

**Table S4. CD2214-CD2215 regulon compared to the set of genes differentially expressed during biofilm/planktonic growth**

Gene-ID	Name	Function	630Δerm/ CD2214-CD2215 mutant		Biofilm/ planktonic growth Micro-array	c-di-GMP riboswitch <sup>1</sup>				
			Micro-array	qRT-PCR						
<b>Sugar transport</b>										
<b>PTS systems for sugar uptake</b>										
CD3027		PTS system. glucose-like IIA component	12.50		17.17					
CD3028		Putative phosphosugar isomerase	25.00		16.55					
CD3030		PTS system. glucose-like IIBC component	20.00		16.34					
CD3031		Transcription antiterminator. PTS operon regulator	9.09		15.06					
CD2666	<i>ptsG-A</i>	PTS system. glucose-specific IIA component	3.13		16.69					
CD2667	<i>ptsG-BC</i>	PTS system. glucose-specific IIBC component	2.86		13.32					
CD3013		PTS system. mannose-specific IIC component	2.27		15.26					
CD3014		PTS system. mannose-specific IIB component	2.17		10.41					
CD3015		PTS system. mannose-specific IIA component	2.04		10.04					
CD3115	<i>bglA</i>	6-phospho-beta-glucosidase	3.13		4.45					
CD3116	<i>bglF</i>	PTS system. beta-glucoside-specific IIABC component	2.13		4.04					
CD3136	<i>bglA</i>	6-phospho-beta-glucosidase	3.03		7.31					
CD3137	<i>bglF</i>	PTS system. beta-glucoside-specific IIABC component	2.94		6.90					
CD3138	<i>bglG</i>	Transcription antiterminator. PTS operon regulator	2.22							
CD2755	<i>ptsl</i>	PTS system. EI component	2.27							
CD2756	<i>ptsH</i>	PTS system. HPr protein	2.38		3.61					
CD0490		Putative sugar-phosphate dehydrogenase	1.85							
CD0763	<i>srlM</i>	Sorbitol operon activator protein (Glucitol)	2.00							
CD0765	<i>srlEa</i>	PTS system. sorbitol-specific IIB N-terminal (Glucitol)	3.03							
CD0766	<i>srlEb</i>	PTS system. sorbitol-specific IIB C-terminal (Glucitol)	3.03							
CD0767	<i>srlB</i>	PTS system. sorbitol-specific IIA component (Glucitol)	3.85							
CD0768	<i>srlD</i>	Sorbitol 6-phosphate 2-dehydrogenase (Glucitol)	2.86							
CD0491		PTS system. mannose/fructose/sorbose IIA component	1.64							
CD0492		PTS system. mannose/fructose/sorbose IIB component	1.64							
CD0493		PTS system. mannose/fructose/sorbose IIC component	1.49							
<b>ABC uptake system</b>										
CD0873		ABC-type transport system. sugar-family extracellular SBP	2.63		6.03					
CD0874		ABC-type transport system. sugar-family ATP-binding protein	2.38		4.36					
<b>Other transport system</b>										
CD3169		Putative malate transporter	0.66		0.47					

