

## The *cue* regulon controls both aerobic and anaerobic copper resistance in *Salmonella*

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Copper resistance in Gram-negative bacteria is primarily controlled by the *cue* regulon. This regulon is composed by the Cu(I) sensor/regulator CueR that induces the expression of two target genes, *copA* and *cueO*, coding for an integral inner-membrane Cu-transporting P-type ATPase, and a periplasmic oxygen-dependent multicopper-Cu(I) oxidase, respectively. *Escherichia coli* also relies on the *cus* system to increase copper resistance under anaerobic conditions. This system is composed by the CusCFBA efflux pump, which is transcriptionally controlled by the two-component system CusR/CusS. Interestingly, *Salmonella* harbours all the *cue* components but lacks the *cus* locus. Despite of this, *Salmonella* displays higher resistance to copper than *E. coli* in anaerobic conditions. We have uncovered a novel, *Salmonella*-specific, CueR-regulated gene, *cueP*, coding for a protein that increases resistance to copper both in aerobic and anaerobic conditions. To test whether CueP could functionally substitute the *E. coli* *cus* system for copper resistance, we replaced the entire *E. coli* chromosomal *cus* locus for the wild-type copy of the *Salmonella* *cueP*, including its own promoter. CueR-dependent expression of *cueP* increased resistance to copper in this engineered strain. Moreover, overexpression of CueP completely restored resistance to copper in a *cueR* *cus* double mutant strain in aerobic conditions. Atomic absorption spectrometry, as well as UV-visible spectroscopy demonstrated that CueP is able to bind two Cu(II) equivalents per monomer, supporting a role of CueP as a copper-resistance factor. Our results indicate that in contrast to other enterobacterial species *Salmonella* has evolved a single pathway to respond to copper excess both in aerobic and anaerobic conditions.

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