Additive effects of metal excess and superoxide, a highly toxic mixture in bacteria.

SUPPORTING INFORMATION

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Figure S1: A 22 kDa protein is induced in the Cd\textsuperscript{2+}-ATPase deficient mutants under respiration. Induction of SodB in *R. gelatinosus* wild-type (WT), *cadR* and *ΔcadA* strains with the increase of CdCl\textsubscript{2} concentration. Cells were grown under respiration. Total protein extracts from the same amount of cells (OD\textsubscript{680nm} = 1) were separated on 15% SDS-PAGE and analyzed by western blot using the His-Probe.
Figure S2: XseA is not required for menadione tolerance. A- Menadione disc-diffusion assay (as described in Experimental procedures) results showing the complementation by pBxseAsodB and pBsodB. pBTc: empty plasmid. B- sodB constructs used in this work. ΔsodB was used to generate the ΔsodB mutant. pB derivatives (pBxseAsodB and pBsodB) were used for complementations of ΔsodB strain.
Figure S3: Genetic organization of xseA sodB orfs. A- xseA-sodB gene fusion. xseA coding sequence is in blue and sodB in red. No stop codon was found between the two orfs. Putative promoters and transcripts and primers used in RT-PCR are indicated. B- Protein sequence of the fusion. The five His detected by the His-probe in SodB are shown in black and bold style. C-Analysis of sodB expression by RT-PCR. Total RNA from wild-type (WT) and ΔsodB strains grown under respiration was extracted according to (Steunou et al. 2004). 2 μg of total RNA was reverse transcribed with the superscript II (Invitrogen) using the specific sodB primer 348. 2 μl of the RT reaction was used for the PCR. We used either the primers 433 - 434 specific to xseA or the 347 - 348 primers specific to sodB. Both PCRs were positive showing the presence of the xseAsodB transcript (arrow). Genomic DNA and 16S rRNA were used for controls. This experiment suggested the presence of xseAsodB transcript but does not exclude the presence of an internal promoter upstream of sodB. Putative promoters are shown in gray.
**Fig. S4** : **A**- Menadione disc-diffusion assay. Plates were incubated at 30 °C overnight either under photosynthetic (PS) or respiratory (RES) conditions.

**B**- Western blot analysis of WT, ΔsodB, ΔcadA, and ΔcadAsodB strains grown by photosynthesis with (50 µM) or without (-) cadmium, showing the induction of SodB

**C**- Effect of Cd²⁺ and menadione stress in ΔsodB, and ΔcadAsodB strains. Menadione disc-diffusion assay in plates supplemented or not with 25 mM CdCl₂. Plates were incubated at 30 °C overnight under aerobic respiration conditions. The simultaneous presence of Cd²⁺ and menadione is deleterious for the ΔcadAsodB hyper-sensitive strain.
Figure S5: Effect of Cu$^{2+}$ and menadione stress in the wild-type (WT), ΔsodB, copA$, and ΔsodBcopA$ strains. Menadione disc-diffusion assay in plates supplemented or not with 25 mM CuSO$_4$ (as described in Experimental procedures). Plates were incubated at 30 °C overnight under aerobic respiration conditions. The simultaneous presence of Cu$^{2+}$ and menadione is deleterious for the ΔsodBcopA$ hyper-sensitive strain.
### Table SI: Bacterial strains and plasmids

