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Supplementary Material

Supplementary Material

Carbon monoxide-releasing antibacterial molecules target respiration and global transcriptional regulators

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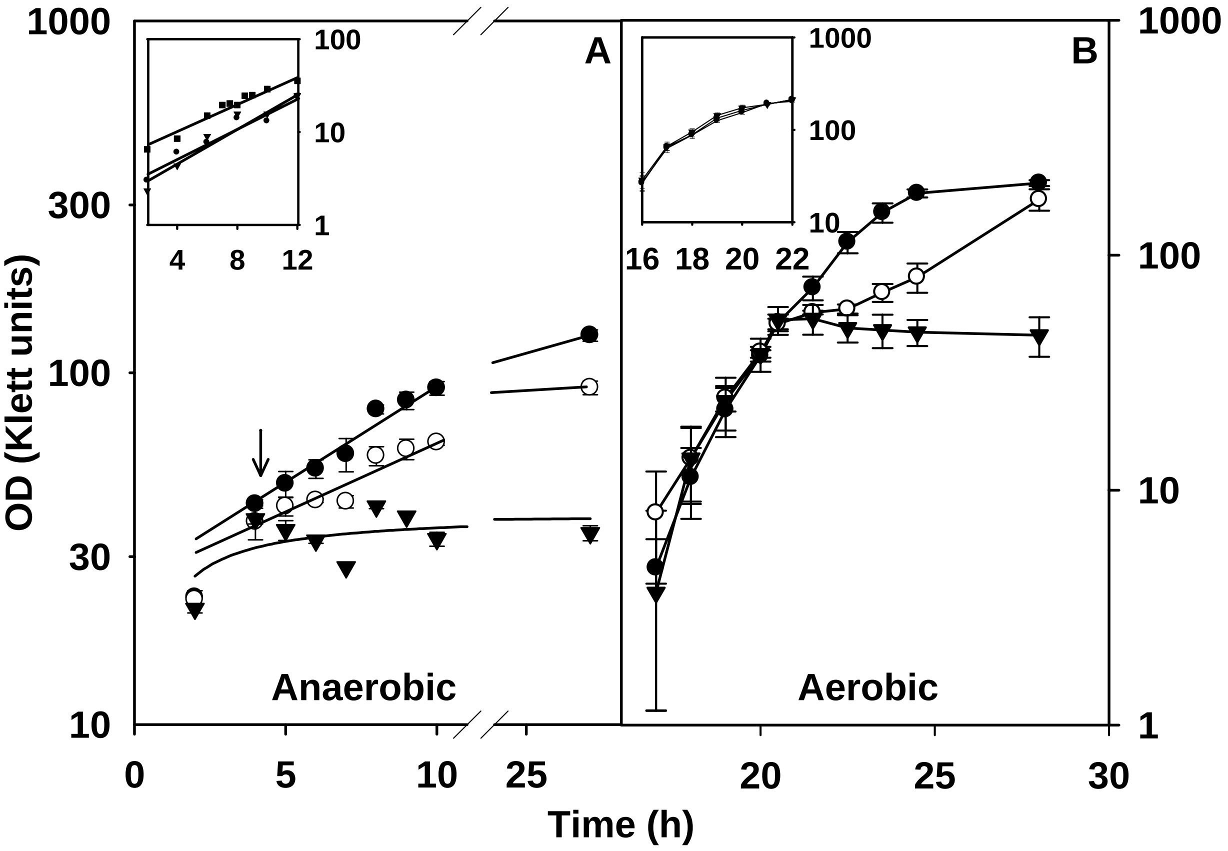
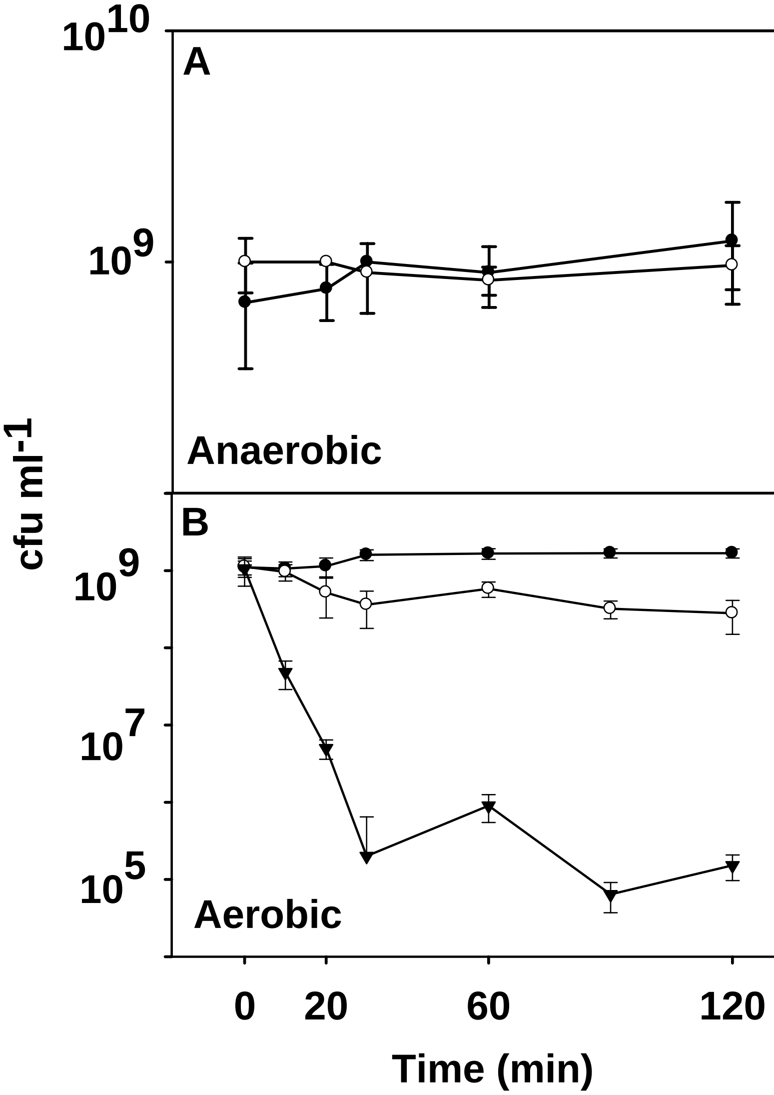
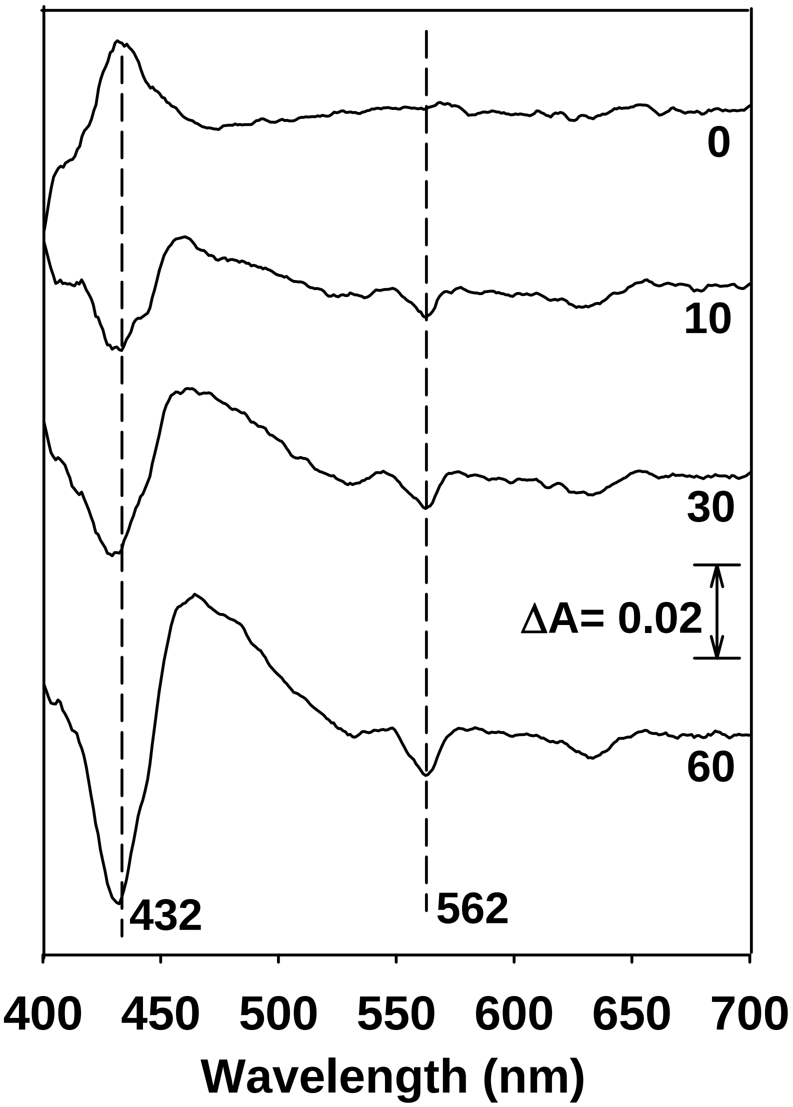
Supplementary Figure S1. Inhibition by CORM-3 of *E. coli* cultures grown in defined medium anaerobically (A) and aerobically (B). Compounds were added at the times indicated by the arrows. Main panel in A shows addition of 100 µM (-○-) and 200 µM (-▼-) CORM-3; main panel in B shows addition of 30 µM (-○-) and 100 µM (-▼-) CORM-3. Controls are shown by closed circles (-●-). Inset A shows, prior to inoculation, addition of 250 µM $RuCl_2(DMSO)_4$ (-▼-) or CO gas-saturated solution (-■-), and comparison with the control (-●-). Inset B shows addition, prior to inoculation, of 250 µM $RuCl_2(DMSO)_4$ (-▼-) and CO gas-saturated solution (-■-), and the control (-●-). Data points are means plus standard deviations.



