Supplementary Figure 1.



Selection of mutants conferring increased tellurite resistance. a) Representative growth curves of strains BW25113, EM40, EM41 and EM2 in LB medium in the presence of the indicated tellurite concentrations. **b**) Representative growth curves of strains EM42, EM43, 44, 64, 70, and 71 in M9 medium in the presence of the indicated tellurite concentrations. Values represent the mean of 3 biological replicates. In all figures, error bars represent SD. **c**) Strains EM 44, EM64, EM70 and EM71 were grown in M9 medium and the heme content was measured in cell extracts. For comparison, the level of heme in strains BW25113 (magenta line), EM43 (blue line) and EM2 (black line) are shown. Values represent the mean of three biological replicates. Error bars represent SD. TeO₃²⁻ : tellurite.

Supplementary Figure 2.



Representative growth curves of strains BW25113 and EM2 with tellurite. Representative growth curves of strains BW25113 and EM2 in M9 medium in the presence of the indicated tellurite concentrations. Values represent the mean of 3 biological replicates. Error bars represent SD. $\text{TeO}_3^{2^2}$: tellurite.

Supplementary Figure 3.



Increased thiols, H₂O₂ detoxification or resistance are not required for tellurite resistance. a) Strains BW25113 and EM2 were grown in M9 medium with or without 25 μ g/mL ALA to an OD 600nm of ~0.4 and treated with 2 mM H_2O_2 (left panel) or tellurite (middle and right panel, 25 μ g/mL for strain BW25113 and 400 μg/mL for strain EM2) and catalase (left and middle panels) or superoxide dismutase (SOD, right panel) activity were determined at the indicated time points. SOD activity was normalized by mg of protein. Values represent the mean of three biological replicates. Error bars represent SD. b) Overnight cultures of strains BW25113 and EM2 were inoculated into M9 medium supplemented or not with 25 μ g/mL ALA, challenged or not with 2 mM H₂O₂ and CFU/mL were quantified at the indicated time points. Values represent the mean of three biological replicates. Error bars represent SD. c) Representative growth curves of strains BW25113, EM2, $\Delta hemN$ and $\Delta hemF$ in M9 medium with 0.3 mM H₂O₂. Values represent the mean of three biological replicates. Error bars represent SD. d) Strains BW25113 and EM2 were grown as in a), exposed to tellurite and tellurite reductase (TR) activity was determined with NADH or NADPH as cofactor. Values represent the mean of three biological replicates. Error bars represent SD. e) Strains BW25113, $\Delta hemN$, hemN⁺ and EM2 were grown as in a), exposed to 25 µg/mL tellurite and total intracellular thiols were determined and normalized by mg of protein. Strain EM2 was also treated with 400 µg/mL tellurite, and thiols were determined (blue dashed line). Values represent the mean of three biological replicates. Error bars represent SD.TeO₃²⁻ : tellurite; RSH: μ M thiols.

Supplementary Figure 4.



Representative growth curves of strains $\Delta hemF$, $hemN^{+}$ and $\Delta hemN$ with tellurite. Representative growth curves of strains $\Delta hemF$, $hemN^{+}$ and $\Delta hemN$ in M9 medium in the presence of the indicated tellurite concentrations. Values represent the mean of 3 biological replicates. Error bars represent SD. TeO₃²⁻ : tellurite.



Supplementary Figure 5.

Representative growth curves of strains BW25113, EM2, $\Delta hemN$ and $\Delta hemF$ in M9 medium supplemented with 25 µg/mL ALA and varying tellurite concentrations. Values represent the mean of 3 biological replicates. TeO₃²⁻ : tellurite.

Supplementary Figure 6.



Representative growth curves of strains BW25113 and EM2 in M9 medium supplemented with the indicated concentrations of tellurite and varying concentrations of ALA. Values represent the mean of 3 biological replicates. $TeO_3^{2^-}$: tellurite.

Supplementary Figure 7.

