maintaining antioxidant tolerance under starvation [50]. The glycolysis enzyme gpmA has been previously identified by a Tn-seq experiment following H₂O₂ exposure in the Gram-negative bacteria Salmonella enterica serovar Typhimurium [20].

When tested with diverse oxidants that damage bacteria through different modes of action, ∆gpmA was specifically more sensitive to H₂O₂, like the ΔkatG strain. However, it was not through a differential expression of H₂O₂-scavenging genes or a decreased catalase activity of the strain, suggesting a different mode of action. Moreover, the upregulation of gpmA by sublethal exposure of H₂O₂ suggests the importance of gpmA in H₂O₂ tolerance. Under oxidative stress, some enzymes of the central metabolism have been shown to be upregulated. The glucose-6-phosphate isomerase encoded by pgi have been shown to be regulated by the oxidative stress sensitive regulators SoxRS [51]. Similarly, in the TCA cycle, the aconitate acnA and the fumarase fumC are regulated by SoxRS and are upregulated under H₂O₂ exposure [52,53]. The hypersensitivity to H₂O₂ of ∆gpmA mutant could be complemented with the low-copy plasmid pWSK29 expressing the WT gpmA gene under its native promoter but not if the histidine 11 was mutated to an alanine. This strongly suggests that the function of gpmA affects E. coli tolerance to H₂O₂.
Surprisingly, addition of other metabolites or the absence of oxygen did not abolish the difference in $H_2O_2$ sensitivity between the WT and the $\Delta gpmA$ mutant. These data contrast with previous work on Salmonella enterica serovar Typhimurium, where other metabolites entering metabolism downstream of gpmA reaction (for a scheme of glycolysis, see Supplementary Figure S2) could complement the increased sensitivity of a $\Delta gpmA$ mutant and where anoxic environment abolished the difference of $H_2O_2$ susceptibility between WT and $\Delta gpmA$ mutant [20]. In the same study, metabolomics approach showed that $H_2O_2$ exposure led to an increase of glycolysis and fermentation that was important in Salmonella $H_2O_2$ tolerance. This contrasts with previous metabolomics analysis on E. coli after $H_2O_2$ treatment which reported a decrease of metabolites related to glycolysis and TCA cycle, changes that were common to other stress conditions such as heat shock and cold stress [54]. Altogether, this suggests a different metabolic adaptation to $H_2O_2$ stress between E. coli and Salmonella and a difference of gpmA function. More research is needed to better understand the mechanisms of gpmA effects in $H_2O_2$ tolerance in E. coli and in other organisms.

Contrary to vertebrates that only possess one phosphoglycerate mutase, some eubacteria, among which relevant pathogens including E. coli, encode two enzymes that display no sequence similarity [55]. The 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase (or dPGM), encoded by gpmA is common to bacteria and vertebrates, whereas 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (or iPGM) encoded by gpmM is shared by bacteria and higher plants. As the double deletion of gpmA and gpmM have been suspected non-viable in E. coli, the glycolysis function is assumed by gpmM in the gpmA-deleted strain and vice versa [56]. The deletion of E. coli gpmM did not affect $H_2O_2$ sensitivity, suggesting that only gpmA function has a role under $H_2O_2$ exposure. This led to the hypothesis that gpmM could be damaged by $H_2O_2$ and its function is replaced by gpmA under $H_2O_2$ exposure. This happens for other enzymes of the TCA cycle, the aconitase and the fumarase, where oxidative-resistant isoforms (acnA, fumC) replace oxidative-sensitive isoforms (acnB, fumA, fumB), after $H_2O_2$ exposure [52,53]. Cysteine residues can be more prone to oxidation by $H_2O_2$ than other amino acids [57]. GpmM possesses two cysteine residues, which can result in $H_2O_2$-induced damage from oxidation of these residues. As GpmA does not possess cysteine residues, it could be more resistant to $H_2O_2$ than GpmM. The cysteine residues of GpmM are not implicated in active sites described in current models (Ecocyc, Uniprot). While Cys397 seems buried and is not conserved in Gram-positive bacteria, Cys424 seems to be more accessible on the protein models and is present in both Gram-negative (P. aeruginosa, Salmonella enterica, K. pneumoniae) and Gram-positive bacteria (S. aureus, B. subtilis). Additional studies are needed to evaluate their potential implication in oxidative stress susceptibility.

5. Conclusions

This work was aimed at expanding the knowledge of which genes are implicated in $H_2O_2$ tolerance. The main finding of this study was that a functional gpmA gene is required for tolerance to $H_2O_2$. This is the first time that gpmA was highlighted as an important contributor to the E. coli tolerance to $H_2O_2$, and it links defense against oxidative stress to central metabolism.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/antiox11102053/s1, Table S1: Primers used to validate the gene replacement by the kanamycin cassette from the Keio collection, Figure S1: Sensitivity of the deletion mutants of the TraDIS exposed to various oxidants, Figure S2: Schematic diagram of $H_2O_2$-induced transcriptional changes of glycolysis and TCA cycle, Figure S3: The deletion of gpmA did not affect bacterial growth.
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