

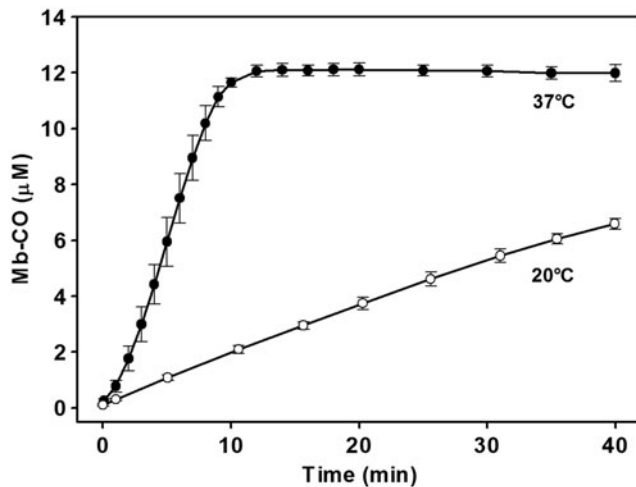
Supplementary Data

SUPPLEMENTARY TABLE S1. LIST OF THE THIRTY MOST HIGHLY REGULATED GENES, BOTH AEROBICALLY AND ANAEROBICALLY

		Time (min, after CORM-401 addition)					
Gene		2.5	5	10	20	40	80
Aerobic	<i>spy</i>	34.3	124.4	357.1	330.3	420.8	250.2
	<i>uhpT</i>	3.3	18.0	76.2	124.2	216.7	142.8
	<i>mdtA</i>	12.4	29.6	46.3	63.2	41.8	17.1
	<i>baeS</i>	1.4	2.3	25.1	45.4	67.4	19.7
	<i>mdtB</i>	4.4	24.3	32.6	42.5	30.2	10.8
	<i>kdpC</i>	0.4	1.5	32.8	33.8	18.2	5.5
	<i>yhdV</i>	3.1	5.5	7.9	29.0	12.5	5.7
	<i>yebE</i>	6.6	20.7	39.8	28.9	19.7	9.4
	<i>kdpF</i>	0.9	6.2	20.8	28.0	1.8	2.0
	<i>mdtC</i>	1.0	9.8	24.7	22.5	24.2	8.6
	<i>kdpA</i>	0.9	2.9	11.3	15.6	1.5	1.5
	<i>kdpB</i>	0.7	1.3	9.8	13.8	7.5	4.8
	<i>ycfS</i>	3.4	10.6	19.9	13.6	9.8	5.1
	<i>cpxP</i>	12.3	17.2	17.7	13.6	16.8	10.6
	<i>b3914</i>	9.6	17.4	18.0	13.4	15.1	10.3
	<i>cpxP</i>	9.7	17.7	18.4	12.6	16.1	10.6
	<i>ybfA</i>	2.9	5.1	6.1	11.8	7.4	3.0
	<i>htpX</i>	5.1	9.2	12.7	11.7	11.7	7.4
	<i>znuA</i>	1.0	2.6	6.5	11.2	10.0	5.2
	<i>acrD</i>	4.8	10.7	15.2	11.2	10.9	6.9
	<i>chaA</i>	2.2	7.0	14.4	9.8	5.8	4.1
	<i>ybdZ</i>	1.6	4.1	4.1	9.3	2.8	2.1
	<i>metF</i>	0.9	0.0	2.4	9.0	3.7	1.0
	<i>yobB</i>	4.7	9.1	11.9	8.4	6.4	4.9
	<i>ybdZ</i>	1.7	3.8	3.5	8.3	2.0	2.0
	<i>yaaX</i>	2.9	4.2	7.5	8.3	6.3	3.3
	<i>glvC</i>	0.5	1.0	1.0	7.9	1.3	3.3
	<i>entA</i>	0.8	1.8	5.3	7.8	4.7	1.8
	<i>degP</i>	2.2	1.2	1.9	7.5	7.4	4.1
	<i>yedX</i>	2.2	6.0	4.3	7.4	5.0	4.2