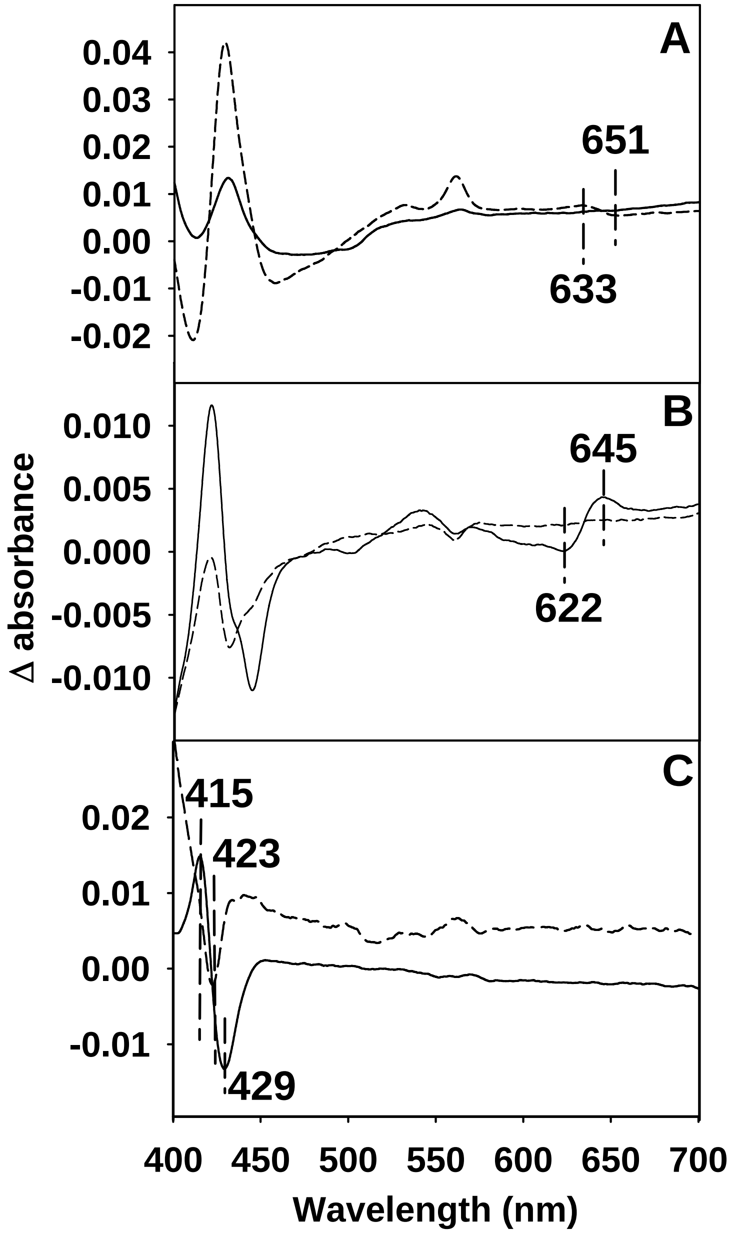
Supplementary Figure S2. Viability assays showing survival of anaerobically (A) and aerobically (B) *E. coli* in defined growth medium. After entry into log phase, CORM-3 was added (t = 0) and samples taken for determination of colony-forming units (cfu). A shows addition of 100 μM CORM-3 (-○-); B shows addition of 30 μM (-○-) and 125 μM (-▼-) CORM-3. Controls are shown by closed circles (-●-).