| Strains          | Relevant characteristics                                                                 | Source                                      |
|------------------|-------------------------------------------------------------------------------------------|---------------------------------------------|
| **E. coli**       |                                                                                            |                                             |
| JM109            | el4 (McrA), recA1, endA1, gyra69, thi-1, hsdR17 (rk-mk+) supE44, recA1, Δ(lac-proAB) [F’traD36, proAB, lacIZΔM15](R·pir) | Stratagene                                  |
| SM10 Δpir        | SM10 Δpir / pEYY345                                                                       | Laboratory Stock                            |
| bEYY1980         | Wild type (WT), background of KEIO collection                                             | (Baba et al, 2006)                         |
| BW25113          | Wild type (WT), background of KEIO collection                                             | (Baba et al, 2006)                         |
| copA             | BW25113 copA::FRT::Km (copA::FRT::Km-FRT)                                                 | (Baba et al, 2006)                         |
| zntA             | BW25113 zntA::FRT::Km (zntA::FRT::Km-FRT)                                                 | (Baba et al, 2006)                         |
| sodA             | BW25113 sodA::FRT::Km (sodA::FRT::Km-FRT)                                                 | (Baba et al, 2006)                         |
| sodB             | BW25113 sodB::FRT::Km (sodB::FRT::Km-FRT)                                                 | (Baba et al, 2006)                         |
| **V. cholerae**  |                                                                                            |                                             |
| C6706            | WT O1 El Tor; Sm'                                                                         | (Cameron et al, 2008)                      |
| ΔcopA            | C6706 Δvc2115 (copA)                                                                      | This work                                   |
| sodB             | C6706 vc2045 (sodB::Tn)                                                                   | (Cameron et al, 2008)                      |
| ΔcopAsodB        | C6706 vc2045 (sodB::Tn) Δvc2115 (copA)                                                    | This work                                   |
| **P. aeruginosa**|                                                                                            |                                             |
| PAO1             | WT                                                                                        | (Jacobs et al, 2003)                        |
| PA3920           | phoAbp01q1G01, Tc'                                                                        | (Jacobs et al, 2003)                        |
| PA3690           | phoAbp01q3B12                                                                            | (Jacobs et al, 2003)                        |
| **P. putida**    |                                                                                            |                                             |
| PNL-MK25         | WT strain PNL-MK25                                                                        | (Adaiikkalam & Swarup, 2007)               |
| CEM1             | Mini-Tn5::gfp inserted into (cueA) copA the copper-transporting ATPase in the wild-type strain ; Km' | (Adaiikkalam & Swarup, 2007)               |
| **B. subtilis**  |                                                                                            |                                             |
| l68              | WT, trpC2                                                                                 | (Kunst et al, 1997)                        |
| CSP105           | ΔcopA::spc'                                                                               | (Chillappagari et al, 2010)                |
| **R. gelatinosus**|                                                                                            |                                             |
| Strain S1        | WT                                                                                        | (Uffen, 1976)                               |
| ΔsodB            | sodB deleted strain (ΔsodB::Km)                                                           | This work                                   |
| cadR             | cadR inserted strain (cadR::Km)                                                           | This work                                   |
| ΔcadA            | cadA deleted strain (ΔcadA::Tp)                                                           | (Steunou et al, 2019)                      |
| copA             | copA inserted strain (copA::Km)                                                           | (Azzouzi et al, 2013)                      |
| ΔcadAsodB        | cadA (ΔcadA::Tp) and sodB (ΔsodB::Km) deleted strain                                     | This work                                   |
| ΔsodBcopA        | copA (copA::Km) and sodB (sodB::Km) deleted strain                                       | This work                                   |
| **Plasmids**     |                                                                                            |                                             |
| pGEM-T           | Cloning vector (Ap')                                                                      | Promega                                    |
| pUC4K            | Plasmid bearing the Km cartridge (Ap' Km')                                                | Pharmacia                                  |
| pBRR1MCS-3       | (mob', Tc') expression vector                                                             | (Kovach et al, 1994)                       |
| pGsodB           | pGEM-T + 1.7 kb PCR fragment containing sodB                                                | This work                                   |
| pGsodB::Km       | Km cartridge cloned into mscI-stu1 site inactivating sodB                                  | This work                                   |
| pGxseAsodB       | pGEM-T + 2.4kb PCR fragment containing xseAsodB gene                                       | This work                                   |
| pBxseAsodB       | xseAsodB subcloned from pGxseAsodB digested by xcmI-saci into pBRR1MCS-3 at smaI and sacI sites | This work                                   |
| pBsodB           | 0.8 kb deletion of xseA by digestion of pBxseAsodB with sacI and smal sites               | This work                                   |
| pEYY345          | Allelic exchange plasmid for Δvc2115                                                      | This work                                   |
Ap<sup>r</sup>, ampicillin resistant, Km<sup>r</sup>, kanamycin resistant, Tp<sup>r</sup>, trimethoprim resistant, Tc<sup>r</sup>, tetracyclin resistant, spc<sup>r</sup>, spectinomycin resistant.

**Table SII:** Primers used in this work

| Primer       | Sequence 5’ to 3’                      |
|--------------|---------------------------------------|
| RG347_SodB RTF | ACGTCGTGAACCTGAACAAC                 |
| RG348_SodB RTR | TCGATGTAATGCACTTCGTC                |
| RG433_XseARTF | GAGAACTGCAACTCGTCGTC                |
| RG434_XseARTR | ATGGAAGCCACACAGATCCT                |
| RG345_SodBF  | AGACCACATCACGCCTCG                   |
| RG346_sodBR  | AGTAATTGCCTCTTTGTCT                  |
| RG369_xseA_F | GCCCGTGTATGATGACAG                   |
| RG370_sodB_R | CAACTGAGCCGACATTGTT                  |
| oYo848       | CCGCATGCGATATCGAGCTCCCCTTTGTCAGCAGG |
| oYo849       | ATTTGCGATTGCTGACCACCACGCAGCCTAAAGCAA|
| oYo850       | TTGCTTTAGCGCTGCTGTGGTCAGCATTGCCAAT  |
| oYo851       | CGGATAACAAATTGTGGAATTCACCAGCGCTTTGTGTCTAGC |

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