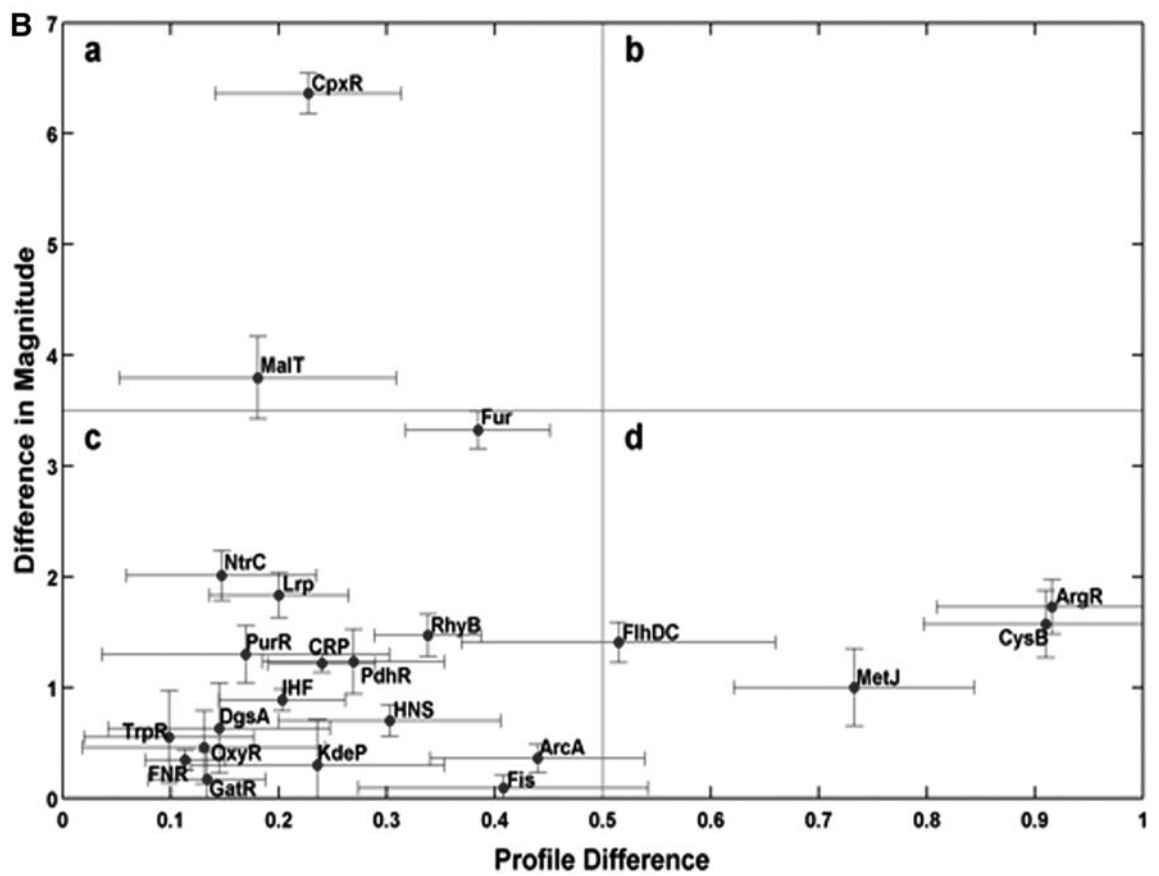
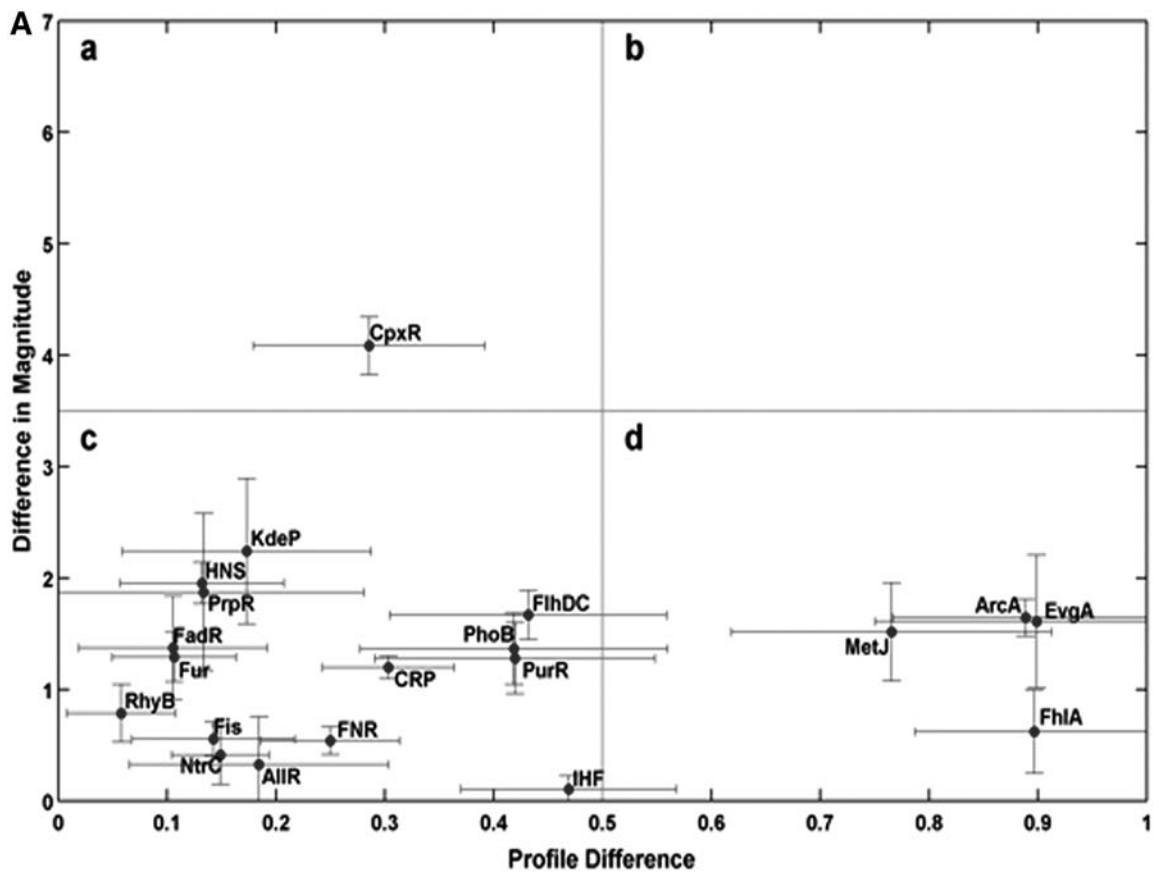
		Time (min, after CORM-401 addition)					
Gene		2.5	5	10	20	40	80
Anaerobic	<i>spy</i>	14.3	200.4	585.0	505.5	672.6	556.4
	<i>uhpT</i>	1.1	8.3	89.5	112.6	112.1	106.3
	<i>mdtA</i>	12.4	35.2	63.0	59.9	60.2	39.3
	<i>baeS</i>	0.9	1.1	31.0	38.9	25.2	14.3
	<i>yebE</i>	2.4	17.2	39.1	32.0	28.0	11.6
	<i>mdtC</i>	1.0	7.9	22.4	29.8	21.5	17.0
	<i>marA</i>	3.6	11.4	16.7	28.6	47.8	20.4
	<i>mdtB</i>	1.1	10.4	24.8	23.0	20.8	18.4
	<i>marB</i>	3.3	5.6	13.9	21.7	26.7	10.3
	<i>ycfS</i>	2.1	25.7	42.2	20.1	20.7	6.1
	<i>degP</i>	0.8	2.0	18.9	18.5	23.9	12.0
	<i>htpX</i>	2.4	10.5	16.1	14.5	12.5	7.6
