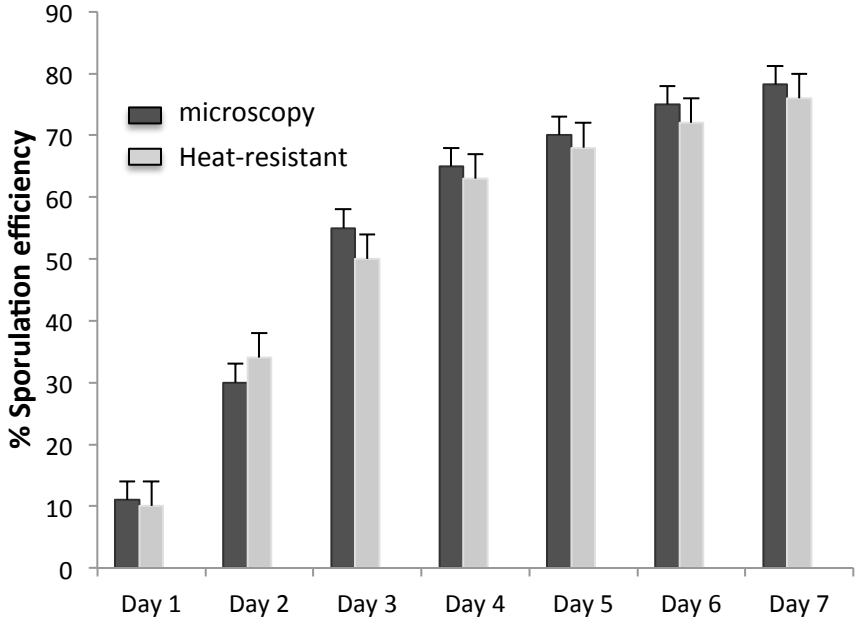


Supplementary Figure 1: Sporulation of *C. difficile* on solid medium.
C. difficile 630 was grown on agar at 37°C. Each day samples were removed and examined for the number of spores by either phase contrast microscopy using a haemocytometer to count phase bright spores and vegetative cells, or, by heating the spore suspension for 60°C for 20 min and plating for CFU/ml with comparison to untreated CFU/ml.

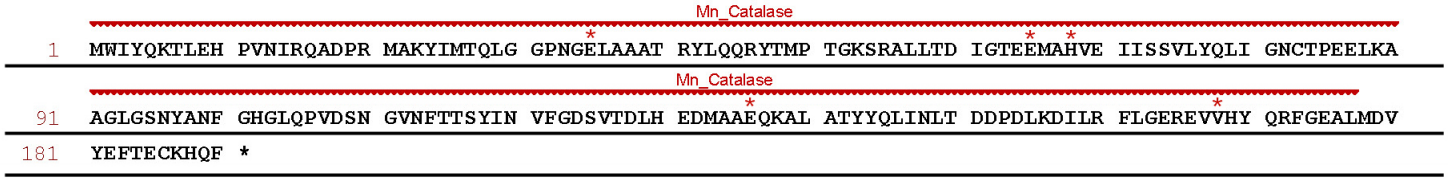
Proteins	Orthologues															
	C.d	*B.s.*	*B.li.*	*B.a.*	*B.c.*	*B.t.*	*B.cl.*	*B.h.*	*G.k.*	*O.i.*	*C.p.*	*C.a.*	*C.t.*	*C.th.*	*C.n.*	*C.c.*
CotA	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CotB	+	-	+	+	+	+	-	+	-	-	+	+	+	+	-	+
CotCA	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+
CotCB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
CotD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

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

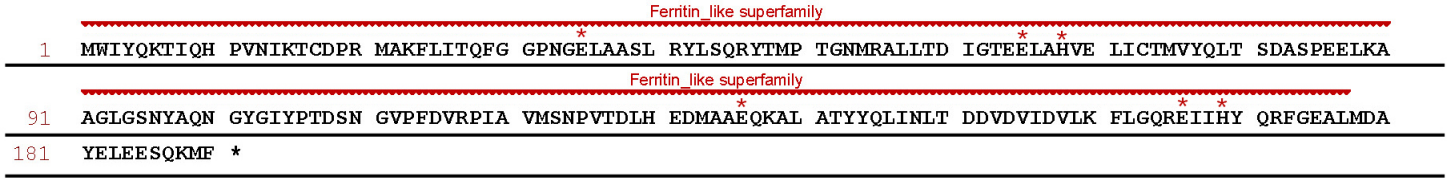
B.a., *Bacillus anthracis* Sterne
B.c., *B. cereus* ATCC 10987
B.cl., *B. clausii* KSM-K16
B.h., *B. halodurans* C-125
B.l., *B. licheniformis* ATCC 14580
B.s., *B. subtilis* 168
B.t., *B. thuringiensis* serovar konkukian str. 97-27
C.a., *Clostridium acetobutylicum* ATCC 824
C.c., *Clostridium cellulolyticum* H10
C.d., *Clostridium difficile* 630
C.n., *Clostridium novyi* NT
C.p., *Clostridium perfringens* ATCC 13124
C.t., *Clostridium tetani* E88 (asporogeneous)
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.