Gene-ID	Name	Function	630Δerm/ CD2214-CD2215 mutant		Biofilm/ planktonic growth Micro-array	c-di-GMP riboswitch <sup>1</sup>				
			Micro-array	qRT-PCR						
<b>Carbohydrate metabolism</b>										
<b>Glycolysis</b>										
CD3285	<i>pgi</i>	Glucose-6-phosphate isomerase (GPI) (Phosphoglucose isomerase)	1.43		2.68					
CD0403	<i>fba</i>	Fructose-1,6-bisphosphate aldolase			3.18					
CD3174	<i>gapA</i>	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	7.14		3.42					
CD3173	<i>pgk</i>	Phosphoglycerate kinase	3.70							
CD3172	<i>tpi</i>	Triosephosphate isomerase	3.85							
CD3171	<i>gpmI</i>	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	3.57							
CD3170	<i>eno</i>	Enolase	2.94							
CD3394	<i>pyk</i>	Pyruvate kinase (PK)	2.63		3.18					
CD3395	<i>pfkA</i>	6-phosphofructokinase	2.27							
CD0582		Putative pyruvate phosphate dikinase. PEP/pyruvate-binding	7.14		2.79					
CD3448		Putative ketose-bisphosphate aldolase class II putative tagatose-b	2.33							
<b>Pyruvate conversion into acetyl-CoA</b>										
CD0758	<i>pflA</i>	Pyruvate formate-lyase activating enzyme	2.04		4.78					
CD0759	<i>pflB</i>	Formate acetyltransferase (Pyruvate formate-lyase)	2.44		3.62					
<b>Lactate fermentation</b>										
CD2966	<i>adhE</i>	Aldehyde-alcohol dehydrogenase	5.00		0.23					
<b>Succinate uptake and utilization</b>										
CD2338	<i>4hbD</i>	4-hydroxybutyrate dehydrogenase	7.14		4.56					
CD2339	<i>cat2</i>	4-hydroxybutyrate CoA transferase	6.25		3.37					
CD2340		Conserved hypothetical protein	6.67		4.19					
CD2341	<i>abfD</i>	g-aminobutyrate metabolism dehydratase/isomerase	7.69		3.22					
CD2342	<i>sucD</i>	Succinate-semialdehyde dehydrogenase (NAD(P)+)	10.00		3.47					
CD2343	<i>cat1</i>	Succinyl-CoA:coenzyme A transferase	11.11	7.69	2.99					
CD2344		Succinate permease. putative membrane protein	3.70		3.61					
<b>Wood Ljungdahl pathway</b>										
CD0718	<i>fhs</i>	Formate--tetrahydrofolate ligase	0.68		0.25					
CD0719	<i>fchA</i>	Methenyltetrahydrofolate cyclohydrolase (5,10-methenyltetrahydrofolate cyclohydrolase)	0.66		0.2					
CD0721		Conserved hypothetical protein	0.61		0.23					
CD0723		Bifunctional CO dehydrogenase/acetyl-CoA synthase. dihydrolipidate reductase	0.65		0.21					
CD0724		Bifunctional CO dehydrogenase/acetyl-CoA synthase.nickel-insecticidal protein	0.63		0.21					
CD0725		Bifunctional CO dehydrogenase/acetyl-CoA synthase. subunit delta	0.61		0.19					
CD0726		Bifunctional CO dehydrogenase/acetyl-CoA synthase. delta subunit	0.61		0.22					
CD0727		Bifunctional CO dehydrogenase/acetyl-CoA synthase. methyltransferase	0.64		0.26					
CD0728		Bifunctional CO dehydrogenase/acetyl-CoA synthase. subunit alpha	0.67		0.33					
CD0716	<i>cooS</i>	Bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthase			0.14					
CD0717		Bifunctional CO dehydrogenase/acetyl-CoA synthase. accessory	0.58		0.13					
<b>Other</b>										
CD3091	<i>treA</i>	Trehalose-6-phosphate hydrolase	1.89		4.14					
CD0582		Putative pyruvate phosphate dikinase. PEP/pyruvate-binding	7.14		2.79					
<b>Energy</b>										
<b>ATSynthase/ATPase (V-type)</b>										
CD2954	<i>ntpD</i>	V-type ATP synthase subunit D	0.58		0.07					
CD2955	<i>ntpB</i>	V-type ATP synthase beta chain (V-type ATPase subunit B)	0.62		0.06					
CD2956	<i>ntpA</i>	V-type ATP synthase alpha chain (V-type ATPase subunit A)	0.62		0.06					
CD2956A	<i>ntpF</i>	V-type ATP synthase subunit F	0.56		0.01					
CD2957	<i>ntpC</i>	V-type ATP synthase subunit C	0.616		0.05					
CD2958	<i>ntpE</i>	V-type ATP synthase subunit E (V-type ATPase subunit E)	0.666		0.05					
CD2959	<i>ntpK</i>	V-type ATP synthase subunit K	0.586		0.04					
CD2960	<i>ntpI</i>	V-type sodium ATP synthase subunit I	0.65		0.07					
<b>Rnf complex</b>										
CD1137	<i>rnfC</i>	Electron transport complex protein	0.58							
CD1138	<i>rnfD</i>	Electron transport complex protein	0.56							
CD1139	<i>rnfG</i>	Electron transport complex protein	0.53							
CD1140	<i>rnfE</i>	Electron transport complex protein	0.56							
CD1141	<i>rnfA</i>	Electron transport complex protein	0.55							
CD1142	<i>rnfB</i>	Electron transport complex protein	0.59		2.67					

