Supplementary Information

Role of the Global Regulator Rex in Control of NAD$^+$-Regeneration in *Clostridioides (Clostridium) difficile*

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Table S1: Microarray analysis of cells grown in TY medium with and without added proline.

Table S2: Microarray analysis of *prdR* mutant and wild-type cells grown in TY medium supplemented with proline.

Table S3: Microarray analysis of *prdR* mutant and wild-type cells grown in TY medium supplemented with proline.

Table S4. Spore formation by JIR8094 and *pdrR* and *rex* mutants.

Table S5: Oligonucleotide primers used in this study (5' to 3').

Fig. S1: Construction the *C. difficile rex* mutants using the TargeTron system.

Fig. S2: Growth curves of the JIR8094 strain and its respective *prdR*, *prdB* and *rex* mutants in both TY medium and TY supplemented with 30 mM L-proline (TYP).

Supplementary Table S1. Microarray analysis of cells grown in TY medium with and without added proline.

Class	Group	Gene ID	Name	Product	TYP/TY	p-value	Rex binding site
Cell Factor	GTP-binding_LepA_CD2467	CD630_24670	lepA	GTP-binding protein LepA	2.01	0.0479	
Cell Factor	Signaling-protein_CD2965	CD630_29650		Putative signaling protein	0.46	0.0140	
Cell Factor	Peptidase_M20D_CD3221	CD630_32210		Putative peptidase, M20D family	0.48	0.0032	
Cell Factor	Cyclase_CD1968-1970	CD630_19680		Putative membrane protein	0.43	0.0036	
Cell Factor	Cyclase_CD1968-1970	CD630_19690		Putative oligonucleotide binding regulator	0.41	0.0195	
Cell Factor	Cyclase_CD1968-1970	CD630_19700		Putative enzyme, cyclase family	0.45	0.0065	
Cell Factor	Hydrogenase_CD0894	CD630_08940		Putative iron-dependent hydrogenase	0.53	0.0017	
Cell Wall	Cell-surface_protein_CD2518	CD630_25180	cwp29	Cell surface protein	0.5	0.0203	
Cell Wall	Membrane-protein_CD0830	CD630_08300		Putative membrane protein	0.17	0.0000	
Cell Wall	Peptidoglycan_synthesis_CD0829	CD630_08290		Putative metallo-beta-lactamase superfamily protein	0.5	0.0200	
Cell Wall	Peptidoglycan_CD1469	CD630_14690	cwp20	Putative cell surface protein putative penicillin-binding protein	0.31	0.0000	
Cell Wall	Cell-surface_protein_CD0514	CD630_05140	cwpV	Cell surface protein	0.47	0.0023	
Fermentation	Alcohol_production_CD2966	CD630_29660	adhE	Aldehyde-alcohol dehydrogenase	0.05	0.0273	Yes
Fermentation	Butyrate_production_CD1054-1059	CD630_10540	bcd2	Butyryl-CoA dehydrogenase	0.04	0.0000	Yes
Fermentation	Butyrate_production_CD1054-1059	CD630_10550	etfB	Electron transfer flavoproteins subunit beta	0.03	0.0000	
Fermentation	Butyrate_production_CD1054-1059	CD630_10560	etfA	Electron transfer flavoprotein subunit alpha	0.04	0.0000	
Fermentation	Butyrate_production_CD1054-1059	CD630_10570	crt2	3-hydroxybutyryl-CoA dehydratase (Crotonase)	0.05	0.0000	
Fermentation	Butyrate_production_CD1054-1059	CD630_10580	hbd	3-hydroxybutyryl-CoA dehydrogenase	0.03	0.0000	
Fermentation	Butyrate_production_CD1054-1059	CD630_10590	thlA1	Acetoacetyl-CoA thiolase 1	0.03	0.0000	
Fermentation	Formate_production_CD3282-3283	CD630_32820	pflD	Pyruvate formate-lyase	0.35	0.0016	
Fermentation	Formate_production_CD3282-3283	CD630_32830	pflE	Pyruvate formate-lyase (activating enzyme)	0.3	0.0000	
Fermentation	Alcohol_dehydrogenase_CD3006	CD630_30060		Alcohol dehydrogenase	3.43	0.0001	
Fermentation	Succinate_CD2338-2344	CD630_23380	4hbD	4-hydroxybutyrate dehydrogenase (4-hydroxybutanoate:NAD+ oxidoreductase)	0.17	0.0000	
Fermentation	Succinate_CD2338-2344	CD630_23390	cat2	4-hydroxybutyrate CoA transferase	0.15	0.0000	
Fermentation	Succinate_CD2338-2344	CD630_23400		Conserved hypothetical protein	0.15	0.0000	
Fermentation	Succinate_CD2338-2344	CD630_23410	abfD	Gamma-aminobutyrate metabolism dehydratase/isomerase [includes: 4- hydroxybutyryl-coa dehydratase vinylacetyl-coa-delta-isomerase]	0.13	0.0000	
Fermentation	Succinate_CD2338-2344	CD630_23420	sucD	Succinate-semialdehyde dehydrogenase (NAD(P)+)	0.05	0.0000	
Fermentation	Succinate_CD2338-2344	CD630_23430	cat1	Succinyl-CoA:coenzyme A transferase	0.04	0.0000	
Fermentation	Succinate_CD2338-2344	CD630_23440		Putative membrane protein	0.1	0.0000	Yes
Membrane Transport	ABC_Cobalt_CD0324-0327	CD630_03240	cbiM	Cobalamin biosynthesis protein	0.27	0.0003	
Membrane Transport	ABC_Cobalt_CD0324-0327	CD630_03250	cbiN	ABC-type transport system, cobalt-specific extracellular solute-binding protein	0.25	0.0006	
Membrane Transport	ABC_Cobalt_CD0324-0327	CD630_03260	cbiQ	ABC-type transport system, cobalt-specific permease	0.29	0.0010	
Membrane Transport	ABC_Cobalt_CD0324-0327	CD630_03270	cbiO	ABC-type transport system, cobalt-specific ATP-binding protein	0.28	0.0015	
Membrane Transport	Symporter_Sodium-dicarboxylate_CD2541	CD630_25410		Sodium:glutamate/aspartate symporter family	0.37	0.0086	
Membrane Transport	Carbohydrate_transport_CD3017	CD630_30170		Putative glucose uptake protein	1.96	0.0014	
Membrane Transport	MFS_CD3036	CD630_30360		Transporter, Major Facilitator Superfamily (MFS)	6.03	0.0001	
Membrane Transport	Antiporter_CD2102	CD630_21020		Putative Na(+)/H(+) antiporter	2.07	0.0080	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29540	atpD	V-type ATP synthase subunit D	0.19	0.0001	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29550	atpB	V-type ATP synthase beta chain (V-type ATPase subunit B)	0.15	0.0000	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29560	atpA	V-type ATP synthase alpha chain (V-type ATPase subunit A)	0.13	0.0000	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29561	atpF	V-type ATP synthase subunit F	0.14	0.0001	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29570	atpC	V-type ATP synthase subunit C	0.15	0.0000	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29580	atpE	V-type ATP synthase subunit E (V-type ATPase subunit E)	0.14	0.0000	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29590	atpK	V-type ATP synthase subunit K	0.13	0.0000	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29600	atpI	V-type sodium ATP synthase subunit I	0.16	0.0001	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29610		Conserved hypothetical protein	0.16	0.0000	
Membrane Transport	Permease_CD1555	CD630_15550		Putative amino acid permease	2.33	0.0021	
Membrane Transport	Antiporter_CD2874	CD630_28740		Putative drug/sodium antiporter, MATE family	0.47	0.0396	
Membrane Transport	ABC_CD0873-CD0875	CD630_08730		ABC-type transport system, sugar-family extracellular solute-binding protein	2.05	0.0196	
Membrane Transport	ABC_CD0873-CD0875	CD630_08740		ABC-type transport system, sugar-family ATP-binding protein	2.34	0.0008	
Metabolism Amino Acid	Cysteine_synthesis_CD1594-CD1595	CD630_15940	cysK	O-acetyl-serine thiol-lyase A (O-acetyl-sulfhydrylase)(OAS-TL)	4.59	0.0015	
Metabolism Amino Acid	Cysteine_synthesis_CD1594-CD1595	CD630_15950	cysE	Serine acetyltransferase (SAT)	5.82	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32360		Putative membrane protein	64.29	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32370	prdF	Proline racemase	54.08	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32380		Putative component of proline reductase prdE-like	41.76	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32390	prdE	Proline reductase PrdE	30.31	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32400	prdD	Proline reductase PrdD	34.1	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32410	prdB	Proline reductase (selenocysteine)	45.54	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32430		Conserved hypothetical protein	61.03	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32440	prdA	D-proline reductase proprotein prdA	51.7	0.0000	
Metabolism Amino Acid	Glycine_decarboxylase_CD1657-1658	CD630_16570	gcvTPA	Bi-functional glycine dehydrogenase/aminomethyl transferase protein	0.37	0.0027	
Metabolism Amino Acid	Glycine_decarboxylase_CD1657-1658	CD630_16580	gcvPB	Glycine decarboxylase	0.41	0.0006	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23480	grdD	Glycine reductase complex component C subunit alpha (Protein PC alpha)	0.05	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23490	grdC	Glycine reductase complex component C subunit beta (Protein PC beta)	0.05	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23510	grdB	Glycine reductase complex component B gamma subunit (selenocysteine)	0.04	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23520	grdA	Glycine reductase complex selenoprotein A (selenocysteine)	0.04	0.0000	

Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23540	grdE	Glycine reductase complex component B subunits alpha and beta (Selenoprotein PB alpha/beta) [Contains: Betaine reductase component B	0.06	0.0000	Yes
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23550	trxA2	Thioredoxin 2 (Trx2)	0.05	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23560	trxB3	Thioredoxin reductase 3	0.05	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23570	grdX	Putative glycine reductase complex component	0.1	0.0001	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23580		Oligoendopeptidase-related, clade2, M3B family	0.49	0.0011	
Metabolism Amino Acid	Glutamate_synthase_CD0828	CD630_08280		Putative oxidative stress glutamate synthase	0.44	0.0447	
Metabolism Amino Acid	Serine_biosynthesis_CD0994-0996	CD630_09940		Putative serine-pyruvate aminotransferase	0.19	0.0011	
Metabolism Amino Acid	Serine_biosynthesis_CD0994-0996	CD630_09950	serA	Putative D-3-phosphoglycerate dehydrogenase	0.11	0.0000	
Metabolism Amino Acid	Serine_biosynthesis_CD0994-0996	CD630_09960		Conserved hypothetical protein	0.21	0.0005	
Metabolism Amino Acid	Aminobutyrate_hydrolase_CD3093	CD630_30930		Putative gamma-glutamyl-gamma-aminobutyrate hydrolase	0.39	0.0006	
Metabolism Carbon	CO_dehydrogenase_CD0716-CD0717	CD630_07160	cooS	Bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH)	0.32	0.0018	
Metabolism Carbon	CO_dehydrogenase_CD0716-CD0717	CD630_07170		Bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthase, accessory protein	0.3	0.0001	
Metabolism Carbon	Nitrogen_degradation_CD1384-CD1387	CD630_13840		Putative lamB-like protein, UPF0271 family	0.32	0.0001	
Metabolism Carbon	Nitrogen_degradation_CD1384-CD1387	CD630_13850		Putative natural resistance-associated macrophage protein	0.27	0.0001	
Metabolism Carbon	Nitrogen_degradation_CD1384-CD1387	CD630_13860		Putative allophanate hydrolase subunit 1	0.38	0.0008	
Metabolism Carbon	Nitrogen_degradation_CD1384-CD1387	CD630_13870		Putative allophanate hydrolase subunit 2	0.29	0.0000	
Metabolism Carbon	Carbohydrate_hydrolase_CD3001-CD3004	CD630_30010		Putative hydrolase/isomerase/hydratase	3.39	0.0000	
Metabolism Carbon	Carbohydrate_hydrolase_CD3001-CD3004	CD630_30020	uxaA	D-galactate dehydratase/Altronate hydrolase	4.12	0.0000	
Metabolism Carbon	Carbohydrate_hydrolase_CD3001-CD3004	CD630_30030		Putative N-terminus region of carbohydrate hydrolase, SAF domain	6.1	0.0033	
Metabolism Carbon	Carbohydrate_hydrolase_CD3001-CD3004	CD630_30040	kdgT2	2-keto-3-deoxygluconate permease 2	6.36	0.0025	
Metabolism Carbon	Polysaccharide_deacetylase_CD3248	CD630_32480		Polysaccharide deacetylase	7	0.0000	
Metabolism Cofactor	Iron-molybdenum_dinitrogenase_CD1693	CD630_16930		Putative dinitrogenase iron-molybdenum cofactor	0.45	0.0023	
Metabolism Cofactor	CoA_reductase_CD2540	CD630_25400		Coenzyme A disulfide reductase	0.24	0.0313	
Metabolism Nucleic Acid	Ribonucleoside_reductase_CD2994-2995	CD630_29940	nrdF	Ribonucleoside-diphosphate reductase subunit beta (Ribonucleotide reductase small subunit)	0.23	0.0001	
Metabolism Nucleic Acid	Ribonucleoside_reductase_CD2994-2995	CD630_29950	nrdE	Ribonucleoside-diphosphate reductase subunit alpha (Ribonucleotide reductase large subunit)	0.24	0.0000	
Metabolism Nucleic Acid	dCMP-deaminase_CD1899	CD630_18990		Putative dCMP deaminase	0.5	0.0014	
Metabolism Nucleic Acid	Xanthine_utilization_CD2099-CD2101	CD630_20990	XdhD	Xanthine dehydrogenase subunit XdhD, molybdopterin-binding	1.62	0.0107	
Metabolism Nucleic Acid	Xanthine_utilization_CD2099-CD2101	CD630_21000	XdhC3	Xanthine dehydrogenase iron-sulfur binding subunit XdhC3	2.14	0.0089	
Metabolism Nucleic Acid	Xanthine_utilization_CD2099-CD2101	CD630_21010	xdhB	Xanthine dehydrogenase subunit XdhB, FAD-binding	1.88	0.0022	
Regulations	TR_ArsR_CD1692	CD630_16920		Transcriptional regulator, ArsR family	0.49	0.0025	
Regulations	Diguanylate_kinase_signaling_protein_CD15	CD630_15380		Putative signaling protein	0.49	0.0155	
Regulations	TR_prdR_CD3245	CD630_32450	prdR	Transcriptional regulator, sigma-54-dependent	3.44	0.0001	
Regulations	TR_LysR_CD2345	CD630_23450		Transcriptional regulator, LysR family	0.34	0.0022	
Regulations	SigA_CD1498	CD630_14980	sigA2	RNA polymerase sigma factor SigA2 (sigma-43)	0.49	0.0107	
Respiration Anaerobic	Electron_transport_CD3247	CD630_32470	prdC	Putative electron transfer protein	36.1	0.0000	
Respiration Anaerobic	NO_reductase_CD1623	CD630_16230		Putative oxidoreductase	0.29	0.0001	
Respiration Anaerobic	Fe-S_oxidoreductase_CD2178-2179	CD630_21780		Putative oxidoreductase, Fe-S subunit	2.34	0.0008	
Respiration Anaerobic	Fe-S_oxidoreductase_CD2178-2179	CD630_21790		Putative anaerobic dehydrogenase	2.28	0.0010	
Respiration Anaerobic	Electron_transport_CD2682	CD630_26820	pfo	Pyruvate-ferredoxin oxidoreductase	0.5	0.0117	
Secretion	Exported-protein_CD0738	CD630_07380		Putative exported protein	2.12	0.0090	
Sporulation	Lysine_synthesis_CD3000	CD630_30000	dapA1	Dihydrodipicolinate synthase 1	2.83	0.0009	
Sporulation	SpoV-Cortex_synthesis_CD3516	CD630_35160	spoVG	Regulator required for spore cortex synthesis	0.46	0.0055	
Stress	Methionine-sulfoxide_reductase_CD2166	CD630_21660	msrAB	Peptide methionine sulfoxide reductase MsrA/MsrB	0.48	0.0028	
Stress	Beta-lactamase_CD0895	CD630_08950		Metallo-beta-lactamase superfamily exported protein	0.46	0.0009	
Stress	Tellurium_resistance_CD1652	CD630_16520		Putative tellurium resistance protein	0.46	0.0054	
Translation	tRNA-Proline_biosynthesis_CD0049	CD630_00490	proS	Prolyl-tRNA synthetase	0.41	0.0002	
Translation	tRNA-Proline_biosynthesis_CD0050	CD630_00500	proS	Prolyl-tRNA synthetase	0.36	0.0002	
Translation	Elongation_CD0022	CD630_00220	fusA1	Elongation factor G (EF-G)	0.42	0.0051	
Translation	tRNA-synthetase_CD1053	CD630_10530		Putative aminoacyl-tRNA synthetase-associated domain	0.36	0.0001	
UNKNOWN	CHP_CD2627	CD630_26270		Conserved hypothetical protein	0.47	0.0005	
UNKNOWN	CHP_CD0519	CD630_05190		Conserved hypothetical protein	0.45	0.0085	
UNKNOWN	CHP_CD1900	CD630_19000		Conserved hypothetical protein	0.49	0.0014	
UNKNOWN	CHP_CD1568	CD630_15680		Conserved hypothetical protein	0.44	0.0005	
UNKNOWN	CHP_CD3325	CD630_33250		Conserved hypothetical protein	0.51	0.0015	
UNKNOWN	CHP_CD2993	CD630_29930		Conserved hypothetical protein	0.22	0.0000	
UNKNOWN	CHP_CD3234	CD630_32340		Conserved hypothetical protein	3.12	0.0275	
UNKNOWN	CHP_CD1778	CD630_17780		Conserved hypothetical protein	0.39	0.0001	
UNKNOWN	CHP_CD1622	CD630_16220		Conserved hypothetical protein	0.48	0.0009	
UNKNOWN	CHP_CD1779	CD630_17790		Conserved hypothetical protein	0.45	0.0017	
UNKNOWN	CHP_CD0807	CD630_08070		Conserved hypothetical protein	0.37	0.0226	
UNKNOWN	CHP_CD0808	CD630_08080		Conserved hypothetical protein	0.43	0.0011	
UNKNOWN	CHP_CD3252	CD630_32520		Conserved hypothetical protein	5.65	0.0000	
UNKNOWN	CHP_CD2962	CD630_29620		Conserved hypothetical protein	0.39	0.0031	
UNKNOWN	CHP_CD1897	CD630_18970		Conserved hypothetical protein	0.44	0.0011	
UNKNOWN	CHP_CD2626	CD630_26260		Conserved hypothetical protein	0.43	0.0008	
UNKNOWN	CHP_CD1660	CD630_16600		Conserved hypothetical protein, DUF 1255 family	2.02	0.0017	