	<i>ykgM</i>	1.5	1.2	2.5	14.3	43.3	40.9
	<i>metB</i>	1.4	1.1	5.6	13.0	2.6	0.8
	<i>cpxP</i>	4.6	11.9	17.0	12.9	11.5	5.9
	<i>b3914</i>	4.1	11.9	17.1	12.4	11.5	5.9
	<i>cpxP</i>	4.7	11.4	13.0	11.7	11.3	6.5
	<i>acrD</i>	1.9	8.9	11.3	11.1	8.2	7.6
	<i>marR</i>	1.8	5.1	8.3	10.9	20.2	10.8
	<i>metF</i>	2.0	1.2	5.9	9.8	1.5	0.7
	<i>yjfN</i>	0.6	5.9	8.2	9.6	9.2	2.1
	<i>chaA</i>	2.2	6.4	12.1	9.5	11.7	4.2
	<i>yhdV</i>	1.3	1.9	5.6	9.2	9.1	12.8
	<i>sdaA</i>	1.4	4.1	9.1	9.2	18.2	6.7
	<i>yodA</i>	0.8	1.9	3.9	9.2	34.6	15.3
	<i>mmuP</i>	1.4	1.3	3.5	8.5	1.8	1.3
	<i>metE</i>	1.4	1.1	1.9	8.1	2.1	0.5
	<i>kdpC</i>	2.8	1.4	1.3	8.0	4.6	2.8
	<i>frmB</i>	1.4	1.5	3.1	7.9	17.8	9.7
	<i>yobB</i>	3.1	8.2	9.6	7.9	6.2	4.0