Gene-ID	Name	Function	630Δerm/ CD2214-CD2215 mutant		Biofilm/ planktonic growth Micro-array	c-di-GMP riboswitch <sup>1</sup>				
			Micro-array	qRT-PCR						
<b>Nitrogen source metabolism</b>										
<b>Uptake systems for oligo-peptides and amino-acids</b>										
CD2670	appF	ABC-type transport system. ATP-binding protein putative oligopeptide transporter	1.64		6.53					
CD2671	appD	ABC-type transport system. ATP-binding protein putative oligopeptide transporter	1.92		6.38					
CD2672	appA	ABC-type transport system. oligopeptide-family solute-binding protein			13.78					
CD2673	appB	ABC-type transport system. oligopeptide-family permease protein			10.09					
CD2674	appC	ABC-type transport system. oligopeptide-family permease protein			11.28					
<b>Dtp systems for di- or tri-peptide uptake</b>										
CD3036		Transporter. Major Facilitator Superfamily (MFS)	5.26		3.66					
<b>Other</b>										
CD2373		Putative CstA-like carbon starvation protein	0.52		0.08					
<b>Peptidases</b>										
CD2697		Putative peptidase. M20D family	1.85		4.87					
CD2698		Putative membrane protein	2.22		2.63					
CD2699		Putative membrane protein	2.08		3.59					
CD0779		Putative amidohydrolase. M20D peptidase family	0.56		0.21					
CD0777		Putative membrane protein	0.57		0.25					
CD0778		Conserved hypothetical protein	0.57		0.19					
CD0780		Conserved hypothetical protein. DUF1177 family	0.53							
CD3269		Putative oligoendopeptidase F. M3B family	1.96							
CD0528		Putative amidohydrolase	1.92		3.14					
CD0529		Putative membrane protein	2.08		2.57					
CD3458		Putative membrane protein	0.66		0.15					
<b>Histidine biosynthesis</b>										
CD1549	hisC	Histidinol-phosphate aminotransferase (Imidazole acetol-phosphate transaminase)			0.33					
CD1550	hisB	Imidazoleglycerol-phosphate dehydratase			0.35					
CD1551	hisH	Imidazole glycerol phosphate synthase subunit HisH	0.67		0.3					
CD1552	hisA	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-phosphate synthase	0.63		0.34					
CD1553	hisF	Imidazole glycerol phosphate synthase subunit HisF			0.41					
<b>Leucine biosynthesis</b>										
CD0991	leuD	3-isopropylmalate dehydratase small subunit			0.30					
CD0992	leuB	3-isopropylmalate dehydrogenase	1.82		0.20					
<b>Sulfur source transport</b>										
CD1482	ssuC	ABC-type transport system. sulfonates-family permease			0.33					
CD1483	ssuB	ABC-type transport system. sulfonates-family ATP-binding protein			0.21					
CD1484	ssuA	ABC-type transport system. alkanesulfonates-family extracellular	0.69		0.21					
<b>Cysteine / sulfur metabolism</b>										
CD3029	malY	Bifunctional protein: cystathionine beta-lyase / repressor	14.29	100,00	20.32					
CD3598	luxS	S-ribosylhomocysteine lyase (AI-2 synthesis protein)	1.69							
CD1279	iscS2	Cysteine desulfurase			0.26					
CD1278	iscR	Transcriptional regulator. Rrf2 family	1.75		0.4					
<b>Fermentations (amino acids) - Stickland reactions</b>										
<b>Glycine reduction</b>										
CD2348	grdD	Glycine reductase complex component C subunit alpha (Protein PC alpha)			0.09					
CD2349	grdC	Glycine reductase complex component C subunit beta (Protein P)	1.52		0.08					
CD2351	grdB	Glycine reductase complex component B gamma subunit (selenocysteine)	1.69		0.05					
CD2352	grdA	Glycine reductase complex selenoprotein A (selenocysteine)	1.69		0.04					
CD2354	grdE	Glycine reductase complex component B subunits alpha and beta (Selenoprotein PB alpha/beta)			0.05					
CD2355	trxA2	Thioredoxin 2 (Trx2)			0.07					
CD2356	trxB3	Thioredoxin reductase 3			0.09					
CD2357	grdX	Putative glycine reductase complex component			0.32					