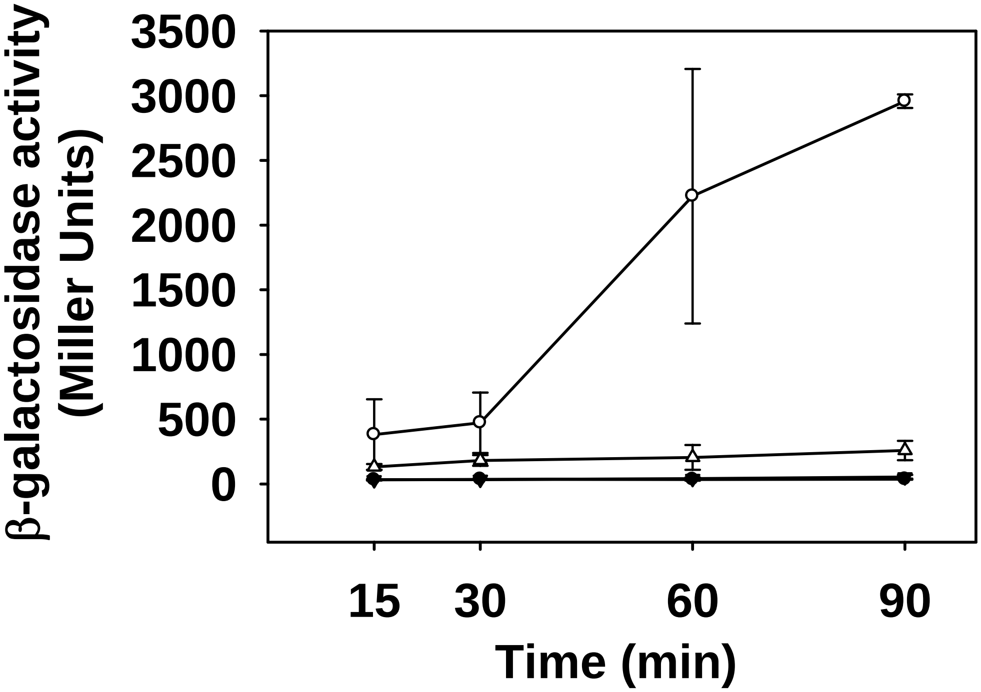
Supplementary Figure S3. Reaction of terminal oxidases *in vivo* on addition of $RuCl_2(DMSO)_4$ to intact cells in a dual-wavelength spectrophotometer. CO difference spectra (reduced + CO *minus* reduced) were taken at times (shown in min) after addition of the compound.



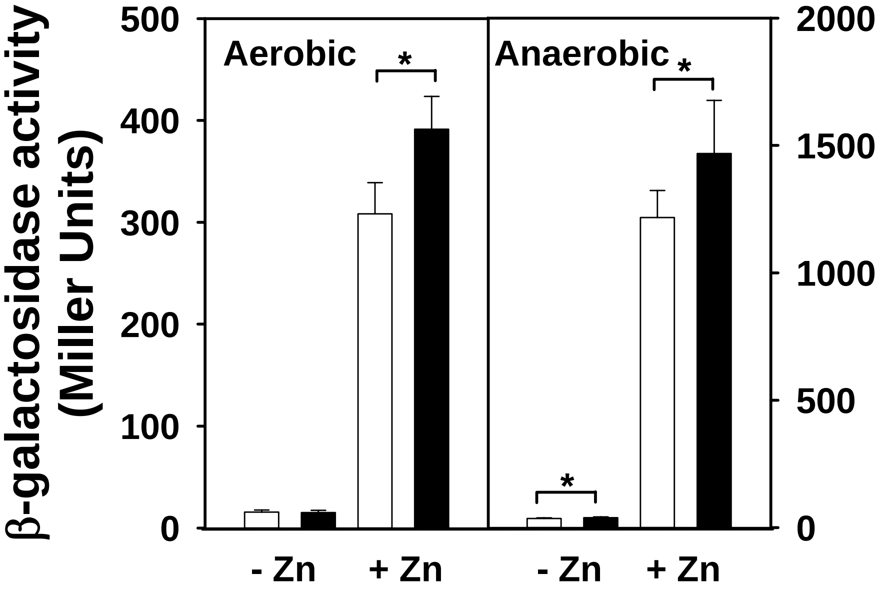
Supplementary Figure S4. CORM-3 generates carbonmonoxycytochrome *bd* in vivo and depresses synthesis of cytochrome *bo'*. A shows the reduced *minus* oxidized difference spectra of a *cyo* mutant grown aerobically in the presence of 30 µM CORM-3 (dashed line) or its absence (solid line), and B shows the CO difference spectrum of the mutant grown in the presence of CORM-3 (dashed line) or its absence (solid line). C presents deconvoluted CO difference spectra to reveal cytochrome *bo'* in cells grown in the absence of CORM-3 (solid line) and its absence in cells grown with CORM-3 (dashed line).



Supplementary Figure S5. Expression of *spy-lacZ* activity and the effects of CORM-3. A strain harbouring Φ (*spy-lacZ*) was grown anaerobically and exposed at mid-logarithmic phase (30-40 Klett units) to 100 µM CORM-3 (open circles), 200 µM $CuSO_4$ (open triangles), 100 µM $RuCl_2(DMSO)_4$ (solid triangles) or left as a control (solid circles).



Supplementary Figure S6. Expression of *zntA-lacZ* activity and the effects of CORM-3. A strain harbouring Φ (*zntA-lacZ*) was grown aerobically and anaerobically and exposed at mid-logarithmic phase (30-40 Klett units) to 30 μM CO-RM (aerobic) or 100 μM CO-RM (anaerobic), shown by the black bars, either in the presence (0.5 mM) or absence of additional $ZnSO_4$. White bars show control cultures. Asterisks show significant difference from control at $P < 0.05$.