Campylobacter jejuni 1	MYYCISFTHKNTDIALREKLSFSNEAKKGEFLKIISTHENIEECLVISTCNRVEIVAF	59
Mycobacterium leprae 1	-MSILLFGVSHRSAPVSVLEQLSLDRSDQIK-IVDRVLQSPLVTEAMVLSTCNRVEVYAV	59
Listeria monocytogenes 1	-MFILTMGLNHHTAPIDIREKLVFKETEEEM-ALVTLQQEKSILENVIISTCNRTEIVAV	59
Bacillus cereus 1	-MHILVVSVNYRTAPVEFREKLTFQAAEIER-AMTTLQNQKSVLENVIVSTCNRTEIYAV	59
Bacillus anthracis 1	-MHILVVSVNYRTAPVEFREKLTFQAAELER-AMTTLQNQKSVLENVIVSTCNRTEIYAV	59
Staphylococcus aureus 1	-MHFIAISINHRTADVALREQVTFRDDALRI-AHEDLYETKSILENVILSTCNRTEVYAV	59
Staphylococcus epidermidis 1	-MHFVAISINHRTADVTLREQVAFRDDALRL-AHEDLYETKAILENVILSTCNRTEVYAI	59
Methanopyrus kandleri 1	MEDLVCVGITHKEAEVEELEKARFESDE-AVRDIVESFGLSGCVLLQTCNRVEVYAS	57
Neisseria meningitidis 1	-MQLTAVGLNHQTAPLSIREKLAFAAAALPK-AVRNLARSNAATEAVILSTCNRTELYCV	59
Neisseria gonorrhoeae 1	-MQLTAVGLNHQTAPLSIREKLAFAAAALPE-AVRNLARSNAATEAVILSTCNRTELYCV	59
Pseudomonas aeruginosa 1	-MAFIALGINHKTASVAVRERVAFTFEQMVE-ALQQLCRLTTSREAAILSTCNRSELYLE	59
Vibrio cholerae 1	-MSLLAIGINHNTASVELREKVAFGPEKLSL-ALNQLSTSSHVKGGVILSTCNRTEIYCD	59
Klebsiella pneumoniae 1	-MTLLALGINHKTAPVALRERVTFSPETLDK-ALESLLAQPMVQGGVVLSTCNRTELYLS	59
Enterobacter cloacae 1	-MTLLALGINHKTAPVSLRERVTFSPDTLDL-ALDSLLAQPMVQGGVVLSTCNRTELYLS	59
Salmonella Typhimurium 1	-MTLLALGINHKTAPVSLRERVTFSPDTLDQ-ALDSLLAQPMVQGGVVLSTCNRTELYLS	59
Escherichia coli 0104:H4 1	-MTLLALGINHKTAPVSLRERVSFSPDKLDQ-ALDSLLAQPMVQGGVVLSTCNRTELYLS	59
EM2 1	-MTLLALGINHKTAPVSLRERVSFSPDKLDQ-ZLDSLLAQPMVQGGVVLSTCNRTELYLS	59
Escherichia coli 1	-MTLLALGINHKTAPVSLRERVSFSPDKLDQ-ALDSLLAQPMVQGGVVLSTCNRTELYLS	59
	*: :	

Conservation of HemA residue mutated in strain EM2. The amino acid sequences of HemA from strain EM2 was aligned against that of other organisms. Residue that was mutated in strain EM2 is indicated by a magenta box along all the sequences.

Supplementary Figure 8.



¹H NMR spectra of ALA in M9 medium with or without tellurite. All the measurements were carried out in M9 medium with 5 mg ALA and with or without 5 mg tellurite. $TeO_3^{2^2}$: tellurite.

Supplementary Figure 9.



Evaluation of the effect of ALA and tellurite on selected pathogens. Representative growth curves of selected pathogens in M9 medium supplemented with 25 μ g/mL ALA in the presence of the indicated tellurite concentrations. TeO₃²⁻ : tellurite.

Supplementary Figure 10.