Values shown are fold changes relative to the pre-CORM culture sampled at the time points shown.



SUPPLEMENTARY FIG. S1. CO release from CORM-401 in 0.1 M KPi (pH 7.4) at 37°C or 20°C. Assays were performed in the presence of excess dithionite with myoglobin (20 μ M) and 5 μ M CORM-401. Under these conditions, CORM-401 releases 2.4 mole equivalent of CO with a $t_{1/2}$ of 5 min. $n = 3 \pm \text{SEM}$.

SUPPLEMENTARY FIG. S2. TFInfer correlation profiles (coherence plots) showing TFs involved in the response to CORM-401 versus CO gas in *E. coli* cells. The x -coordinate of each point is presented with error bars and represents the profile difference between CORM-401 and CO treatments, computed as 1 minus the absolute Pearson correlation coefficient between the two profiles; the y -coordinate represents the change in magnitude of the response, computed as the difference of the norm of the two profiles. Data from aerobic (**A**, top) and anaerobic (**B**, bottom) conditions are shown. TFs whose response is similar for both CORM-401 and CO gas, both in magnitude and kinetics, will be located near the origin of each coherence plot in quadrant c, while TFs in quadrant b of each plot respond differently in both kinetics and amplitude. For example, CpxR, both aerobically and anaerobically, has a similar response in terms of the shape of the profile, but different magnitudes in (**A**) and (**B**), while ArcA (**A**) and ArgR (**B**) show similar magnitudes, but major differences in response profiles. TF, transcription factor.



A

Gene	Function	Relevant regulatory proteins	Fold change down-regulated						Fold change up-regulated				
			30+	20-30	10-20	5-10	2-5	0.0	2-5	5-10	10-20	20-30	30+
			CORM-401 AEROBIC						CORM-401 ANAEROBIC				
Time						Time							
2.5	5.0	10.0	20.0	40.0	80.0	2.5	5.0	10.0	20.0	40.0	80.0		
<i>cyoA</i>	cytochrome <i>bo'</i> terminal oxidase subunit II	ArcA (-)			0.44	0.20		0.44	0.11	0.09	0.20		
<i>cyoB</i>	cytochrome <i>bo'</i> terminal oxidase subunit I	ArcA (-)				0.23		0.50	0.33	0.32	0.38		
<i>cyoC</i>	cytochrome <i>bo'</i> terminal oxidase subunit III	ArcA (-)				0.20			0.22	0.26	0.34		
<i>cyoD</i>	cytochrome <i>bo'</i> terminal oxidase subunit IV	ArcA (-)				0.20			0.23	0.21	0.26	0.48	
<i>cyoE</i>	heme <i>o</i> synthase	ArcA (-)				0.21			0.33	0.36	0.37	0.48	
<i>cydA</i>	cytochrome <i>bd-I</i> terminal oxidase subunit I	ArcA (+), FNR (-)	2.88	2.42		2.86	3.07	2.01		0.32	0.26	0.25	0.38
<i>cydB</i>	cytochrome <i>bd-I</i> terminal oxidase subunit II	ArcA (+), FNR (-)	2.54	2.37	2.07	2.83	2.70			0.42	0.30	0.22	0.39
<i>nuoA</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)		0.49		0.43				0.39	0.29	0.27	0.39
<i>nuoB</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)									0.43	0.39	
<i>nuoC</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)				0.49				0.46	0.33	0.35	
<i>nuoE</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)				0.44				0.45	0.33	0.34	
<i>nuoF</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)								0.47	0.34	0.31	
<i>nuoG</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)							0.50	0.32	0.26	0.48	
<i>nuoH</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)									0.41	0.30	
<i>nuoI</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)									0.43	0.32	
<i>nuoJ</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)									0.47	0.34	
<i>nuoK</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)									0.39	0.31	
<i>nuoL</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)									0.47	0.35	
<i>nuoM</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)									0.35	0.28	0.48
<i>nuoN</i>	NADH:ubiquinone oxidoreductase I	ArcA (-), FNR (-)										0.36	
<i>ndh</i>	NADH dehydrogenase II	FNR (-)			2.27	2.50	2.45	2.04					

