



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 | | | | | | | | | | | | | | | |
|----------------------|-------------|-------------|--------------|-------------|-------------|-------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|-------------|-------------|
| | <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> |
| 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)

| | Mn_Catalase | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| 1 | MW | YQ | KT | LE | H | P | V | N | I | R | Q | A | D | P | R | M | A | K | Y | I | M | T | Q | L | G | P | N | G | E | L | A | A | A | T | R | Y | L | Q | Q | R | Y | T | M | P | T | G | K | S | R | A | L | L | T | I | G | T | E | E | M | A | H | V | E | I | I | S | S | V | L | Y | Q | L | I | G | N | C | T | P | E | E | L | K | A | | | | | | |
| 91 | A | G | L | G | S | N | Y | A | N | F | G | H | G | L | Q | P | V | D | S | N | G | V | N | F | T | T | S | Y | I | N | V | F | G | D | S | V | T | D | L | H | E | D | M | A | E | Q | K | A | L | A | T | Y | Y | Q | L | I | N | L | T | D | D | P | D | L | K | D | I | L | R | F | L | G | E | R | E | V | V | H | Y | Q | R | F | G | E | A | L | M | D | V |
| 181 | Y | E | F | T | E | C | K | H | Q | F | * | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

B (CotD)

| | Ferritin_like superfamily | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| 1 | MW | YQ | KT | I | Q | H | P | V | N | I | K | T | C | D | P | R | M | A | K | F | L | I | T | Q | F | G | P | N | G | E | L | A | A | S | L | R | Y | L | S | Q | R | Y | T | M | P | T | G | K | S | T | G | N | M | R | A | L | L | T | I | G | T | E | E | L | A | H | V | E | L | I | C | T | M | V | Y | Q | L | T | S | D | A | S | E | E | L | K | A |
| 91 | A | G | L | G | S | N | Y | A | Q | N | G | Y | G | I | P | T | D | S | N | G | V | P | D | V | R | P | I | A | V | M | S | N | P | V | T | D | L | H | E | D | M | A | E | Q | K | A | L | A | T | Y | Y | Q | L | I | N | L | T | D | D | V | D | V | I | D | V | L | K | F | L | G | Q | R | E | I | I | H | Y | Q | R | F | G | E | A | L | M | D | A |
| 181 | Y | E | L | E | S | Q | K | M | F | * | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

C (CotCB vs CotD alignment)