Supplementary Table S2. Microarray analysis of *prdR* mutant and wild-type cells grown in TY medium supplemented with proline.

Class	Group	Gene ID	Name	Product	prdR/WT	pvalue	Rex binding site
Cell Factor	Hydrogenase_CD0894	CD630_08940		Putative iron-dependent hydrogenase	2.02	0.0015	
Cell Factor	Redox_CD3018	CD630_30180		Putative redox-active protein	0.3	0.0001	
Cell Factor	Hydrolase_CD2480	CD630_24800		Putative hydrolase	2.75	0.0003	
Cell Wall	Peptidoglycan_synthesis_CD2664	CD630_26640	murE	UDP-N-acetylmuramyl-tripeptide synthetase	0.27	0.0000	
Cell Wall	Membrane-protein_CD0830	CD630_08300		Putative membrane protein	3.72	0.0001	
Cell Wall	Teichoic-acid_biosynthesis_CD2851-2854	CD630_28510	dltC	D-alanine--poly(phosphoribitol) ligase subunit 2 (D-alanyl carrier protein) (DCP)	0.45	0.0008	
Cell Wall	Teichoic-acid_biosynthesis_CD2851-2854	CD630_28520	dltB	D-alanyl transferase DltB, MBOAT family	0.39	0.0004	
Cell Wall	Teichoic-acid_biosynthesis_CD2851-2854	CD630_28530	dltA	D-alanine--poly(phosphoribitol) ligase subunit 1	0.33	0.0001	
Cell Wall	Teichoic-acid_biosynthesis_CD2851-2854	CD630_28540	dltD	D-alanine transferase DltD	0.41	0.0016	
Cell Wall	Membrane-protein_CD1413	CD630_14130		Putative membrane protein	6.11	0.0000	
Fermentation	Succinate_CD2338-2344	CD630_23380	4hbD	4-hydroxybutyrate dehydrogenase (4-hydroxybutanoate:NAD+ oxidoreductase)	3.33	0.0012	
Fermentation	Succinate_CD2338-2344	CD630_23390	cat2	4-hydroxybutyrate CoA transferase	4.39	0.0046	
Fermentation	Succinate_CD2338-2344	CD630_23400		Conserved hypothetical protein	3.1	0.0063	
Fermentation	Succinate_CD2338-2344	CD630_23410	abfD	Gamma-aminobutyrate metabolism dehydratase/isomerase [includes: 4- hydroxybutyryl-coa dehydratase vinylacetyl-coa-delta-isomerase]	4.12	0.0016	
Fermentation	Succinate_CD2338-2344	CD630_23420	sucD	Succinate-semialdehyde dehydrogenase (NAD(P)+)	10.44	0.0000	
Fermentation	Succinate_CD2338-2344	CD630_23430	cat1	Succinyl-CoA:coenzyme A transferase	10.4	0.0000	
Fermentation	Succinate_CD2338-2344	CD630_23440		Putative membrane protein	4.52	0.0002	Yes
Fermentation	Alcohol_dehydrogenase_CD3006	CD630_30060		Alcohol dehydrogenase	0.22	0.0000	
Fermentation	Butyrate_production_CD2379-2382	CD630_23790	buk	Butyrate kinase (Branched-chain carboxylic acid kinase) (BK)	0.26	0.0000	
Fermentation	Butyrate_production_CD2379-2382	CD630_23800	iorB	Indole pyruvate ferredoxin/flavodoxin oxidoreductase	0.22	0.0000	
Fermentation	Butyrate_production_CD2379-2382	CD630_23810	iorA	Indole pyruvate ferredoxin/flavodoxin oxidoreductase	0.23	0.0000	
Fermentation	Butyrate_production_CD2379-2382	CD630_23820		Putative pyridoxal phosphate-dependent transferase	0.22	0.0000	
Fermentation	Alcohol_production_CD2966	CD630_29660	adhE	Aldehyde-alcohol dehydrogenase	12.77	0.0000	Yes
Fermentation	Butyrate_production_CD1054-1059	CD630_10540	bcd2	Butyryl-CoA dehydrogenase	6.68	0.0000	Yes
Fermentation	Butyrate_production_CD1054-1059	CD630_10550	etfB	Electron transfer flavoproteins subunit beta	7.1	0.0000	
Fermentation	Butyrate_production_CD1054-1059	CD630_10560	etfA	Electron transfer flavoprotein subunit alpha	8.33	0.0000	
Fermentation	Butyrate_production_CD1054-1059	CD630_10570	crt2	3-hydroxybutyryl-CoA dehydratase (Crotonase)	5.92	0.0001	
Fermentation	Butyrate_production_CD1054-1059	CD630_10580	hbd	3-hydroxybutyryl-CoA dehydrogenase	7.01	0.0000	
Fermentation	Butyrate_production_CD1054-1059	CD630_10590	thlA1	Acetoacetyl-CoA thiolase 1	9.26	0.0000	
Membrane Transport	Symporter_Sodium-dicarboxylate_CD2541	CD630_25410		Sodium:glutamate/aspartate symporter family	2.11	0.0013	
Membrane Transport	ABC_Cobalt_CD0324-0327	CD630_03240	cbiM	Cobalamin biosynthesis protein	8.04	0.0000	
Membrane Transport	ABC_Cobalt_CD0324-0327	CD630_03250	cbiN	ABC-type transport system, cobalt-specific extracellular solute-binding protein	12.07	0.0000	
Membrane Transport	ABC_Cobalt_CD0324-0327	CD630_03260	cbiQ	ABC-type transport system, cobalt-specific permease	9.47	0.0000	
Membrane Transport	ABC_Cobalt_CD0324-0327	CD630_03270	cbiO	ABC-type transport system, cobalt-specific ATP-binding protein	9.86	0.0000	
Membrane Transport	ABC_Amino-Acid_CD2172-2177	CD630_21720		ABC-type transport system, cystine/aminoacid-family ATP-binding protein	0.34	0.0002	
Membrane Transport	ABC_Amino-Acid_CD2172-2177	CD630_21730		Putative peptidase, M20D family	0.35	0.0026	
Membrane Transport	ABC_Amino-Acid_CD2172-2177	CD630_21740		ABC-type transport system, cystine/aminoacid-family extracellular solute-binding protein	0.24	0.0000	
Membrane Transport	ABC_Amino-Acid_CD2172-2177	CD630_21750		ABC-type transport system, cystine/aminoacid-family permease	0.27	0.0008	
Membrane Transport	ABC_Amino-Acid_CD2172-2177	CD630_21760		ABC-type transport system, cystine/aminoacid-family permease	0.3	0.0003	
Membrane Transport	ABC_Amino-Acid_CD2172-2177	CD630_21770		ABC-type transport system, cystine/aminoacid-family extracellular solute-binding protein	0.24	0.0000	
Membrane Transport	ABC_CD0873-CD0875	CD630_08730		ABC-type transport system, sugar-family extracellular solute-binding protein	0.29	0.0001	
Membrane Transport	ABC_CD0873-CD0875	CD630_08740		ABC-type transport system, sugar-family ATP-binding protein	0.37	0.0001	
Membrane Transport	PTS_Beta-glucoside_CD3115-3117	CD630_31150	bglA	6-phospho-beta-glucosidase	0.23	0.0002	
Membrane Transport	PTS_Beta-glucoside_CD3115-3117	CD630_31160	bglF	PTS system, beta-glucoside-specific IIABC component	0.26	0.0024	
Membrane Transport	PTS_Beta-glucoside_CD3115-3117	CD630_31170	bglG	Transcription antiterminator, PTS operon regulator	0.2	0.0009	
Membrane Transport	PTS_Mannose_CD2566-2569	CD630_25660		PTS system, mannose-specfic IIA component	0.45	0.0015	
Membrane Transport	PTS_Mannose_CD2566-2569	CD630_25670		PTS system, mannose-specfic IIB component	0.28	0.0000	
Membrane Transport	PTS_Mannose_CD2566-2569	CD630_25680		PTS system, mannose-specfic IIC component	0.32	0.0000	
Membrane Transport	PTS_Mannose_CD2566-2569	CD630_25690		Putative glycoside hydrolase, family 38 Putative alpha-mannosidase	0.55	0.0023	
Membrane Transport	Carbohydrate_transport_CD3017	CD630_30170		Putative glucose uptake protein	0.31	0.0004	
Membrane Transport	PTS_Beta-glucoside_CD3136-3138	CD630_31360	bglA	6-phospho-beta-glucosidase	0.11	0.0005	
Membrane Transport	PTS_Beta-glucoside_CD3136-3138	CD630_31370	bglF	PTS system, beta-glucoside-specific IIABC component	0.12	0.0002	
Membrane Transport	PTS_Beta-glucoside_CD3136-3138	CD630_31380	bglG	Transcription antiterminator, PTS operon regulator	0.29	0.0002	
Membrane Transport	ABC_Oligopeptide_CD2670-2674	CD630_26700		ABC-type transport system, ATP-binding protein putative oligopeptide transport system	0.26	0.0001	
Membrane Transport	ABC_Oligopeptide_CD2670-2674	CD630_26710		ABC-type transport system, ATP-binding protein putative oligopeptide transport system	0.25	0.0000	
Membrane Transport	ABC_Oligopeptide_CD2670-2674	CD630_26720	appA	ABC-type transport system, oligopeptide-family solute-binding protein	0.21	0.0000	
Membrane Transport	ABC_Oligopeptide_CD2670-2674	CD630_26730	appB	ABC-type transport system, oligopeptide-family permease protein	0.29	0.0000	
Membrane Transport	ABC_Oligopeptide_CD2670-2674	CD630_26740	appC	ABC-type transport system, oligopeptide-family permease protein	0.33	0.0002	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29540	atpD	V-type ATP synthase subunit D	3.07	0.0003	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29550	atpB	V-type ATP synthase beta chain (V-type ATPase subunit B)	3.62	0.0000	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29560	atpA	V-type ATP synthase alpha chain (V-type ATPase subunit A)	3.43	0.0001	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29561	atpF	V-type ATP synthase subunit F	3.19	0.0002	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29570	atpC	V-type ATP synthase subunit C	2.9	0.0007	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29580	atpE	V-type ATP synthase subunit E (V-type ATPase subunit E)	3.49	0.0000	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29590	atpK	V-type ATP synthase subunit K	3.12	0.0002	
Membrane Transport	ATP_synthase_CD2954-2961	CD630_29600	atpI	V-type sodium ATP synthase subunit I	3.15	0.0007	