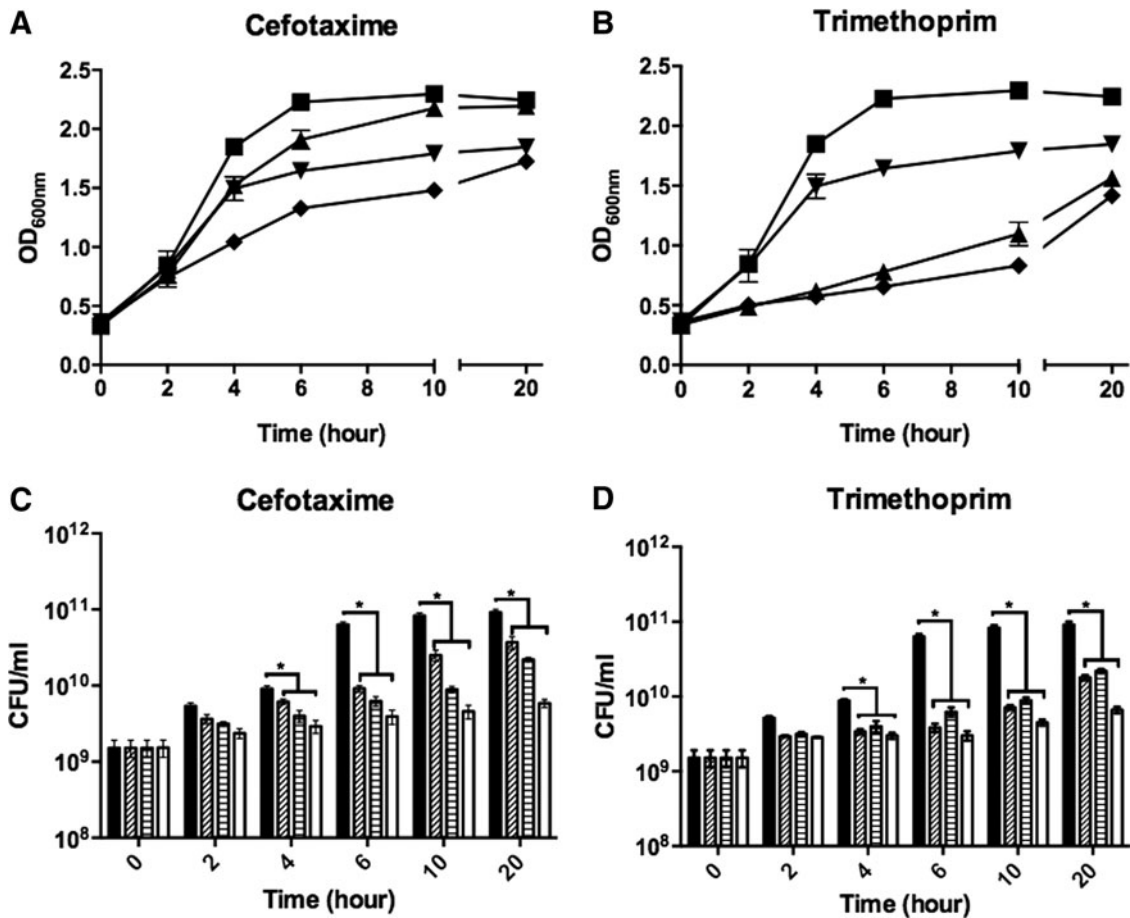
B

Gene	Product/Function	Relevant regulatory proteins	Fold change down-regulated						Fold change up-regulated					
			30+	20-30	10-20	5-10	2-5	0.0	2-5	5-10	10-20	20-30	30+	
			CORM-401 AEROBIC						CORM-401 ANAEROBIC					
Time						Time								
2.5	5.0	10.0	20.0	40.0	80.0	2.5	5.0	10.0	20.0	40.0	80.0			
<i>chaA</i>	Na ⁺ : K ⁺ /H ⁺ antiporter		2.2	7.0	14.4	9.8	5.8	4.1	2.2	6.4	12.1	9.5	11.7	4.2
<i>kdpA</i>	K ⁺ transporting ATPase subunit	KdpE (+)		2.9	11.3	15.6						2.1	2.3	
<i>kdpB</i>	K ⁺ transporting ATPase subunit	KdpE (+)			9.8	13.8	7.5	4.8				3.7	3.4	2.0
<i>kdpC</i>	K ⁺ transporting ATPase subunit	KdpE (+)	0.4		32.8	33.8	18.2	5.5	2.8				4.6	2.8
<i>kdpD</i>	regulator of the K ⁺ transporting ATPase						2.1					2.5		
<i>kdpE</i>	regulator of the K ⁺ transporting ATPase	KdpD-P (+)					2.7	2.6	2.0			2.4	2.3	2.0