Supplementary Table S1. Genes down-regulated >2-fold after addition of 30 µM CORM-3 aerobically

Gene	Alternative name	Fold change	P Value	Description
aceA		-2.02	0.0050	Isocitrate lyase; acetate utilization
aceB		-2.43	0.0234	Malate synthase A; glyoxylate shunt enzyme
acnA		-3.31	0.0003	Aconitase A; stationary phase induced
acnB		-5.82	0.0003	Aconitase B; apo-enzyme binds mRNA for negative translational autoregulation; iron-sulfur cluster
acs		-18.50	0.0000	Acetyl CoA synthetase
actP	*yjcG*	-10.90	0.0000	Acetate transporter; member of solute:sodium symporter (SSS) family
aldA		-17.90	0.0000	Subunit of aldehyde dehydrogenase A, NAD-linked
arcA		-3.15	0.0014	Response regulator prot ein represses aerobic genes under anaerobic growth conditions, activates some anaerobic genes; phosphorylated by ArcB
argT		-8.44	0.0000	Lysine/arginine/ornithine transporter subunit
aroC		-2.17	0.0037	Chorismate synthase
aspA		-2.23	0.0285	L-Aspartate ammonia-lyase (L-aspartase)
atpC		-2.04	0.0184	Membrane-bound ATP synthase; F1 sector; epsilon-subunit
atpD		-2.69	0.0001	Membrane-bound ATP synthase; F1 sector; beta-subunit
atpI		-2.16	0.0062	Membrane-bound ATP synthase subunit; F1-F0-type proton-ATPase
b0725		-2.32	0.0302	Unknown function, cytoplasmic
bcsC	*yhjL*	-2.24	0.0436	Oxidase involved in cellulose synthesis
betI		-2.12	0.0259	Regulatory gene; perhaps repressor for choline regulation of bet genes
bfr		-2.76	0.0016	Bacterioferritin; negatively regulated by *ryhB* RNA as part of indirect positive regulation by Fur
btuB		-2.03	0.0361	Vitamin B12/cobalamin outer membrane transporter; member of outer membrane receptor family(OMR); receptor for E colicins; phage BF23
can	*yadF*	-2.99	0.0176	Carbonic anhydrase 2
cfa		-3.04	0.0284	Cyclopropane fatty acid synthase
cmoA	*yecO*	-2.40	0.0433	Predicted methyltransferase
crl		-2.29	0.0003	Regulatory protein for curli (cryptic *csgA*)
cspC		-2.16	0.0187	Cold shock protein homolog; multicopy suppresses *mukB* mutants; constitutively expressed at 37°C; affects *rpoS* and *uspA* expression
cspE		-2.90	0.0007	Cold shock protein homolog
cstA		-13.30	0.0000	Starvation-induced protein involved in peptide utilization during carbon starvation
cynX		-2.07	0.0211	Predicted cyanate transporter; member of major facilitator superfamily (MFS)
cyoA		-17.30	0.0000	Cytochrome *o* oxidase subunit II; cytochrome bo(3) ubiquinol oxidase subunit II
cyoB		-22.10	0.0000	Cytochrome *o* oxidase subunit I; cytochrome bo(3) ubiquinol oxidase subunit I
cyoC		-14.00	0.0000	Cytochrome *o* oxidase subunit III; cytochrome bo(3) ubiquinol oxidase subunit III
cyoD		-12.70	0.0000	Cytochrome *o* oxidase subunit IV; cytochrome bo(3) ubiquinol oxidase subunit IV
cyoE		-10.40	0.0000	Cytochrome *o* oxidase subunit; protoheme IX farnesyltransferase
dadX		-2.88	0.0038	Alanine racemase; homodimeric
dctA		-4.72	0.0001	C4-dicarboxylic acid; orotate and citrate transport protein; member of dicarboxylate/amino acid: cation (Na or proton) symporter
degS		-2.10	0.0007	Serine endoprotease degrades periplasmic RseA; activating RpoE; multicopy supplement of prc periplasm, may be anchored to inner membrane; essential gene
dppA		-2.53	0.0021	Dipeptide transporter subunit; periplasmic-binding protein; member of ABC superfamily
dppD		-2.06	0.0042	Dipeptide transporter subunit; ATP-binding component; member of ABC superfamily
efeO	*ycdF*	-2.93	0.0061	Conserved protein, subunit of EfeU/EfeO/EfeB ferrous iron transporter; cryptic

efeU	*ycdN*	-3.64	0.0031	Hypothetical protein of the OFeT transport family , subunit of EfeU/EfeO/EfeB ferrous iron transporter; cryptic
elaB		-2.97	0.0233	Function unknown, membrane anchored
elbB		-2.17	0.0258	Involved in isoprenoid biosynthesis
fadB		-3.49	0.0001	Alpha subunit of the fatty acid oxidation multienzyme complex
fadE	*yafH*	-3.34	0.0017	Medium-long-chain fatty acyl-CoA dehydrogenase; *fadR* regulon; starvation inducible
fadI		-3.32	0.0110	Beta-ketoacyl-CoA thiolase; anaerobic beta-oxidation complex II; in FadR regulon
fadJ		-2.09	0.0090	Anaerobic B-oxidation complex II + 3-hydroxyacyl-CoA dehydrogenase; putative beta-hydroxybutyryl-CoA epimerase; enoyl-CoA hydratase activity; FadR regulated
fadL		-2.20	0.0094	Fatty acid transport protein; outer membrane
fdoG		-8.98	0.0000	Formate dehydrogenase-O subunit; major
fdoH		-7.70	0.0000	Formate dehydrogenase-O subunit; Fe-S
fdoI		-6.59	0.0001	Formate dehydrogenase-O subunit cytochrome b556
fliC		-3.81	0.0005	Flagellin; structural gene; flagellar regulon
folE		-2.39	0.0021	GTP cyclohydrolase I
fumA		-4.66	0.0026	Fumarase A; aerobic; negatively regulated by *ryhB* RNA as part of indirect positive regulation by Fur
fumC		-4.77	0.0101	Fumarase C; aerobic; member of *soxRS* regulon
gabD		-3.67	0.0011	Succinate-semialdehyde dehydrogenase; NADP dependent
gabP		-2.30	0.0447	Gamma-aminobutyrate transporter; member of amino-acid-polyamine-organocation superfamily (APC)
gabT		-5.09	0.0007	Aminobutyrate aminotransferase
gatA		-2.36	0.0001	Galactitol-specific enzyme IIA component of PTS; member of PTS galactitol (Gat) family
gatR_2		-2.48	0.0031	Malate synthase G
glcB		-5.82	0.0002	Malate synthase G
glcC		-4.53	0.0376	Regulatory gene for *glc* operon
glcD		-2.22	0.0152	Regulatory protein; transcriptional activator
glcF		-3.99	0.0010	Glycolate oxidase subunit; FeS protein
glcG		-4.06	0.0000	Protein of unknown function encoded by a gene within the glycolate utilization operon
glpA		-2.84	0.0003	Glycerol-3-phosphate dehydrogenase (anaerobic) large subunit
glpB		-2.82	0.0051	sn-Glycerol-3-phosphate dehydrogenase (anaerobic) subunit; membrane anchor
glpD		-2.95	0.0000	Glycerol-3-phosphate dehydrogenase (aerobic)
glpE		-2.36	0.0025	Thiosulfate:cyanide sulfurtransferase (rhodanase); in glpEGR operon; induced by glycerol
glpF		-3.73	0.0001	Glycerol facilitator; member of major intrinsic protein family (MIP)
glpK		-2.48	0.0003	Glycerol kinase
glpQ		-4.26	0.0183	Glycerol-3-phosphate diesterase; periplasmic
glpT		-5.54	0.0000	sn-glycerol-3-phosphate transporter; member of major facilitator superfamily (MFS)
glpX		-3.13	0.0003	Fructose 1;6 bisphosphatase; induced by glycerol; in *glpFKX* operon; not required for growth on glycerol
gltA		-2.96	0.0000	Citrate synthase
gltI	*ybeJ*	-4.54	0.0082	Glutamate and aspartate transporter subunit; periplasmic-binding protein; member of ABC superfamily
gltK		-2.25	0.0086	Glutamate and aspartate transporter subunit; membrane component; member of ABC superfamily
gpmA		-2.01	0.0005	Phosphoglycerate mutase 1; 2;3-bisphosphoglycerate-dependent; Fur regulon; dimeric
hcaR		-2.19	0.0112	Transcriptional LysR-type activator of the *hca* operon; autoregulatory
hcaR-r		-2.61	0.0035	
idnD		-2.94	0.0109	L-idonate 5-dehydrogenase
lhgO	*ygaF*	-6.65	0.0007	L-2-hydroxyglutarate oxidase
ilvB		-2.16	0.0133	Acetohydroxy acid synthase I (AHAS-I); acetolactate synthase I (ALS-I);