Membrane Transport	ATP_synthase_CD2954-2961	CD630_29610		Conserved hypothetical protein	3.02	0.0002	
Membrane Transport	ABC_Iron_CD2997-CD2999	CD630_29970		ABC-type transport system, iron-family ATP-binding protein	2.1	0.0012	
Membrane Transport	ABC_Iron_CD2997-CD2999	CD630_29980		ABC-type transport system, iron-family permease	2.26	0.0010	
Membrane Transport	ABC_Iron_CD2997-CD2999	CD630_29990		ABC-type transport system, iron-family extracellular solute-binding protein	2.43	0.0008	
Membrane Transport	PTS_Glucose_CD2666-2667	CD630_26660	ptsG-A	PTS system, glucose-specific IIA component	0.04	0.0000	
Membrane Transport	PTS_Glucose_CD2666-2667	CD630_26670	ptsG-BC	PTS system, glucose-specific IIBC component	0.04	0.0001	
Membrane Transport	PTS_Sorbitol_CD0762-0768	CD630_07620	srlR	PTS operon regulator	0.4	0.0012	
Membrane Transport	PTS_Sorbitol_CD0762-0768	CD630_07630	srlM	Sorbitol operon activator protein (Glucitol)	0.29	0.0001	
Membrane Transport	PTS_Sorbitol_CD0762-0768	CD630_07640	srlA	PTS system, sorbitol-specific IIC component (Glucitol)	0.35	0.0105	
Membrane Transport	PTS_Sorbitol_CD0762-0768	CD630_07650	srlEa	PTS system, sorbitol-specific IIB N-terminal component (Glucitol)	0.22	0.0001	
Membrane Transport	PTS_Sorbitol_CD0762-0768	CD630_07660	srlEb	PTS system, sorbitol-specific IIB C-terminal component (Glucitol)	0.28	0.0024	
Membrane Transport	PTS_Sorbitol_CD0762-0768	CD630_07670	srlB	PTS system, sorbitol-specific IIA component (Glucitol)	0.26	0.0007	
Membrane Transport	PTS_Sorbitol_CD0762-0768	CD630_07680	srlD	Sorbitol 6-phosphate 2-dehydrogenase (Glucitol)	0.21	0.0008	
Membrane Transport	Transporter_CD3019	CD630_30190		Putative transporter	0.37	0.0011	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32360		Putative membrane protein	0.05	0.0001	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32370	prdF	Proline racemase	0.03	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32380		Putative component of proline reductase prdE-like	0.03	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32390	prdE	Proline reductase PrdE	0.03	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32400	prdD	Proline reductase PrdD	0.04	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32410	prdB	Proline reductase (selenocysteine)	0.04	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32430		Conserved hypothetical protein	0.03	0.0000	
Metabolism Amino Acid	Proline_utilization_CD3236-3244	CD630_32440	prdA	D-proline reductase proprotein prdA	0.04	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23480	grdD	Glycine reductase complex component C subunit alpha (Protein PC alpha)	24.69	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23490	grdC	Glycine reductase complex component C subunit beta (Protein PC beta)	28.37	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23510	grdB	Glycine reductase complex component B gamma subunit (selenocysteine)	64.16	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23520	grdA	Glycine reductase complex selenoprotein A (selenocysteine)	51.54	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23540	grdE	Glycine reductase complex component B subunits alpha and beta (Selenoprotein PB alpha/beta) [Contains: Betaine reductase component B	46.46	0.0000	Yes
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23550	trxA2	Thioredoxin 2 (Trx2)	22.66	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23560	trxB3	Thioredoxin reductase 3	21.48	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23570	grdX	Putative glycine reductase complex component	6.61	0.0000	
Metabolism Amino Acid	Glycine_utilization_CD2348-2358	CD630_23580		Oligoendopeptidase-related, clade2, M3B family	3.21	0.0005	
Metabolism Amino Acid	Aminotransferase_CD3664	CD630_36640		Putative aminotransferase	0.38	0.0002	
Metabolism Amino Acid	Amidohydrolase_CD3099-CD3100	CD630_30990		Putative amidohydrolase, M20D family	3.96	0.0000	
Metabolism Amino Acid	Amidohydrolase_CD3099-CD3100	CD630_31000		Putative C4-dicarboxylate anaerobic carrier, DcuC family	4.23	0.0001	
Metabolism Carbon	Hydrolase_transaminase_CD2849	CD630_28490	phnXW	Bifunctional phosphonoacetaldehyde hydrolase/aminoethylphosphonate transaminase [Includes: Phosphonoacetaldehyde hydrolase 2-ami	0.46	0.0021	
Metabolism Carbon	Glycolysis_CD3171-3174	CD630_31710	gpmI	2,3-bisphosphoglycerate-independent phosphoglycerate mutase (Phosphoglyceromutase) (BPG-independent PGAM) (iPGM)	2.08	0.0011	
Metabolism Carbon	Glycolysis_CD3171-3174	CD630_31720	tpi	Triosephosphate isomerase (TIM) (Triose-phosphate isomerase)	2.16	0.0016	
Metabolism Carbon	Glycolysis_CD3171-3174	CD630_31730	pgk	Phosphoglycerate kinase	2.51	0.0014	
Metabolism Carbon	Glycolysis_CD3171-3174	CD630_31740	gapA	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	4.78	0.0000	
Metabolism Carbon	Nitrogen_degradation_CD1384-CD1387	CD630_13840		Putative lamB-like protein, UPF0271 family	2.5	0.0005	
Metabolism Carbon	Nitrogen_degradation_CD1384-CD1387	CD630_13850		Putative natural resistance-associated macrophage protein	2.86	0.0003	
Metabolism Carbon	Nitrogen_degradation_CD1384-CD1387	CD630_13860		Putative allophanate hydrolase subunit 1	2.44	0.0005	
Metabolism Carbon	Nitrogen_degradation_CD1384-CD1387	CD630_13870		Putative allophanate hydrolase subunit 2	2.62	0.0001	
Metabolism Carbon	Dehydrogenase_GA3P_CD1767	CD630_17670	gapB	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	0.07	0.0000	
Metabolism Carbon	Glycolysis_CD3394-3395	CD630_33940	pyk	Pyruvate kinase (PK)	3.38	0.0010	
Metabolism Carbon	Glycolysis_CD3394-3395	CD630_33950	pfkA	6-phosphofructokinase (Phosphofructokinase) (Phosphohexokinase)	2.43	0.0009	
Metabolism Carbon	Enolase_CD3170	CD630_31700	eno	Enolase (2-phosphoglycerate dehydratase) (2-phospho-D-glycerate hydro-lyase)	2.2	0.0007	
Metabolism Carbon	Gluconeogenesis_regulators_CD2410-2412	CD630_24100	ppdK	Pyruvate phosphate dikinase (ATP:pyruvate, phosphate phosphotransferase)	0.23	0.0003	
Metabolism Carbon	Gluconeogenesis_regulators_CD2410-2412	CD630_24110		Putative positive regulator of gluconeogenesis putative phosphotransferase	0.48	0.0014	
Metabolism Carbon	Gluconeogenesis_regulators_CD2410-2412	CD630_24120		Putative negative regulator of gluconeogenesis	0.4	0.0005	
Metabolism Carbon	Carbohydrate_hydrolase_CD3001-CD3004	CD630_30010		Putative hydrolase/isomerase/hydratase	0.28	0.0000	
Metabolism Carbon	Carbohydrate_hydrolase_CD3001-CD3004	CD630_30020	uxaA	D-galactate dehydratase/Altronate hydrolase	0.2	0.0000	
Metabolism Carbon	Carbohydrate_hydrolase_CD3001-CD3004	CD630_30030		Putative N-terminus region of carbohydrate hydrolase, SAF domain	0.23	0.0001	
Metabolism Carbon	Carbohydrate_hydrolase_CD3001-CD3004	CD630_30040	kdgT2	2-keto-3-deoxygluconate permease 2	0.23	0.0001	
Metabolism Carbon	Polysaccharide_deacetylase_CD3248	CD630_32480		Polysaccharide deacetylase	0.17	0.0004	
Metabolism Cofactor	Fe-S_binding-protein_CD2168-2169	CD630_21680	hcp	Hydroxylamine reductase	2.81	0.0002	
Metabolism Cofactor	Fe-S_binding-protein_CD2168-2169	CD630_21690		Putative iron-sulfur binding protein	2.56	0.0004	
Metabolism Cofactor	CoA_reductase_CD2540	CD630_25400		Coenzyme A disulfide reductase	2.8	0.0002	
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19060		Conserved hypothetical protein	0.97	0.9700	
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19070	eutG	Ethanolamine iron-dependent Alcohol dehydrogenase	8.22	0.0002	
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19080	eutS	Ethanolamine carboxysome structural protein, BMC family	8.78	0.0001	
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19090	eutP	Ethanolamine utilization protein, GTPase family	9.02	0.0000	
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19100	eutV	Two-component response regulator, Ethanolamine specific	6.83	0.0001	
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19110	eutW	Two-component sensor histidine kinase, Ethanolamine specific	5.55	0.0002	
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19120	eutA	Ethanolamine reactivating factor for ammonia lyase eutBC	32.97	0.0001	
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19130	eutB	Ethanolamine ammonia lyase large subunit	71.88	0.0000	
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19140	eutC	Ethanolamine ammonia lyase small subunit	19.41	0.0000	
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19150	eutL	Ethanolamine carboxysome structural protein, BMC family	23.95	0.0000	

Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19160	eutM	Ethanolamine carboxysome structural protein, BMC family	39.12	0.0000
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19170	eutE	Ethanolamine acetaldehyde oxydoreductase	31.16	0.0000
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19180	eutK	Ethanolamine carboxysome structural protein, BMC family	61.95	0.0000
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19190	eutT	Ethanolamine corrinoid cobalamin adenosyltransferase	20.28	0.0000
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19200	eutD	Putative phosphotransacetylase	14.37	0.0000
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19210		Putative ethanolamine utilization protein	14.41	0.0003
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19220	eutN	Ethanolamine carboxysome structural protein, BMC family	18.6	0.0000
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19230		Ethanolamine carboxysome structural protein, BMC family	12.41	0.0000
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19240	eutH	Ethanolamine transport protein	20.14	0.0000
Metabolism Lipids	Ethanolamine_utilization_CD1906-1925	CD630_19250	eutQ	Ethanolamine utilization protein, Cupin barrels family	13.76	0.0001
Regulations	TR_HxlR_CD3165-CD3166	CD630_31650		Conserved hypothetical protein	0.35	0.0008
Regulations	TR_HxlR_CD3165-CD3166	CD630_31660		Transcriptional regulator, hxlR family	0.29	0.0000
Regulations	TR_licT_CD2668	CD630_26680		Transcription antiterminator, licT family	0.19	0.0001
Regulations	PEBP_regulatory_protein_CD3167	CD630_31670		Putative phosphatidylethanolamine-binding regulatory protein	0.48	0.0016
Regulations	Diguanylate_kinase_signaling_protein_CD15	CD630_15380		Putative signaling protein	2.72	0.0005
Regulations	Histidine_kinase_CD1811	CD630_18110		Fragment of putative two-component sensor histidine kinase (N-terminal region)	5.25	0.0007
Regulations	Sig54_modulation-protein_CD2444	CD630_24440		Putative ribosome-associated sigma 54 modulation protein	0.43	0.0004
Regulations	TR_DeoR_CD2850	CD630_28500		Transcriptional regulator, DeoR family	0.5	0.0019
Regulations	TR_CggR_CD3175	CD630_31750	cggR	Transcriptional regulator, SorC family	3.93	0.0000
Regulations	TCS_VirR_CD3255	CD630_32550	rgaR	Two-component response regulator VirR-like	0.38	0.0012
Respiration Anaerobic	NO_reductase_CD1623	CD630_16230		Putative oxidoreductase	2.31	0.0004
Respiration Anaerobic	Fe-S_oxidoreductase_CD2178-2179	CD630_21780		Putative oxidoreductase, Fe-S subunit	0.25	0.0000
Respiration Anaerobic	Fe-S_oxidoreductase_CD2178-2179	CD630_21790		Putative anaerobic dehydrogenase	0.24	0.0001
Respiration Anaerobic	Electron_transport_CD3247	CD630_32470	prdC	Putative electron transfer protein	0.03	0.0000
Respiration Anaerobic	Electron_transport_CD1137-1142	CD630_11370	rnfC	Electron transport complex protein	0.34	0.0001
Respiration Anaerobic	Electron_transport_CD1137-1142	CD630_11380	rnfD	Electron transport complex protein	0.3	0.0002
Respiration Anaerobic	Electron_transport_CD1137-1142	CD630_11390	rnfG	Electron transport complex protein	0.39	0.0013
Respiration Anaerobic	Electron_transport_CD1137-1142	CD630_11400	rnfE	Electron transport complex protein	0.32	0.0013
Respiration Anaerobic	Electron_transport_CD1137-1142	CD630_11410	rnfA	Electron transport complex protein	0.33	0.0001
Respiration Anaerobic	Electron_transport_CD1137-1142	CD630_11420	rnfB	Electron transport complex protein	0.34	0.0001
Secretion	Exported-protein_CD0739	CD630_07390		Putative exported protein	0.29	0.0004
Secretion	Exported-protein_CD0738	CD630_07380		Putative exported protein	0.31	0.0002
Sporulation	Lysine_synthesis_CD3000	CD630_30000	dapA1	Dihydrodipicolinate synthase 1	0.28	0.0002
Stress	Vancomycin-protein_CD2149	CD630_21490		Putative vancomycin resistance protein, vanW family	0.43	0.0005
Stress	Beta-lactamase_CD0895	CD630_08950		Metallo-beta-lactamase superfamily exported protein	2.21	0.0007
Translation	tRNA-binding_protein_CD2392	CD630_23920		Putative tRNA-binding protein	0.41	0.0005
UNKNOWN	CHP_CD2251	CD630_22510		Conserved hypothetical protein	0.43	0.0009
UNKNOWN	CHP_CD2369	CD630_23690		Conserved hypothetical protein	0.34	0.0008
UNKNOWN	CHP_CD3072	CD630_30720		Conserved hypothetical protein	0.32	0.0014
UNKNOWN	CHP_CD2368	CD630_23680		Conserved hypothetical protein	0.43	0.0018
UNKNOWN	CHP_CD3617	CD630_36170		Conserved hypothetical protein	2.62	0.0012
UNKNOWN	CHP_CD3325	CD630_33250		Conserved hypothetical protein	2.09	0.0016
UNKNOWN	CHP_CD2752	CD630_27520		Conserved hypothetical protein	0.15	0.0009
UNKNOWN	CHP_CD0281	CD630_02810		Conserved hypothetical protein	2.88	0.0007
Virulence Factors	P-cresol_production_CD0153-CD0155	CD630_01530	hpdB	4-hydroxyphenylacetate decarboxylase, catalytic subunit	0.33	0.0003
Virulence Factors	P-cresol_production_CD0153-CD0155	CD630_01540	hpdC	4-hydroxyphenylacetate decarboxylase, regulatory subunit	0.35	0.0003
Virulence Factors	P-cresol_production_CD0153-CD0155	CD630_01550	hpdA	4-hydroxyphenylacetate decarboxylase, activating subunit	0.35	0.0169