<i>kdpF</i>	K ⁺ transporting ATPase subunit			6.2	20.8	28.0		2.0		0.5				
<i>kefA (mscK)</i>	Potassium-dependent mechanosensitive channel					2.5	2.6					2.0		
<i>kefB</i>	K ⁺ : H ⁺ antiporter			0.3	0.1	0.1								
<i>kefC</i>	K ⁺ : H ⁺ antiporter													
<i>kefF</i>	Regulator of the Kef transporter					0.2								
<i>kefG</i>	Protein required for KefB activity				0.4	0.5								
<i>trkA</i>	K ⁺ transporter			2.3	2.6									
<i>znuA</i>	Zn ²⁺ ABC transporter	Zur (-)		2.6	6.5	11.2	10.0	5.2			3.4	4.3	7.2	6.4
<i>mscL</i>	mechanosensitive channel	RpoS (+)				0.4	0.4							
<i>mscS</i>	mechanosensitive channel	RpoS (+)		2.9	4.7	3.9					2.3	4.6	3.8	2.6
<i>aqpZ</i>	Aquaporin				0.3	0.4	0.4							
<i>ompC</i>	Outer membrane porin C	CpxR (-), OmpR (-)			2.1		3.1	2.5						
<i>ompF</i>	Outer membrane porin F	CpxR (-), OmpR (-)	0.5	0.2	0.2	0.0	0.1	0.2			0.2	0.0	0.0	0.0