				valine sensitive; large subunit
ilvC		-4.09	0.0001	Ketol-acid reductoisomerase
ilvE		-2.60	0.0030	Branched-chain amino acid aminotransferase
ivbL		-2.76	0.0072	*ilvB* operon leader peptide
katE		-2.76	0.0001	Catalase hydroperoxidase III
kgtP		-6.69	0.0001	Alpha-ketoglutarate transporter; member of major facilitator superfamily (MFS)
lamB		-2.62	0.0001	Maltose outer membrane porin (maltoporin); member of sugar porin family (SP); phage lambda receptor protein
lipA		-2.17	0.0084	Lipoate synthase; lipoic acid biosynthesis; iron-sulfur protein; SAM-dependent chemistry
livJ		-3.96	0.0000	Leucine/isoleucine/valine transporter subunit; periplasmic-binding protein; member of ABC superfamily
lldP		-5.47	0.0028	L-lactate permease; member of lactate permease family (LctP)
lldR		-2.25	0.0362	Regulatory gene for *lld* operon
lpdA		-6.19	0.0314	Lipoamide dehydrogenase (NADH); glycine cleavage system L protein; binds Zn(II)
lsrG		-2.05	0.0099	Function unknown; part of an operon for autoinducer-2 (AI-2) uptake in Salmonella regulated by LsrR and LuxS; in Salmonella; *ygiN* paralog
maeB		-4.09	0.0001	Putative NADP+-linked malic enzyme
malK		-4.13	0.0001	Maltose transporter subunit; ATP-binding component; member of ABC superfamily
manY		-2.64	0.0005	Mannose-specific enzyme IIC component of PTS; member of PTS mannose-fructose-sorbose (Man) family
mdh		-3.20	0.0027	Malate dehydrogenase; NAD-dependent
mglA		-5.22	0.0000	Methyl-galactoside transporter subunit; ATP-binding component; member of ABC superfamily
mglB		-8.30	0.0000	Methyl-galactoside transporter subunit; periplasmic-binding protein; member of ABC superfamily; receptor for galactose taxis
mglC		-5.82	0.0001	Methyl-galactoside transporter subunit; membrane component; member of ABC superfamily
mhpR		-2.04	0.0157	Regulatory gene for MHP utilization
modF		-2.74	0.0043	Molybdate transporter subunit; ATP-binding component; member of ABC superfamily
msrB	*yeaA*	-3.96	0.0161	Methionine sulfoxide reductase B; specific for met-R-(o) diastereoisomers within proteins; mutant cadmium sensitive; free met-R-(o) is reduced by MsrB
ndk		-13.70	0.0000	Nucleoside diphosphate kinase
nikC		-2.12	0.0404	Nickel transporter subunit; membrane component; member of ABC superfamily
nlpD		-2.19	0.0005	New lipoprotein may function in cell wall formation; has M37 metalloprotease domain
nmpC		-2.36	0.0466	Outer membrane porin; in cryptic prophage DLP12; interrupted by IS5B in K-12
nuoA		-3.24	0.0056	NADH:quinone oxidoreductase subunit A; complex I; NADH dehydrogenase I
nuoB		-2.29		NADH:quinone oxidoreductase subunit B; complex I; NADH dehydrogenase I
nuoC		-3.67	0.0003	NADH:quinone oxidoreductase subunit C; complex I; NADH dehydrogenase I
nuoE		-2.83	0.0003	NADH:quinone oxidoreductase subunit E; complex I; NADH dehydrogenase I
nuoF		-2.38	0.0036	NADH:quinone oxidoreductase subunit F; complex I; NADH dehydrogenase I
nuoG		-2.74		NADH:quinone oxidoreductase subunit G; complex I; NADH dehydrogenase I
nuoH		-3.04	0.0002	NADH:quinone oxidoreductase subunit H; complex I; NADH dehydrogenase I
nuoI		-2.50	0.0000	NADH:quinone oxidoreductase subunit I; complex I; NADH dehydrogenase I
nuoJ		-2.57	0.0009	NADH:quinone oxidoreductase subunit J; complex I; NADH dehydrogenase I
nuoK		-2.23		NADH:quinone oxidoreductase subunit K; complex I; NADH dehydrogenase I
nuoL		-2.33	0.0103	NADH:quinone oxidoreductase subunit L; complex I; NADH dehydrogenase