Supplementary Table S3. Genes regulated ≥2-fold by the presence of proline and by inactivation of PrdR.

Gene ID	Name	Product	TYP/TY	prdR/WT
CD630_03240	cbiM	Cobalamin biosynthesis protein	0.27	8.04
CD630_03250	cbiN	ABC-type transport system, cobalt-specific extracellular solute-binding protein	0.25	12.07
CD630_03260	cbiQ	ABC-type transport system, cobalt-specific permease	0.29	9.47
CD630_03270	cbiO	ABC-type transport system, cobalt-specific ATP-binding protein	0.28	9.86
CD630_07380		Putative exported protein	2.12	0.31
CD630_08070		Conserved hypothetical protein	0.37	0.29
CD630_08300		Putative membrane protein	0.17	3.72
CD630_08730		ABC-type transport system, sugar-family extracellular solute-binding protein	2.05	0.29
CD630_08740		ABC-type transport system, sugar-family ATP-binding protein	2.34	0.37
CD630_08940		Putative iron-dependent hydrogenase	0.66	2.02
CD630_08950		Metallo-beta-lactamase superfamily exported protein	0.46	2.21
CD630_10540	bcd2	Butyryl-CoA dehydrogenase	0.04	6.68
CD630_10550	etfB	Electron transfer flavoproteins subunit beta	0.03	7.1
CD630_10560	etfA	Electron transfer flavoprotein subunit alpha	0.04	8.33
CD630_10570	crt2	3-hydroxybutyryl-CoA dehydratase (Crotonase)	0.05	5.92
CD630_10580	hbd	3-hydroxybutyryl-CoA dehydrogenase	0.03	7.01
CD630_10590	thlA1	Acetoacetyl-CoA thiolase 1	0.03	9.26
CD630_13840		Putative lamB-like protein, UPF0271 family	0.32	2.5
CD630_13850		Putative natural resistance-associated macrophage protein	0.27	2.86
CD630_13860		Putative allophanate hydrolase subunit 1	0.38	2.44
CD630_13870		Putative allophanate hydrolase subunit 2	0.29	2.62
CD630_15380		Putative signaling protein	0.49	2.72
CD630_16230		Putative oxidoreductase	0.29	2.31
CD630_21780		Putative oxidoreductase, Fe-S subunit	2.34	0.25
CD630_21790		Putative anaerobic dehydrogenase	2.28	0.24
CD630_23380	4hbD	4-hydroxybutyrate dehydrogenase (4-hydroxybutanoate:NAD+ oxidoreductase)	0.17	3.33
CD630_23390	cat2	4-hydroxybutyrate CoA transferase	0.15	2.99
CD630_23400		Conserved hypothetical protein	0.15	3.105
CD630_23410	abfD	Gamma-aminobutyrate metabolism dehydratase/isomerase [includes: 4- hydroxybutyryl-coa dehydratase vinylacetyl-coa-delta-isomerase]	0.13	4.12
CD630_23420	sucD	Succinate-semialdehyde dehydrogenase (NAD(P)+)	0.05	10.44
CD630_23430	cat1	Succinyl-CoA:coenzyme A transferase	0.04	10.4
CD630_23440		Putative membrane protein	0.1	4.52
CD630_23480	grdD	Glycine reductase complex component C subunit alpha (Protein PC alpha)	0.05	24.69
CD630_23490	grdC	Glycine reductase complex component C subunit beta (Protein PC beta)	0.05	28.37
CD630_23510	grdB	Glycine reductase complex component B gamma subunit (selenocysteine)	0.04	64.16
CD630_23520	grdA	Glycine reductase complex selenoprotein A (selenocysteine)	0.04	51.54
CD630_23540	grdE	Glycine reductase complex component B subunits alpha and beta (Selenoprotein PB alpha/beta) [Contains: Betaine reductase component B subunit beta Betaine reductase	0.06	46.46
CD630_23550	trxA2	Thioredoxin 2 (Trx2)	0.05	22.66
CD630_23560	trxB3	Thioredoxin reductase 3	0.05	21.48
CD630_23570	grdX	Putative glycine reductase complex component	0.1	6.61
CD630_23580		Oligoendopeptidase-related, clade2, M3B family	0.49	3.21
CD630_25410		Sodium:glutamate/aspartate symporter family	0.37	2.11
CD630_29540	atpD	V-type ATP synthase subunit D	0.19	3.07

