

Supplementary Figure 1: Sporulation of *C. difficile* on solid medium.

C. difficile 630 was grown on agar at 37°C. Each day samples were removed and examined for the number of spores by either phase contrast microscopy using a haemocytometer to count phase bright spores and vegetative cells, or, by heating the spore suspension for 60°C for 20 min and plating for CFU/ml with comparison to untreated CFU/ml.

Proteins

	Orthologues															
Proteins	<i>C.d.</i>	<i>B.s.</i>	<i>B.li.</i>	<i>B.a.</i>	<i>B.c.</i>	<i>B.t.</i>	<i>B.cl.</i>	<i>B.h.</i>	<i>G.k.</i>	<i>O.i.</i>	<i>C.p.</i>	<i>C.a.</i>	<i>C.t.</i>	<i>C.th.</i>	<i>C.n.</i>	<i>C.c.</i>
<i>CotA</i>	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>CotB</i>	+	-	+	+	+	+	-	+	-	-	+	+	+	+	-	+
<i>CotCA</i>	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+
<i>CotCB</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
<i>CotD</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
<i>CotE-perox-chitinase</i>	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>CotE-peroxiredoxin</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
<i>CotE-chitinase</i>	+	+	+	+	+	+	+	+	-	+	-	-	-	+	-	+

Orthologues were identified through BLASTP searches (www.ncbi.nlm.nih.gov) using protein sequences against the following genomes:

B.a., *Bacillus anthracis* Sterne

B.c., *B. cereus* ATCC 10987

B.cl., *B. clausii* KSM-K16

B.h., *B. halodurans* C-125

B.l., *B. licheniformis* ATCC 14580

B.s., *B. subtilis* 168

B.t., *B. thuringiensis* serovar konkukian str. 97-27

C.a., *Clostridium acetobutylicum* ATCC 824

C.c., *Clostridium cellulolyticum* H10

C.d., *Clostridium difficile* 630

C.n., *Clostridium novyi* NT

C.p., *Clostridium perfringens* ATCC 13124

C.t., *Clostridium tetani* E88 (asporogenous)

C.th., *Clostridium thermocellum* ATCC 27405

G.k., *Geobacillus kaustophilus* HTA426

O.i., *Oceanobacillus iheyensis* HTE831

Supplementary Fig 2: Orthologues of *C. difficile* 630 spore surface proteins CotA-E in other spore formers

The table shows possible orthologues of CotA-E found in *Geobacillus kaustophilus* and *Oceanobacillus iheyensis* as well as other common *Clostridium* and *Bacillus* spore formers. For CotE also shown are orthologues to the individual peroxiredoxin and chitinase domains. Hypothetical proteins are also included.

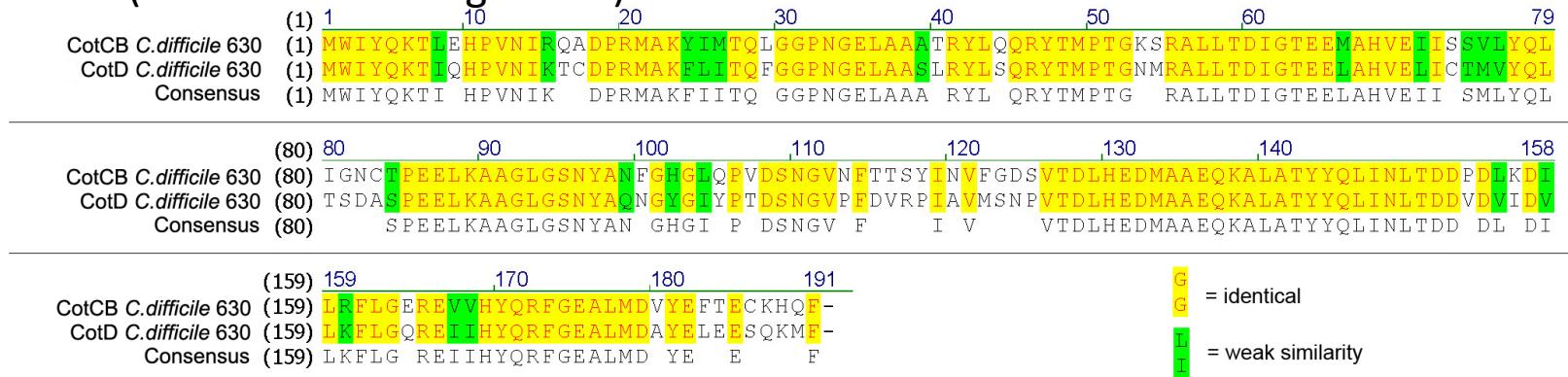
A (CotCB)