Gene-ID	Name	Function	630Δerm/ CD2214-CD2215 mutant		Biofilm/ planktonic growth	c-di-GMP riboswitch <sup>1</sup>
			Micro-array	qRT-PCR	Micro-array	
<b>Envelope biogenesis</b>						
<b>Fatty acid biosynthesis</b>						
CD1062	acpP	Acyl carrier protein (ACP)	1.69		3.24	
<b>Cell wall</b>						
CD0119	glmM	Phosphoglucosamine mutase	1.52		4.01	
CD0120	glmS	Glucosamine-fructose-6-phosphate aminotransferase	1.612		4.09	
CD2239		Putative Na <sup>+</sup> /solute symporter, SSS family	2.78		4.52	
CD2240	nanA	Acetylneuraminate lyase			5.56	
CD2241	nanE	N-acetylmannosamine-6-phosphate 2-epimerase (ManNAc-6-P epimerase)			3.57	
CD1010	nagA	N-acetylglucosamine-6-phosphate deacetylase	2.70			
CD1011	nagB	Glucosamine-6-phosphate deaminase	2.44			
CD2136	uppS	Undecaprenyl pyrophosphate synthetase	0.66		0.3	
<b>D-Alanylation of wall polysaccharides</b>						
CD2851	dltC	D-alanine--poly(phosphoribitol) ligase subunit 2 (D-alanyl carrier protein) (DCP)			3.42	
CD2852	dltB	D-alanyl transferase DltB. MBOAT family	1.45		5.21	
CD2853	dltA	D-alanine--poly(phosphoribitol) ligase subunit 1			2.97	
CD2854	dltD	D-alanine transferase DltD			2.38	
<b>Exported proteins</b>						
CD2831		Putative adhesin	2.17	3,13	2.76	Type II
CD3513		Putative pilin protein	2.63	3,45	2.61	Type II
CD2305		Putative pilin protein	3.33	3,13	3.5	
CD0873		Adhesin and Sugar-binding lipoprotein (ABC transport system)	2.63		6.03	
CD3246		Putative surface protein	2.04			Type II
CD2830	zmp1	Extracellular Zinc metalloprotease			0.21	Type I
Gene-ID	Name	Function	630Δerm/ CD2214-CD2215 mutant		Biofilm/ planktonic growth	c-di-GMP riboswitch <sup>1</sup>
			Micro-array	qRT-PCR	Micro-array	
<b>Transcriptional regulator</b>						
CD0312		Transcriptional regulator. ArsR family	1.49		3.01	
CD1755A		Transcriptional regulator. HTH-type	1.82		0.35	
CD2214		Transcriptional regulator. HTH-type. homologous to B. subtilis SirR	3.702			
CD2215		Transcriptional regulator. HTH-type	4.127			
CD0581		Transcriptional regulator. TetR family	3.13	6,25		
CD2463	hrcA	Transcriptional regulator. Heat-inducible repressor HrcA	2.00			
CD2564		Transcriptional regulator. MerR family	2.94			
CD3175	cggR	Transcriptional regulator. SorC family	5.88			
CD1998		Transcriptional regulator. TetR family	2.04			
CD2004	effR	Transcriptional regulator. MarR family	3.03			
CD1009		Transcriptional regulator. GntR family	3.03			
CD2665		Transcriptional regulator. AraC family	3.23			
CD2345		Transcriptional regulator. LysR family	1.89			
CD0470	blaR	Beta-lactamase-inducing penicillin-binding protein	2.27			
CD0471	blal	Transcriptional regulator. Penicillinase repressor	2.38			
CD3158		Transcriptional regulator. TRAP family	3.85			
<b>Two component system family</b>						
CD3265		Two-component response regulator	2.27		2.73	
CD3267		Two-component response regulator	2.33		3.68	Type II
<b>Signaling proteins</b>						
CD1420	dccA	diguanylate cyclase	2.13		2.37	
CD1421		Putative phosphodiesterase	2.44		2.04	
CD2384		Putative diguanylate cyclase	0.68		4.06	
CD1515		Putative phosphodiesterase	0.68			
CD1616		Putative phosphodiesterase	2.70	4,55		
<b>Other</b>						
CD1893		Putative oligonucleotide binding regulator	0.62		0.37	
CD1498	sigA2	RNA polymerase sigma factor SigA2 (sigma-43)	0.65		0.34	