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ompW		-2.69	0.0298	Outer membrane protein; colicin S4 receprtor
osmE		-3.19	0.0110	Promoter overlaps *nadE* promoter; regulated by growth phase as well as osmotic pressure
osmY		-2.37	0.0238	Periplasmic; RpoS dependent protein (stationary phase)
pckA		-4.07	0.0006	Phosphoenolpyruvate carboxykinase (ATP)
pfkB		-2.19	0.0046	Phosphofructokinase; Pfk-2 (PFK II);promoter activation mutation increases expression and suppresses *pfkA* mutations; tetrameric; allosteric: inhibited by ATP
pheP		-2.04	0.0429	Phenylalanine transporter; member of amino-acid-polyamine-organocation superfamily (APC)
phoH		-4.55	0.0043	ATP-binding protein of unknown function; *pho* regulon
ppc		-2.28	0.0011	Phosphoenolpyruvate carboxylase
ptsG		-2.42		Glucose-specific enzyme IIBC component of PTS; member of PTS glucose-glucoside (Glc) Family
purU		-2.08	0.0005	Formyltetrahydrofolate hydrolase; mutation causes transient glycine starvation
PuuD	*ycjL*	-2.32	0.0456	Gamma-glutamyl-γ-aminobutyrate hydrolase , subunit of γ-glutamyl-γ-aminobutyrate hydrolase
pyrE		-2.35	0.0153	Orotate phosphoribosyltransferase
rbsB		-2.63	0.0008	D-ribose transporter subunit; periplasmic-binding protein; member of ABC superfamily
rbsK		-2.42	0.0132	Ribokinase
rpoS		-2.11	0.0020	Sigma S subunit of RNA polymerase; stationary phase regulator
rsd	*yjaE*	-2.15	0.0470	Stationary phase protein; binds sigma 70 RNA polymerase subunit
sdhA		-19.00	0.0000	Succinate dehydrogenase (SQR) flavoprotein subunit; negatively regulated by *ryhB* RNA as part of indirect positive regulation by Fur
sdhA-r		-12.10	0.0000	
sdhB		-14.80	0.0000	Succinate dehydrogenase iron-sulfur protein; negatively regulated by *ryhB* RNA as part of indirect positive regulation by Fur
sdhC		-22.50	0.0000	Succinate dehydrogenase membrane anchor subunit; cytochrome b556; negatively regulated by *ryhB* RNA as part of indirect positive regulation by Fur
sdhD		-16.10	0.0000	Succinate dehydrogenase hydrophobic subunit; negatively regulated by *ryhB* RNA as part of indirect positive regulation by Fur
slpA		-2.40	0.0075	FKBP-type peptidyl-prolyl cis-trans isomerase/Integrase gene within defective prophage CP4-57
sodA		-3.83	0.0077	Member of SoxRS regulon; superoxide dismutase; Mn
sodC		-2.12	0.0262	Superoxide dismutase; Cu; Zn
sstT		-3.21	0.0090	Sodium:serine/threonine symporter; member of dicarboxylate/amino acid:cation symporter family (DAACS)
stfE	*b1157*	-2.54	0.0272	Phage lambda *stf* gene homolog in prophage e14
sucA		-11.80	0.0000	Alpha-ketoglutarate dehydrogenase; E1 component
sucB		-9.31	0.0005	Dihydrolipoamide succinyltransferase component of alpha-ketoglutarate dehydrogenase complex (E2); acid-inducible
sucC		-6.46	0.0000	Succinyl CoA synthetase beta-subunit; acid-inducible
sucD		-8.98	0.0000	Succinyl CoA synthetase alpha-subunit
talA		-4.79	0.0089	Transaldolase A; *creBC* regulon
thiC		-2.09	0.0065	Hydroxymethylpyrimidine synthesis; thiamin pyridine moiety biosynthesis
thiG		-2.29	0.0040	Thiamin-thiazole moiety synthesis; complexes with ThiS
thiH		-2.74	0.0021	Thiamin-thiazole moiety synthesis
tnaA		-4.74	0.0002	Tryptophanase
tpx		-6.10	0.0000	Thioredoxin-linked thiol peroxidase; induced by acid or base
ubiC		-2.52	0.0013	Ubiquinone
ucpA		-2.03	0.0021	Short-chain dehydrogenase/reductase homolog
udhA		-2.66	0.0080	Soluble pyridine nucleotide transhydrogenase
wrbA		-2.98	0.0045	Affects association between Trp repressor and operators in stationary phase
yagU		-6.10	0.0003	Function unknown, integral membrane protein

yahK		-2.53	0.0253	Predicted oxidoreductase, Zn-dependent and NAD(P)-binding
yahO		-3.19	0.0014	Function unknown; Salmonella ortholog regulated by RpoS
ybiC		-5.02	0.0000	Predicted dehydrogenase
yccJ		-2.51	0.0363	Function unknown, cytoplasmic
yceA		-2.87	0.0078	Function unknown, cytoplasmic
ycgB		-2.31	0.0062	Function unknown; Salmonella ortholog regulated by RpoS, cytoplasmic; putative sporulation protein
ychH		-4.05	0.0006	Function unknown; putative membrane protein transcribed divergently from pth; two transmembrane helices predicted
yciE		-2.38	0.0455	Function unknown, cytoplasmic
ydcA		-5.03	0.0002	Function unknown
ydcI	*b1422*	-5.06	0.0003	Putative transcriptional regulator LysR-type
ydcV		-2.13	0.0095	Predicted spermidine/putrescine transporter subunit; membrane component; member of ABC superfamily
ydiZ	*b1724*	-3.15	0.0030	Polypeptide: predicted protein
ydjA		-2.17	0.0139	Predicted oxidoreductase , subunit of predicted oxidoreductase
yeaC	*b1777*	-4.27	0.0002	Polypeptide: conserved protein
yeaQ		-2.49	0.0029	Function unknown
yeiM		-2.12	0.0072	Predicted nucleoside transporter; member of concentrative nucleoside transporter family (CNT)
yejG		-2.58	0.0042	Polypeptide: predicted protein, function unknown, cytoplasmic
ygaM		-2.35	0.0003	Function unknown, membrane anchored
ygjG		-2.34	0.0487	Putrescine transaminase
yhjE		-2.40	0.0063	Predicted transporter; member of major facilitator superfamily (MFS), integral membrane protein
yjcH		-11.60	0.0005	Function unknown, integral membrane protein
ylaC		-2.14	0.0410	Function unknown, integral membrane protein
ynaJ		-2.91	0.0262	Function unknown, integral membrane protein
yqhA		-2.14	0.0402	Function unknown, integral membrane protein

Supplementary Table S2. Genes up-regulated >2-fold after addition of 30 µM CORM-3 aerobically