		Mn Catalase					
1	MWIYQKTLEH PVNIRQADPR MAKYIMTQLG GPNGEELAAAT RYLQQRYTMP TGKSRALLTD IGTEEMAHVE IISSVLYQLI GNCTPEELKA	*	*	*	*		
Mn_Catalase							
91	AGLGSNYANF GHGLQPVDN GVNFTTSYIN VFGDSVTDLH EDMAAEQKAL ATYYQLINIT DDPDIKDILR FLGEREVVHY QRFGEALMDV	*			*		
181	YEFTECKHQF *						

B (CotD)

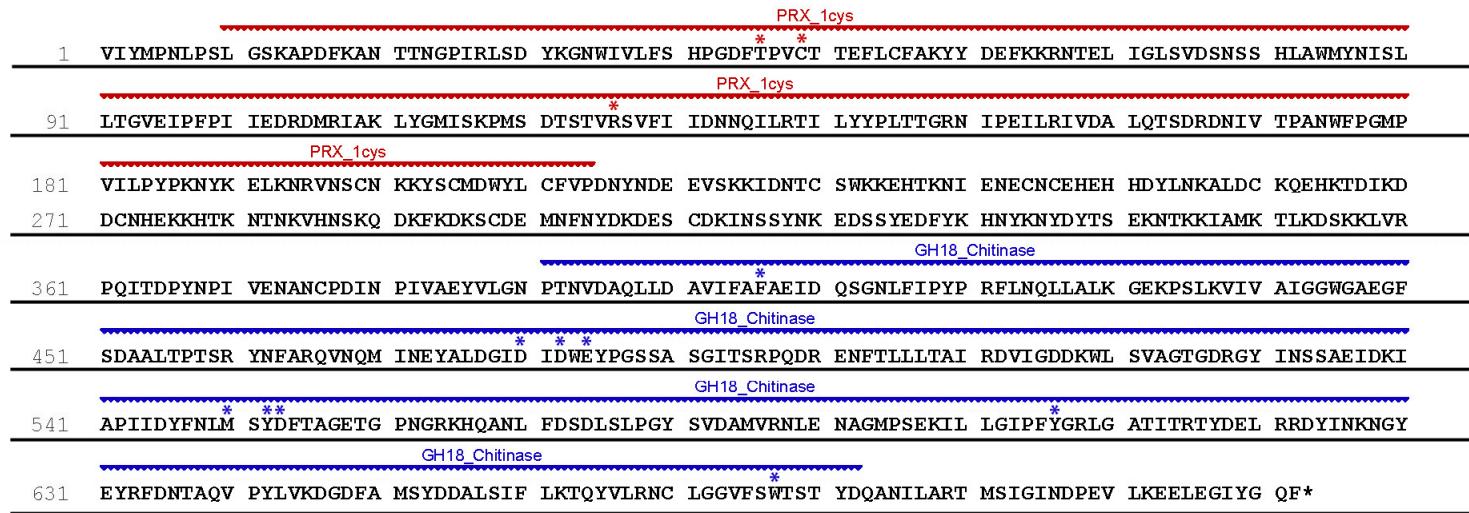
		Ferritin_like superfamily					
1	MWIYQKTIQH PVNIKTCDPR MAKFLITQFG GPNGEELAASL RYLSQRYTMRP TGNMRALLTD IGTEELAHVE LICTMVYQLT SDASPEELKA	*		*	*	*	
Ferritin_like superfamily							
91	AGLGSNYAQN GYGIYPTDSN GVPFDVRPIA VMSNPVTDLH EDMAAEQKAL ATYYQLINLT DDVDVIDVLK FLGQREIIHY QRFGEALMDA	*			*	*	
181	YELEESQKMF *						

C (CotCB vs CotD alignment)



Supplementary Fig 3: CotCB and CotD.

Panel A shows the entire CotCB polypeptide and its similarity with the manganese catalases (a family of ferritin-like diiron enzymes). Residues involved in forming the dimanganese centre are indicated (*). **Panel B** shows the homology of CotD with the ferritin-like family of catalases and amino acids involved in forming the dinuclear metal binding motif (*). **Panel C** shows the amino acid sequence homology between CotCB and CotD which share consensus and identity positions at 80.6% and 70.2% respectively.



~~~~~ = Peroxiredoxin Domain

~~~~~ = Chitinase Domain

* = amino acids involved in catalytic site (chitinase domain)

* = amino acids forming catalytic triad (peroxiredoxin domain)

Supplementary Fig 4: CotE.

Figure shows the entire CotE polypeptide and its amino-terminal 1-cys-peroxiredoxin and carboxy-terminal chitinase domains. Active site residues are indicated.