Gene-ID	Name	Function	630Δerm/ CD2214-CD2215 mutant		Biofilm/ planktonic growth Micro-array	c-di-GMP riboswitch <sup>1</sup>
			Micro-array	qRT-PCR		
<b>Other metabolism</b>						
CD1512	<i>panC</i>	Pantothenate synthetase	0.43		0.35	
CD1513	<i>panB</i>	Ketopantoate hydroxymethyltransferase	0.47		0.25	
CD2737		Putative nitrilase/cyanide hydratase and apolipoprotein N-acyltransferase	0.59		0.28	
CD1508		Putative iron-sulfur binding protein	0.63		0.26	
CD1507A		Conserved hypothetical protein	0.59			
CD0730		Putative iron-sulfur protein	0.67		0.20	
CD0731		Putative radical SAM superfamily protein	0.58		0.17	
CD0732		Putative radical SAM superfamily protein			0.24	
CD0733		Putative biotin/lipoate-protein ligase	0.65		0.17	
CD0184	<i>pyrB</i>	Aspartate carbamoyltransferase )	1.69			
CD0185	<i>pyrK</i>	Dihydroorotate dehydrogenase electron transfer subunit	1.96			
CD0186	<i>pyrD</i>	Dihydroorotate dehydrogenase catalytic subunit	2.17			
CD0594		Conserved hypothetical protein	1.75			
CD0595		Putative nuclelease. ParB-like	2.04			
CD0994		Putative serine-pyruvate aminotransferase	2.44			
CD0995	<i>serA</i>	Putative D-3-phosphoglycerate dehydrogenase	4.17	3,57		
CD0996		Conserved hypothetical protein	3.13			
CD2479		Putative molybdenum cofactor biosynthesis	2.17			
CD3555	<i>coaX</i>	Type III pantothenate kinase	2.50			
CD3556		Putative membrane protein	2.38			
CD3248		Polysaccharide deacetylase	3.85			
<b>Other transport</b>						
CD0313		Putative K/Mg/Cd/Cu/Zn/Na/Ca/Na/H-transporting P-type ATPases	1.92		3.86	
CD3036		Transporter. Major Facilitator Superfamily (MFS)	5.26		3.66	
CD2594	<i>uraA</i>	ABC-type transport system. uracil-specific permease	2.33		2.49	
CD2541		Sodium:glutamate/aspartate symporter family	2.22		2.83	
CD2738		Putative cytosine permease	0.60		0.26	
CD1518	<i>feoA</i>	Ferrous iron transport protein	0.56		0.33	
CD3273	<i>feoA</i>	Ferrous iron transport protein	1.72			
CD3274	<i>feoB</i>	Ferrous iron transport protein B	1.85			
CD2276		Sodium:alanine symporter	3.23			
<b>Stress</b>						
CD0193	<i>groS</i>	10 kDa chaperonin. GroES protein	1.59			
CD0194	<i>groL</i>	60 kDa chaperonin. GroEL protein	1.89		2.98	
CD2149		Putative vancomycin resistance protein. vanW family	1.79		4.63	

Gene-ID	Name	Function	630Δerm/ CD2214-CD2215 mutant		Biofilm/ planktonic growth Micro-array	c-di-GMP riboswitch <sup>1</sup>
			Micro-array	qRT-PCR		
<b>Sporulation</b>						
CD1492		Two-component sensor histidine kinase. sporulation-associated	0.56			
CD1613	cotA	Spore outer coat layer protein CotA	0.51			
CD2400	cotJB2	Spore coat peptide assembly protein CotJB 2	0.63			
CD3349	bclA3	Exosporium glycoprotein BclA3	0.40			
CD0596		Conserved hypothetical protein	0.46			
CD0597	cotJB1	Spore coat peptide assembly protein	0.45			
CD0598	cotCB	Spore-coat protein CotCB manganese catalase	0.42			
CD1067		Conserved hypothetical protein	0.52			
CD1063B		Conserved hypothetical protein	0.54			
CD1063C		Conserved hypothetical protein	0.58			
CD1581		Conserved hypothetical protein	0.33			
CD1845		Putative membrane protein Tn1549-like. CTn5-Orf1	0.63			
<b>Other</b>						
CD3149		DNA helicase	0.67			
<b>Unknown</b>						
CD1768		Conserved hypothetical protein	2.38		5.78	
CD2366		Conserved hypothetical protein	0.65		4.75	
CD3252		Conserved hypothetical protein	1.52		3.6	
CD0172		Conserved hypothetical protein	0.69		0.27	
CD0279		Conserved hypothetical protein	0.61		0.13	
CD1568		Conserved hypothetical protein	0.68		0.53	
CD1622		Conserved hypothetical protein	0.57		0.22	
CD2962		Conserved hypothetical protein	0.63		0.13	
CD3148		Hypothetical protein	0.64			
CD3271		Conserved hypothetical protein	1.75			
CD3622		Conserved hypothetical protein. PhnA family	2.17			
CDP10		Hypothetical protein	2.78			

Gene identification number (ID), names and functions correspond to those indicated in the MaGe database Clostriscope (<https://www.genoscope.cns.fr>). A gene was considered as differentially expressed when the p-value was < 0.05 (see Material and Methods).

#### Reference

- <sup>1</sup> Soutourina, O.A., Monot, M., Boudry, P., Saujet, L., Pichon, C., Sismeiro, O., Semenova, E., Severinov, K., Le Bouguenec, C., Coppee, J.Y., Dupuy, B., and Martin-Verstraete, I. (2013) Genome-wide identification of regulatory RNAs in the human pathogen *Clostridium difficile*. *PLoS Genet* 9, e1003493