Gene	***Alternative name***	***Fold change***	***P Value***	***Description***
acrD		6.31	0.0067	Aminoglycoside/multidrug efflux system; member of resistance-nodulation-cell division superfamily (RND)
ara7-8		2.18	0.0449	
argA		4.42	0.0180	N-acetylglutamate synthase; first step in arginine biosynthesis; amino-acid acetyltransferase; growth on acetylornithine
argC		2.50	0.0193	N-acetyl-gamma-glutamyl-phosphate reductase
argG		3.11	0.0134	Argininosuccinate synthetase
argH		2.37	0.0179	Argininosuccinate lyase
aroF		7.38	0.0000	3-deoxy-d-arabino-heptulosonate-7-phosphate (DAHP) synthase; tyrosine repressible; TyrR regulon
artJ		3.15	0.0081	Arginine transporter subunit; periplasmic-binding protein; member of ABC superfamily
baeR		3.31	0.0264	Response regulator for *mdtABCD* and *acrD*
baeS		4.98	0.0001	Sensor kinase for *mdtABCD* and *acrD* regulation
caiA		3.44	0.0301	Crotonobetaine reductase; CII component
cheR		3.08	0.0107	Protein methyltransferase (in chemotactic response); flagellar regulon
cpxP		23.50	0.0000	Periplasmic protein; CpxA/R activated; induced in alkaline pH; suppresses toxic envelope protein effects
cydA		22.90	0.0019	Cytochrome *d* (bd-I) terminal oxidase subunit I
cydB		2.50		Cytochrome *d* (bd-I) terminal oxidase subunit II
cydD		2.52	0.0170	ATP-binding cassette membrane transporter; *bd*-type oxidase
dacC		2.40	0.0001	Penicillin binding protein
ftnB	*yecI*	6.74	0.0395	Function unknown; ferritin-like protein
glnK		3.90		Regulated through NRI/NRII 2-component regulatory system; potent activator of NRII(GlnL/NtrB) phosphatase; binds to membrane when inhibiting AmtB
gltB		2.55	0.0301	Glutamate synthase (GOGAT); large subunit
hisC		2.56	0.0330	Histidinol-phosphate aminotransferase
hisH		3.09	0.0366	Amidotransferase component of imidazole glycerol phosphate (IGP) synthase
htpX		2.48	0.0000	Heat shock protein
mdtA	*b2074*	6.18	0.0000	Multidrug efflux system subunit of heterodimeric MdtABC system; member of resistance-nodulation-cell division superfamily (RND)
mdtB	*yegN*	13.10	0.0006	Multidrug efflux system subunit of heterodimeric MdtABC system; member of resistance-nodulation-cell division superfamily (RND)
mdtC	*yegO*	7.60	0.0025	Multidrug efflux system subunit of heterodimeric MdtABC system; member of resistance-nodulation-cell division superfamily (RND)
metF		10.50	0.0083	5;10-Methylenetetrahydrofolate reductase
mutL		3.84	0.0016	Methyl-directed mismatch repair
ompC		5.50	0.0000	Outer membrane protein 1b (Ib; c)
ompR		8.78	0.0001	Activator protein for osmoregulation of OmpC and OmpF
ompX		8.59	0.0040	Outer membrane protein; with role in inducing RNAP-sigma E production; induced by acid or base
pspA		3.86	0.0203	Negative regulatory gene for phage-shock-protein *psp* operon; binds PspB and PspC
pspD		3.63	0.0447	Expressed protein in *psp* operon; peripheral inner membrane protein
pstA		3.20		Phosphate transporter subunit; membrane component; member of ABC superfamily
pstB		2.44	0.0127	Phosphate transporter subunit; ATP-binding component; member of ABC superfamily
pstC		5.07	0.0128	Phosphate transporter subunit; membrane component; member of ABC superfamily
pstS		3.52	0.0033	Phosphate transporter subunit; periplasmic-binding protein; member of ABC superfamily
rstA		6.07		Putative response regulator
sbp		2.48	0.0077	Sulfate transporter subunit; periplasmic-binding protein; member of ABC

				superfamily
sdaA		4.70	0.0149	L-Serine deaminase
slt		3.46	0.0328	Lytic transglycosylase; major autolysin FK-506-BP-like lysis protein for phiX174; metal ion-regulated peptidyl-prolyl
spy		2.53	0.0000	Periplasmic protein induced by zinc; part of *cpx* regulon
tgt-r		26.10	0.0248	
tyrA		2.27	0.0021	Chorismate mutase T-prephenate dehydrogenase; bifunctional; TyrR regulon
ybjG		4.96	0.0000	Enzyme: undecaprenyl pyrophosphate phosphatase
yccA		5.54	0.0001	Membrane protein; degraded by FtsH protease
ycfS		4.03	0.0364	Function unknown, periplasmic
ycgK		6.61	0.0021	Function unknown, periplasmic
yebE		4.56	0.0000	Polypeptide: conserved protein, mambrane anchored
yedX		15.00	0.0401	Function unknown, periplasmic
yedY		5.02	0.0138	Periplasmic reductase of unknown function
yfaZ		3.95	0.0157	Predicted outer membrane protein
yhdV		2.86	0.0221	Function unknown, outer membrane lipoprotein
yiaD		3.26	0.0017	Function unknown, outer membrane lipoprotein
yiiM		4.37	0.0100	Protein involved in base analog detoxification
yjfN		3.02	0.0005	Polypeptide: predicted protein, function unknown, periplasmic
ykgM		7.58	0.0004	Predicted ribosomal protein
ymgD	*b1171*	9.82	0.0001	Polypeptide: predicted protein, function unknown, periplasmic
yncJ		33.30	0.0000	Function unknown, periplasmic
yodA	*b1973*	19.10	0.0000	Periplasmic cadmium binding protein; induced by cadmium and peroxide; binds zinc; nickel; cadmium; SoxS and Fur regulated
yqaE		16.10	0.0447	Predicted membrane protein
znuA	*yebL*	3.14	0.0004	Zinc transporter subunit; periplasmic-binding protein; member of ABC superfamily

Supplementary Table S3. Genes down-regulated >2-fold after addition of 100 μM CORM-3 anaerobically