C

Gene	Product/Function	Relevant regulatory proteins	Fold change down-regulated						Fold change up-regulated					
			30+	20-30	10-20	5-10	2-5	0.0	2-5	5-10	10-20	20-30	30+	
			CORM-401 AEROBIC CONDITIONS						CORM-401 ANAEROBIC CONDITIONS					
Time						Time								
2.5	5.0	10.0	20.0	40.0	80.0	2.5	5.0	10.0	20.0	40.0	80.0			
<i>baeR</i>	DNA-binding response regulator	CpxR (+), BaeR (+)			4.2	5.9	6.6	4.3			3.7	5.3	4.8	3.5
<i>baeS</i>	Sensory histidine kinase	CpxR (+), BaeR (+)		2.3	25.1	45.4	67.4	19.7			31.0	38.9	25.2	14.3
<i>acrD</i>	AcraD-TolC multidrug efflux system; permease subunit	CpxR (+), BaeR (+)	4.8	10.7	15.2	11.2	10.9	6.9		8.9	11.3	11.1	8.2	7.6
<i>marA</i>	DNA-binding transcriptional dual regulator	CpxR (+)	3.0	3.3	4.9	4.7	7.8	2.7	3.6	11.4	16.7	28.6	47.8	20.4
<i>marB</i>	Polypeptide: multiple antibiotic resistance protein	CpxR (+)		2.2		4.4	6.5		3.3	5.6	13.9	21.7	26.7	10.3
<i>marR</i>	DNA-binding transcriptional repressor	CpxR (+)				2.5	3.2			5.1	8.3	10.9	20.2	10.8
<i>mdtA</i>	Multidrug efflux system; subunit A	CpxR (+), BaeR (+)	12.4	29.6	46.3	63.2	41.8	17.1	12.4	35.2	63.0	59.9	60.2	39.3
<i>mdtB</i>	Multidrug efflux system; subunit B	CpxR (+), BaeR (+)	4.4	24.3	32.6	42.5	30.2	10.8		10.4	24.8	23.0	20.8	18.4
<i>mdtC</i>	Multidrug efflux system; subunit C	CpxR (+), BaeR (+)		9.8	24.7	22.5	24.2	8.6		7.9	22.4	29.8	21.5	17.0
<i>mdtD</i>	Predicted transport protein	CpxR (+), BaeR (+)				2.0					2.6	2.7	2.4	2.3
<i>spy</i>	Envelope stress protein	CpxR (+), BaeR (+)	34.3	124.4	357.1	330.3	420.8	250.2	14.3	200.4	585.0	505.5	672.6	556.4
<i>slt</i>	Soluble lytic murein transglycosylase	CpxR (+)		2.5	3.8	3.4	3.5	2.5		2.5	3.8	3.4	3.5	2.5
<i>degP</i>	Serine protease	CpxR (+)		2.2		7.5	7.4	4.1		2.0	18.9	18.5	23.9	12.0
<i>cpxA</i>	Sensory histidine kinase	CpxR (+)				2.0				2.0	2.9	2.8	2.1	2.5
<i>cpxP</i>	Inhibitor of the Cpx response	CpxR (+)	12.3	17.2	17.7	13.6	16.8	10.6	4.6	11.9	17.0	12.9	11.5	5.9
<i>cpxR</i>	Transcriptional dual regulator	CpxR (+)		2.3	3.4	3.2	2.6	2.0		3.1	3.4	3.4	3.3	3.1

SUPPLEMENTARY FIG. S3. Differential expression of genes, both aerobically and anaerobically, in response to 67 μM CORM-401, involved in (A) the respiratory chains, (B) osmoregulation, and (C) general stress responses, metal ion stress, and cell envelope stress. Values within each cell are fold changes in transcript levels relative to the no-CORM condition. The heat map (above) also quantifies the changes elicited in selected genes.



SUPPLEMENTARY FIG. S4. CORM-401 enhances the antimicrobial effects of cefotaxime and trimethoprim with additive effects on growth and viability. In (A) and (B), bacterial growth was followed in Evans medium with glucose until $OD_{600nm} \sim 0.3$ was reached. CORM-401 and antibiotics were then added to the cultures; OD readings and samples for viability were taken at regular intervals. In (A) (cefotaxime) and (B) (trimethoprim), *squares* show control cultures, *triangles* are antibiotic alone ($1 \mu\text{g ml}^{-1}$), *inverted triangles* are CORM-401 alone ($100 \mu\text{M}$), and *diamonds* are antibiotic and CORM combined. In (C) (cefotaxime) and (D) (trimethoprim), *black columns* show control cultures, *diagonal hatched bars* are antibiotic alone ($1 \mu\text{g ml}^{-1}$), *horizontally striped bars* are CORM-401 alone ($100 \mu\text{M}$), and *open bars* are antibiotic and CORM combined. $n = 3 \pm \text{SD}$. *, $p < 0.05$ (T-test).