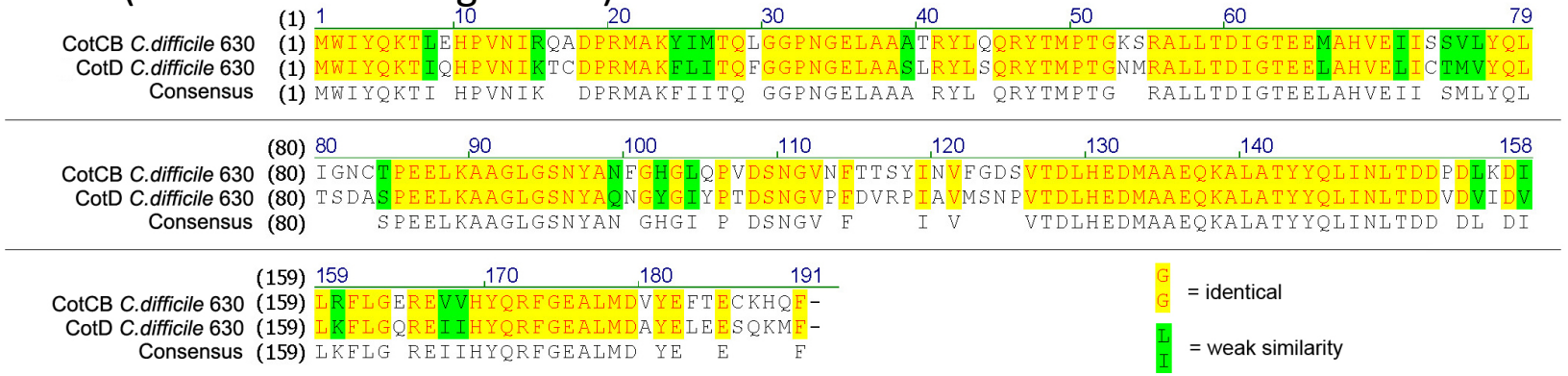
A (CotCB)



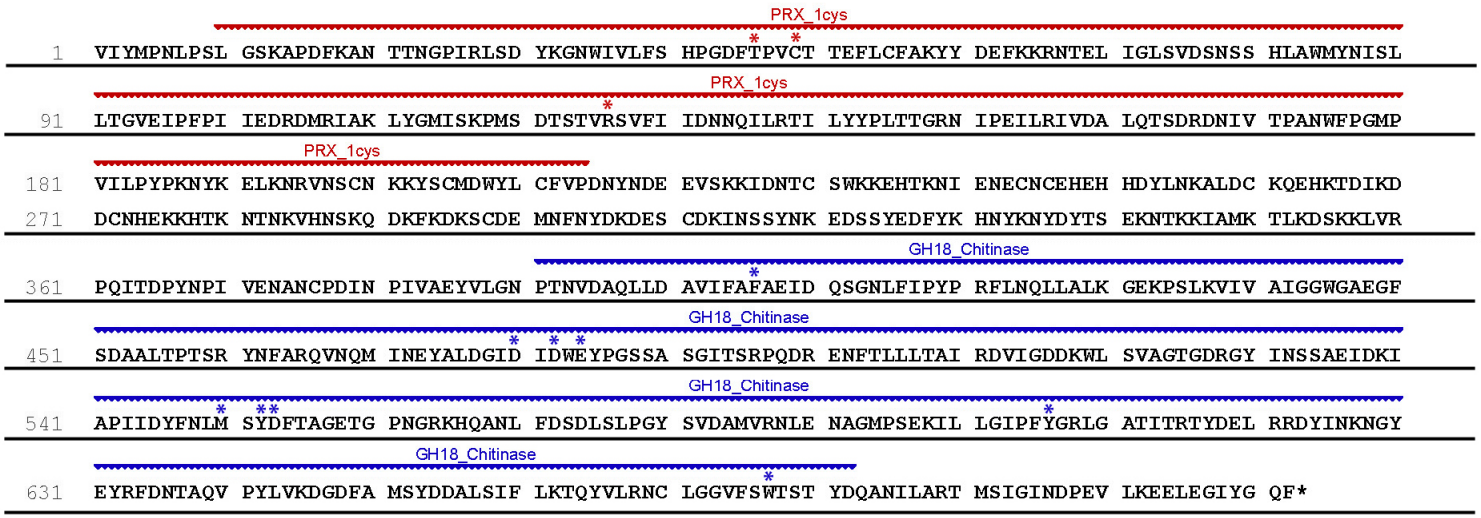
B (CotD)



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 diron 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.



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.