Gene	Alternative name	Fold change	P Value	Description
aceA		-3.15	0.0001	Isocitrate lyase; acetate utilization
aceB		-3.89	0.0048	Malate synthase A; glyoxylate shunt enzyme
aceF		-2.04	0.0000	Pyruvate dehydrogenase (dihydrolipoyltransacetylase component) E2p; acetate requirement
arcA		-2.13	0.0121	Response regulator protein represses aerobic genes under anaerobic growth conditions, activates some anaerobic genes; phosphorylated by ArcB
cld	*wzzB*	-2.03	0.0440	Regulator of lipopolysaccharide O-chain length; gene studied in Salmonella and non-K-12 strains
cspB		-2.44	0.0354	Cold shock protein homolog; cold-inducible
cydA		-2.95	0.0031	Cytochrome *d* (*bd*-I) terminal oxidase subunit I
cyoB		-3.21	0.0362	Cytochrome *o* oxidase subunit I; cytochrome *bo*(3) ubiquinol oxidase subunit I
dctA		-2.14	0.0171	C4-dicarboxylic acid; orotate and citrate transport protein; member of dicarboxylate/amino acid:cation(Na or H+)symporter
dppA		-3.66	0.0280	Dipeptide transporter subunit; periplasmic-binding protein; member of ABC superfamily
fdoG		-2.34	0.0000	Formate dehydrogenase-O subunit; major
fdoH		-2.12	0.0028	Formate dehydrogenase-O subunit; Fe-S
fdoI		-2.17	0.0365	Formate dehydrogenase-O subunit cytochrome b556
ftnA	*ftn*	-3.24	0.0000	Ferritin; negatively regulated by *ryhB* RNA as part of indirect positive regulation by Fur
gadB		-3.13	0.0163	Glutamate decarboxylase B
glpD		-2.76	0.0000	Glycerol-3-phosphate dehydrogenase (aerobic)
gltA		-2.53	0.0000	Citrate synthase
gpmA		-2.32	0.0000	Phosphoglycerate mutase 1; 2;3-bisphosphoglycerate-dependent; Fur regulon; dimeric
gpmA-r		-2.15	0.0016	
gpsA		-2.18	0.0379	sn-Glycerol-3-phosphate dehydrogenase [NAD(P)+]
hdeD		-2.06	0.0010	Putative membrane transporter; H-NS repressed
hyaA		-2.72	0.0229	Hydrogenase 1 small subunit [NiFe]
ivbL		-3.00	0.0058	*ilvB* operon leader peptide
rsd	*yjaE*	-2.13	0.0275	Stationary phase protein; binds sigma 70 RNA polymerase subunit
sdhC		-3.41	0.0002	Succinate dehydrogenase membrane anchor subunit; cytochrome b556; negatively regulated by *ryhB* RNA as part of indirect positive reg by Fur
speB		-3.13	0.0169	Agmatinase
tdh-r		-2.13	0.0183	
tpx		-2.42	0.0008	Thioredoxin-linked thiol peroxidase; induced by acid or base
trmD		-2.77	0.0389	tRNA (guanine-7)-methyltransferase
wrbA		-2.17	0.0009	Affects association between Trp repressor and operators in stationary phase
xasA		-2.42	0.0005	Predicted glutamate:gamma-aminobutyric acid antiporter;memb of aa-polyamine-organocation superfamily (APC)
ybgK		-2.13	0.0249	Putative carboxylase
ybiC		-2.18	0.0085	Function unknown; lactate; malate dehydrogenase family
ychH		-2.16	0.0007	Function unknown;putative memb protein transcribed divergently from pth;two transmembrane helices predicted
ydcW	*b1444*	-3.27	0.0086	Gamma-aminobutyraldehyde dehydrogenase , subunit of γ-aminobutyraldehyde dehydrogenase
ydjA		-2.00	0.0025	Predicted oxidoreductase , subunit of predicted oxidoreductase
yeaC	*b1777*	-2.16	0.0036	Polypeptide: conserved protein
yeaG		-2.37	0.0372	Function unknown; Salmonella ortholog regulated by RpoS; protein kinase
yeiA		-2.22	0.0499	Predicted oxidoreductase
ynaJ		-2.12	0.0057	Function unknown, integral membrane protein
yqjC		-2.47	0.0479	Function unknown; expressed protein

Supplementary Table S4. Genes up-regulated >2-fold after addition of 100 µM CORM-3 anaerobically

Gene	Alternative name	Fold change	P Value	Description
cpxP	*b3914*	7.28	0.0000	Periplasmic protein; CpxA/R activated; induced in alkaline pH; suppresses toxic envelope protein effects
cusA	*ybdE*	4.59	0.0213	Component of copper/silver efflux system; member of resistance-nodulation-cell division superfamily (RND)
cusB	*ylcD*	6.13	0.0169	Cation efflux system subunit; member of membrane fusion protein family (MFP)
cusC	*ylcB*	3.49	0.0139	Predicted component of copper/silver efflux system; TolC-like outer membrane protein
cusF	*ylcC*	7.00	0.0001	Periplasmic copper-binding protein; predicted component of CusCFBA copper/silver efflux system
cysA		5.06	0.0001	Sulfate/thiosulfate transporter subunit; ATP-binding component; member of ABC superfamily
cysH		4.04	0.0010	Phosphoadenylyl sulfate (PAPS) reductase
cysP		2.74	0.0485	Thiosulfate transporter subunit; periplasmic-binding protein; member of ABC superfamily
cysU		3.00	0.0476	Sulfate/thiosulfate transporter subunit; membrane component; member of ABC superfamily
cysW		2.60	0.0065	Sulfate/thiosulfate transporter subunit; membrane component; member of ABC superfamily
htpX		2.85	0.0058	Heat shock protein
mdtA	*b2074*	7.83	0.0003	Multidrug efflux system subunit of heterodimeric MdtABC system; member of resistance-nodulation-cell division superfamily (RND)
mdtC	*yegO*	3.18	0.0043	Multidrug efflux system subunit of heterodimeric MdtABC system; member of resistance-nodulation-cell division superfamily (RND))
mutL		3.48	0.0000	Methyl-directed mismatch repair
phoP		2.82	0.0199	In Salmonella; a sensor in the two-component regulatory system; with *phoQ*
pta		2.31	0.0457	Phosphotransacetylase; creBC regulon; alkali-inducible; binds Zn(II)
spy		112.00	0.0000	Periplasmic protein induced by zinc; part of cpx regulon
ybjG		3.03	0.0032	Enzyme: undecaprenyl pyrophosphate phosphatase
yccA		2.49	0.0231	Membrane protein; degraded by FtsH protease
yceP		2.15	0.0148	Function unknown
ydeH		2.75	0.0404	Function unknown
ymgD	*b1172*	4.13	0.0429	Predicted protein
ymgD	*b1171*	4.74	0.0001	Polypeptide: predicted protein, function unknown, periplasmic
yncJ	*b1436*	2.64	0.0202	Function unknown
yobB	*b1843*	2.29	0.0145	Function unknown
yodA	*b1973*	9.02	0.0012	Periplasmic cadmium binding protein; induced by cadmium and peroxide; binds zinc; nickel; cadmium; SoxS and Fur regulated
znuA	*yebL*	5.85	0.0255	Zinc transporter subunit; periplasmic-binding protein; member of ABC superfamily
zraP	*yjaI*	4.58	0.0066	Zinc-binding periplasmic protein; responsive to Zn(2+) and Pb(2+); regulated by zraSR two-component system; rpoN-dependent