E. coli growth in the presence of high tellurite concentrations results in black deposits of elemental tellurium. Representative growth curves in LB medium containing or not 100 µg/mL tellurite of colonies isolated from each passage during directed evolution experiment. Number at the top of each well indicates the passage at which the corresponding colony was isolated. Strains EM40, EM41 and EM2 were isolated at passages 3, 13, and 26, respectively. Blackening of the growth culture is due to tellurite reduction to tellurium. TeO₃²⁻ : tellurite.

Strain(s)	Gene	Nucleotide change	Amino acid change	
EM 40, EM41, EM2	yigE	$GCC \rightarrow GTC$	A22V	
EM 41, EM2	rpoD	$CGT \rightarrow AGT$	R603S	
EM 2	pspF	$CTC \rightarrow CAC$	L52H	
EM 2	hemA	$GCG \rightarrow GAG$	A31E	
EM 2	hemL	$GCG \rightarrow GTG$	A118V	

Supplementary Table 1. Nonsynonymous mutations found in evolved strains.

Supplementary Table 2. Primers used in this study.

Primer name	SEQUENCE 5' → 3'	Amplified product
Gibson_pKD34F	GTGTAGGCTGGAGCTGCT	Plasmid pKD3
Gibson_pKD34R	AATCGCTCAAGACGTGTAATG	
Gibson_hemAF	CACGTCTTGAGCGATTATGACCCTTTTAGCACTCGG	hemA for cloning
Gibson_hemAR	AGCTCCAGCCTACACTACTCCAGCCCGAGGCTG	in plasmid pKD3
Gibson_hemLF	CACGTCTTGAGCGATTATGAGTAAGTCTGAAAATCTTTAC	hemL for cloning
Gibson_hemLR	AGCTCCAGCCTACACTCACAACTTCGCAAACAC	in plasmid pKD3
Gibson_rpoDF	ATTACACGTCTTGAGCGATTCACCAACCTCATGAAATAAG	<i>rpoD</i> for cloning
Gibson_rpoDR	GAAGCAGCTCCAGCCTACACTTAATCGTCCAGGAAGCTAC	in plasmid pKD3
Gibson_pspFF	ATTACACGTCTTGAGCGATTTTAGCGAATTACACTAACAAG	pspF for cloning in
Gibson_pspFR	GAAGCAGCTCCAGCCTACACCTAAATCTGGTGCTTTTTC	plasmid pKD3
Gibson_hemNF	ATTACACGTCTTGAGCGATTCGTAGCCGCCAGAGACGC	hemN for cloning
Gibson_hemNR	GAAGCAGCTCCAGCCTACACTTAAATCACCCGAGAGAACTGCTGC	in plasmid pKD3
wanner_muthemAF	GATGCAAGCAGACTAACCCT	hemA::cat
wanner_hemAR	AAATGCACCCTGTAAAAAAAGAAAATGATGTACTGC	cassette for
	ATATGAATATCCTCCTTAG	restoring hemA
wanner_muthemLF	GCACCAGTACAAGCAGCCTG	hemL::cat
wanner_hemLR	AGGCGTTCACGCCGCATCCGACAAACCATGCTGGACATATGAATAT	cassette for
	CCTCCTTAG	restoring hemL
wanner_hemN2F	CGTAGCCGCCAGAGACGC	hemN::cat
wanner_hemNR	GTTTTCTACTTTGTAAACGAAGCGCCATTCACTATCATATGAATATCC	cassette for
	TCCTTAG	restoring hemN
wanner_rpoD2F	CACCAACCTCATGAAATAAG	rpoD::cat cassette
wanner_rpoDR	CCGGGTGCGGCGTAACGCCTGATCCGGCCTACCGACATATGAATATC	for restoring rpoD
	CTCCTTAG	
wanner_pspF2F	TTAGCGAATTACACTAACAAG	pspF::cat cassette
wanner_pspFR	ACGCCGCATCCGGCAAGTTGTATTGCTCAACTTCGCATATGAATATCCT	for restoring pspF
	CCTTAG	