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | (1) | 1 | 10 | 20 | 30 | 40 | 50 | 60 | 79 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CotCB <i>C.difficile</i> 630 | (1) | MW | YQ | KT | LE | HP | VNI | RQA | DPR | MAKY | IM | TQL | G | P | N | G | E | L | A | A | A | T | R | Y | L | Q | Q | R | Y | T | M | P | T | G | K | S | R | A | L | L | T | D | I | G | T | E | E | M | A | H | V | E | I | I | S | S | V | L | Y | Q | L | | | | | | | | | | | | | | | | | | |
| CotD <i>C.difficile</i> 630 | (1) | MW | YQ | KT | I | Q | HP | VNI | K | T | C | D | P | R | M | A | K | F | L | I | T | Q | F | G | P | N | G | E | L | A | A | S | L | R | Y | L | S | Q | R | Y | T | M | P | T | G | N | M | R | A | L | L | T | D | I | G | T | E | E | L | A | H | V | E | L | I | C | T | M | V | Y | Q | L | | | | | | | |
| Consensus | (1) | MW | YQ | KT | I | HP | VNI | K | DPR | MAK | F | I | T | Q | F | G | P | N | G | E | L | A | A | A | R | Y | L | S | Q | R | Y | T | M | P | T | G | N | M | R | A | L | L | T | D | I | G | T | E | E | L | A | H | V | E | I | I | S | M | L | Y | Q | L | | | | | | | | | | | | | | | | | |
| | (80) | 80 | 90 | 100 | 110 | 120 | 130 | 140 | 158 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CotCB <i>C.difficile</i> 630 | (80) | I | G | N | C | T | P | E | E | L | K | A | A | G | L | G | S | N | Y | A | N | F | G | H | G | L | Q | P | V | D | S | N | G | V | N | F | T | T | S | I | N | V | F | G | D | S | V | T | D | L | H | E | D | M | A | E | Q | K | A | L | A | T | Y | Y | Q | L | I | N | L | T | D | D | P | D | L | K | D | I | |
| CotD <i>C.difficile</i> 630 | (80) | T | S | D | A | S | P | E | E | L | K | A | A | G | L | G | S | N | Y | A | N | F | G | H | G | L | Q | P | V | D | S | N | G | V | N | F | D | V | R | P | I | A | V | M | S | N | P | V | T | D | L | H | E | D | M | A | E | Q | K | A | L | A | T | Y | Y | Q | L | I | N | L | T | D | D | V | D | V | I | D | V |
| Consensus | (80) | S | P | E | E | L | K | A | A | G | L | G | S | N | Y | A | N | F | G | H | G | I | P | D | S | N | G | V | F | I | V | V | T | D | L | H | E | D | M | A | E | Q | K | A | L | A | T | Y | Y | Q | L | I | N | L | T | D | D | D | L | D | I | | | | | | | | | | | | | | | | | | |
| | (159) | 159 | 170 | 180 | 191 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CotCB <i>C.difficile</i> 630 | (159) | L | R | F | L | G | R | E | R | E | V | V | H | Y | Q | R | F | G | E | A | L | M | D | V | Y | E | F | T | E | C | K | H | Q | F | - | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CotD <i>C.difficile</i> 630 | (159) | L | K | F | L | G | Q | R | E | I | I | H | Y | Q | R | F | G | E | A | L | M | D | A | Y | E | L | E | S | Q | K | M | F | - | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Consensus | (159) | L | K | F | L | G | R | E | I | I | H | Y | Q | R | F | G | E | A | L | M | D | Y | E | E | - | - | - | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

■ = identical

■ = weak similarity

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.

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                                PRX_1cys
1  VIYMPNLPSL GSKAPDFKAN TTNGPIRLSD YKGNWIVLFS HPGDFTPVCT TEFLCFAYK DEFKKRNTTEL IGLSVDSNSS HLAWMYNISL
-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*
                                PRX_1cys
91 LTGVEIPFPI IEDRDMRIAK LYGMISKPMS DTSTVRSVFI IDNNQILRTI LYYPLTTGRN IPEILRIVDA LQTSDRDNIV TPANWFPGMP
-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*
                                PRX_1cys
181 VILPYPKNYK ELKNRVNSCN KKYS CMDWYL CFVPDNYNDE EVSKKIDNTC SWKKEHTKNI ENECNCEHEH HDYLNKALDC KQEHKTDIKD
271 DCNHEKKHTK NTKVHNSKQ DKFKDKSCDE MNFNYDKDES CDKINSSYNK EDSSYEDFYK HNYKNYDYTS EKNTKKIAMK TLKDSKKLVR
-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*
                                                                GH18_Chitinase
361 PQITDPYNPI VENANCPDIN PIVA EYVLGN PTNVDAQLLD AVIFAFAEID QSGNLFIPYP RFLNQLLALK GEKPSLKVIV AIGGWGAEGF
-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*
                                                                GH18_Chitinase
451 SDAALTPTS R YNFARQVNQM INEYALDGD IDWEYPGSSA SGITSRPQDR ENFTLLLTAI RDVIGDDKWL SVAGTGDRGY INSSAEIDKI
-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*
                                                                GH18_Chitinase
541 APIIDYFNLM SYDFTAGETG PNGRKHQANL FSDLSLPGY SVDAMVRNLE NAGMPSEKIL LGIPFYGR LG ATITRTYDEL RRDYINKNGY
-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*
                                                                GH18_Chitinase
631 EYRFDNTAQV PYLVKDG DFA MSYDDALSIF LKTQYVLRNC LGGVFSWTST YDQANILART MSIGINDPEV LKEELEG IYG QF*
-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*

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-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----*-----* = 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.