CD630_29550	atpB	V-type ATP synthase beta chain (V-type ATPase subunit B)	0.15	3.62
CD630_29560	atpA	V-type ATP synthase alpha chain (V-type ATPase subunit A)	0.13	3.43
CD630_29561	atpF	V-type ATP synthase subunit F	0.14	3.19
CD630_29570	atpC	V-type ATP synthase subunit C	0.15	2.9
CD630_29580	atpE	V-type ATP synthase subunit E (V-type ATPase subunit E)	0.14	3.49
CD630_29590	atpK	V-type ATP synthase subunit K	0.13	3.12
CD630_29600	atpI	V-type sodium ATP synthase subunit I	0.16	3.15
CD630_29610		Conserved hypothetical protein	0.16	3.02
CD630_29660	adhE	Aldehyde-alcohol dehydrogenase	0.05	12.77
CD630_30000	dapA1	Dihydrodipicolinate synthase 1	2.83	0.28
CD630_30010		Putative hydrolase/isomerase/hydratase	3.39	0.28
CD630_30020	uxaA	D-galactate dehydratase/Altronate hydrolase	4.12	0.2
CD630_30030		Putative N-terminus region of carbohydrate hydrolase, SAF domain	6.1	0.23
CD630_30040	kdgT2	2-keto-3-deoxygluconate permease 2	6.36	0.23
CD630_30060		Alcohol dehydrogenase	3.43	0.22
CD630_30170		Putative glucose uptake protein	1.96	0.31
CD630_32360		Putative membrane protein	64.29	0.05
CD630_32370	prdF	Proline racemase	54.08	0.03
CD630_32380		Putative component of proline reductase prdE-like	41.76	0.03
CD630_32390	prdE	Proline reductase PrdE	30.31	0.03
CD630_32400	prdD	Proline reductase PrdD	34.1	0.04
CD630_32410	prdB	Proline reductase (selenocysteine)	45.54	0.04
CD630_32430		Conserved hypothetical protein	61.03	0.03
CD630_32440	prdA	D-proline reductase proprotein prdA	51.7	0.04
CD630_32470	prdC	Putative electron transfer protein	36.1	0.03
CD630_32480		Polysaccharide deacetylase	7	0.17
CD630_33250		Conserved hypothetical protein	0.51	2.09

Table S4. Spore formation by JIR8094 and *pdrR* and *rex* mutants

Strain	CFU/ml on BHIS (no heat)	CFU/ml on BHIS+TA (no heat)	CFU/ml on BHIS+TA (+heat)
JIR8094	$6.5\text{x}10^7$	$6.6\text{x}10^7$	$7.9\text{x}10^6$
LB-CD8 (*prdR*)	$3.3\text{x}10^7$	$4.2\text{x}10^7$	$4.8\text{x}10^6$
LB-CD24 (*rex*)	$6.3\text{x}10^7$	$7.4\text{x}10^7$	$6.1\text{x}10^6$

The three strains were streaked on plates of 70:30 medium (1). After 24 hrs, samples of the plate cultures were resuspended in 1-ml liquid 70:30 medium. A portion of the resuspension was heated at 65ºC for 20 min, cooled, diluted in 70:30 medium and plated on BHIS plates (2) supplemented with the germination activator sodium taurocholate (TA). The unheated samples were diluted and plated on BHIS with and without TA.

References:

(1) Shen A, Fimlaid KA, Pishdadian K. Inducing and quantifying *Clostridium difficile* spore formation. Methods Mol Biol. 2016;1476:129-42.

(2) Bouillaut L, McBride SM, Sorg JA. Genetic manipulation of *Clostridium difficile*. Curr Protoc Microbiol. 2011;Chapter 9:Unit 9A 2.

Supplementary Table S5. Oligonucleotide primers used in this study (5’ to 3’)

Name	Sequence
78	AAACGCGTTTAACTATTTATCAATTCCTGCAAT
83	AAACGCGTAATTAGATGCTAAAAATTTGTAATT
EBSu	CGAAATTAGAAACTTGCGTTCAGTAAAC
oKZ69	CCGCTCTAGAGGATCCTGCAGAATTCTTTTTTTTTTTTTTTTTTTTTTTTT
oKZ70	CCGCTCTAGAGGATCCTGCAGAATTC
oLB131	GTATGGATAGGTGGAGAAGTCA
oLB132	CTCTTCCTCTAGTAGCTGTAATGC
oLB170	GGTCAAGTACTAGGAGCTAAGT
oLB171	CTACTTCTTCTTTAGCCTCTCCTG
oLB176	CCCTGGTATCATGTCTAAAGTTG
oLB177	GAGTATACTTAGCTCCTTCTCCAG
oLB184	TCAATTCCTCAACAGGTAACCC
oLB185	CTAAGGATGATGCTACAGTTCC
oLB186	CCCTAATTCAAGACGCATAC
oLB196	TGGCGCTATAATTGTTCCCGC
oLB198	GAGAAAATGTTTACAAATACAAAAAATAAC
oLB240	CCCTCTAGAGTCTTGTTTTAACTAAATTG
oLB273	TAAAGGTAGAGGTTATGTTTCTGCT
oLB274	TTTGACCAACTCTTGTGTTTTCC
oLB281	AAGAATATGGTGGAGAAG
oLB282	GAGCCAAGAGATGTATG
oLB292	AAGCATGCTCTGTATACAATCTATAC
oLB293	CATATGAGCCTCCTCAGTATTTTAT
oLB329	AAAGCTTTTGCAACCCACGTCGATCGTGAATTATTTCCCAACGTGCGCCCAGATAGGGTG
oLB330	CAGATTGTACAAATGTGGTGATAACAGATAAGTCCCCAACATTAACTTACCTTTCTTTGT
oLB331	CGCAAGTTTCTAATTTCGGTTAATAATCGATAGAGGAAAGTGTCT

oLB332	TTGATATAATATAATCAAGG
oLB333	CCTAATATTTCAAAAAGCC
oLB334	AACATATGTTGGGAAATAAAAATATATCAATGG
oLB335	AAGGATCCTACTTTCCTTCTTTCATTAAATACG
oLB337	AAGCTATAGCAAGCCATCC
oLB338	AACTTCATTTTCAGCGAAGC
oLB339	CAAAACTTCTCTGCCTTGAAC
oLB341	GAGAAAGACGAAGCATTTGGTATG
oLB342	AGCAGTAGAAGTTGGATTAGTTGTA
oLB345	AAGGATCCATTTCTACCTCCTTGAACTATC
oLB369	TTATAACCTTAATCAAACTACGC
oLB400	AATAGCATAAGTTTTGTATATTGTTG
oLB401	CAAACATAAGGACAAAAGGATTC
oLB402	GTGATATCTAGTTAAATTAAGTATTTG
oLB403	CCAACTGACATAACTTCATTTTC
oLB404	TTATCTGTAACCAGTGTGAAAGAAATC
JP700	GTTTTGATATAATATAATCAAGGTTATATG
JP701	CCTAATATTTCAAAAAGCCAG
RamF	ACGCGTTATATTGATAAAAATAATAATAGTGGG
RamR	ACGCGTGCGACTCATAGAATTATTTCCTCCCG

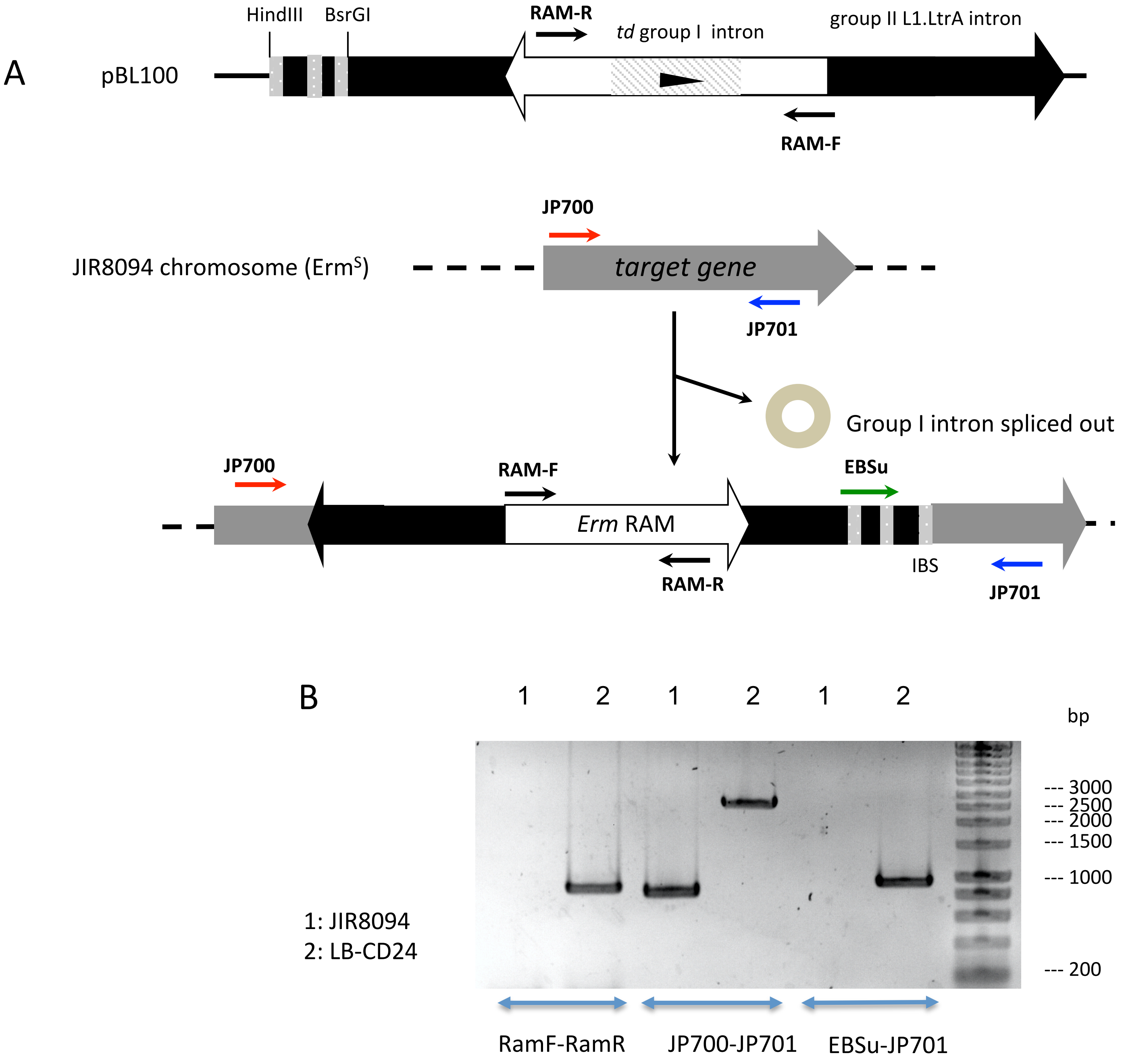
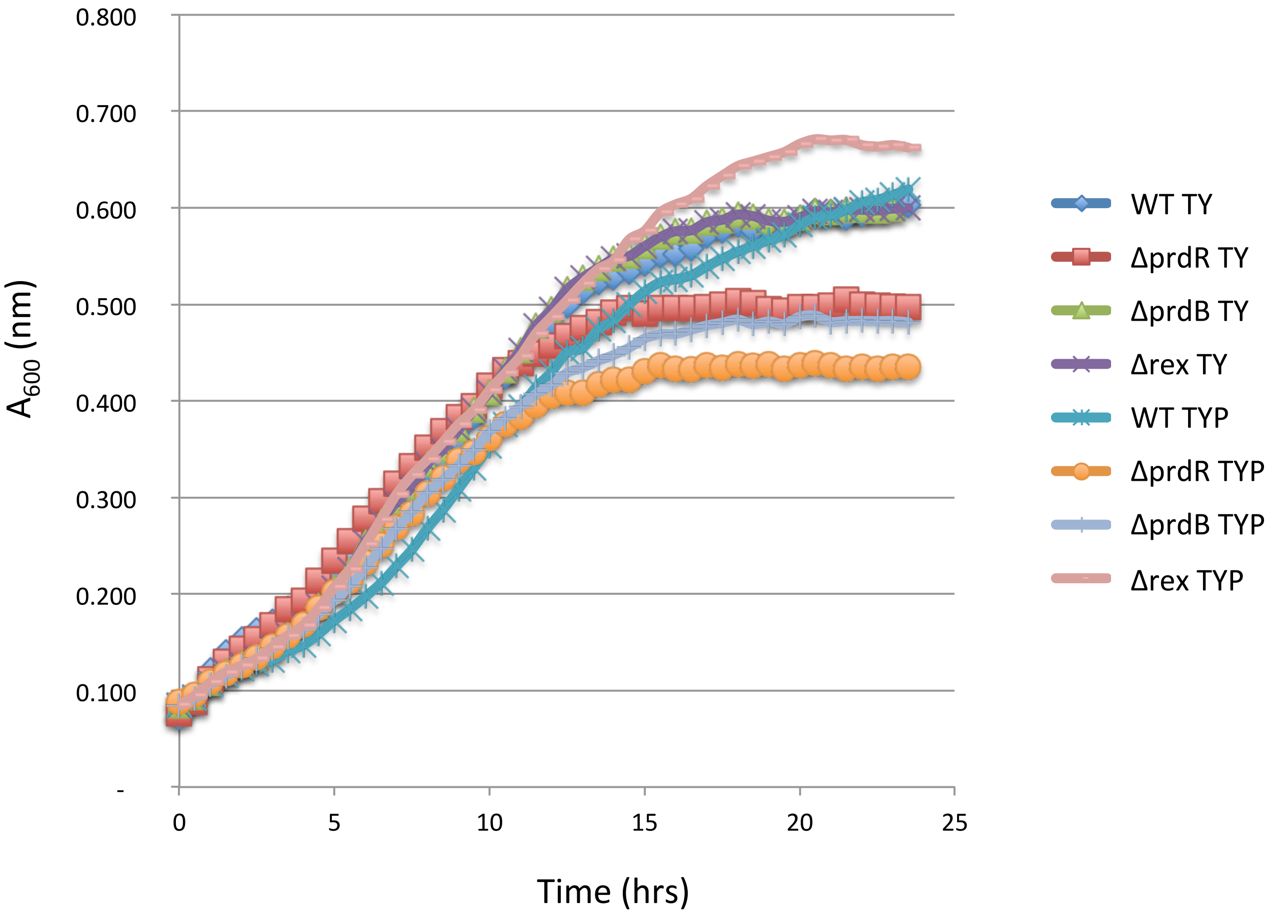

A
pBL100
HindIII
BsrGI
RAM-R
td group I intron
group II L1.LtrA intron
RAM-F
JP700
JIR8094 chromosome (Erm^S)
target gene
JP701
Group I intron spliced out
JP700
RAM-F
EBSu
Erm RAM
RAM-R
IBS
JP701
B
1
2
1
2
1
2
bp
--- 3000
--- 2500
--- 2000
--- 1500
--- 1000
--- 200
1: JIR8094
2: LB-CD24
RamF-RamR
JP700-JP701
EBSu-JP701

Fig. S1: **Construction the *C. difficile rex* mutants using the** TargeTron **system.**

A Schematic diagram of the TargeTron technology. The ClosTron delivery system encoded on plasmid pBL100 consists of a group-II intron (black arrow) with an internal Retrotransposition-Activated Marker (RAM) conferring erythromycin resistance (white arrow) that is itself interrupted by a *td* group-I intron (hatched box). The group-II intron is retargeted to the target gene (grey arrow) by altering the sequence of the IBS/EBS region using overlap PCR. Splicing the group-II intron into the target gene disrupts it, and splicing the *td* group-I intron of the *erm* RAM restores the functional *erm* gene, allowing positive selection of the mutants.
B. Confirmation of the mutant knockout by PCR. PCR using the primer pair Ram-F/Ram-R (lane 1 and 2, black arrows) was performed to confirm the splicing out of the group-I intron in the mutant. To verify the integration of the Ll.LtrB intron in the targeted gene, we performed PCR with three primer pairs: the intron primer EBSu associated with a primer within the *rex* gene (JP701), the RamF and RamR inside the *erm* cassette, and the two primers (JP700-JP701) flanking the insertion site in the targeted gene. Each PCR was performed on chromosomal DNA of the *rex* mutant and strain JIR8094 as a negative control. 1kb Ladder (Eurogentec) was used as a molecular-weight marker.



Supplementary Fig. 2: Growth curves of the JIR8094 strain and its respective *prdR*, *prdB* and *rex* mutants in both TY and TY supplemented with 30 mM L-proline (TYP)