

Supplementary Figure 1A: PCR using ErmRAM primers Erm-F and Erm-R with no template (1) and DNA extracts from 630 Δ erm (2) and *cotA::CT555a* (3), *cotB::CT329a* (4), *cotCB::CT220s* (5), *cotD::CT302s* (6), *cotE::CT220s* (7), *cotE::CT1203s* (8) and *sodA::CT394s* (9).

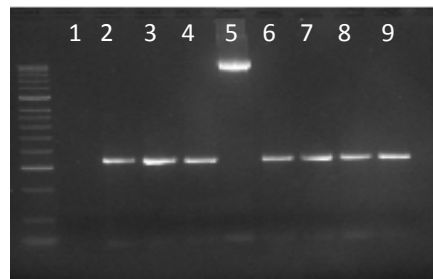
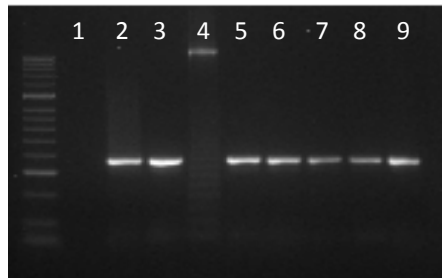
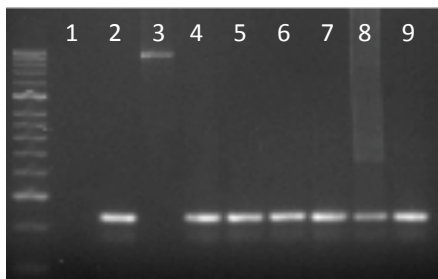
ErmRAM primers	Sequence 5'-3'
Forward (Erm-F)	ACGCGTTATATTGATAAAAATAATAATAGTGGG
Reverse (Erm-R)	ACGCGTGCGACTCATAGAATTATTCCTCCCG



A. *cotA*::CT555

B. *cotB*::CT329

C. *cotCB*::CT220

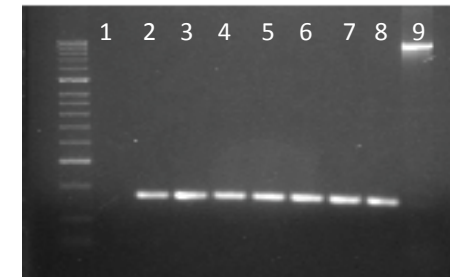
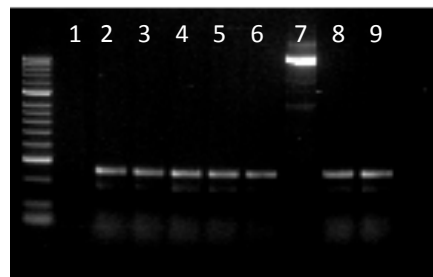
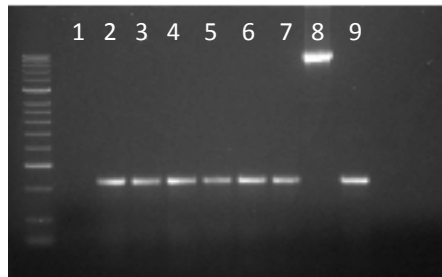
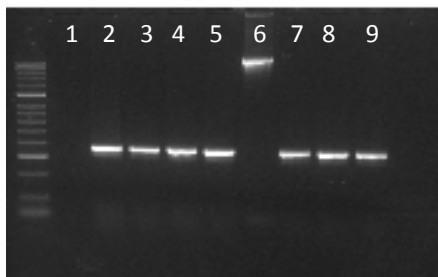


D. *cotD*::CT302

E. *cotE*::CT220

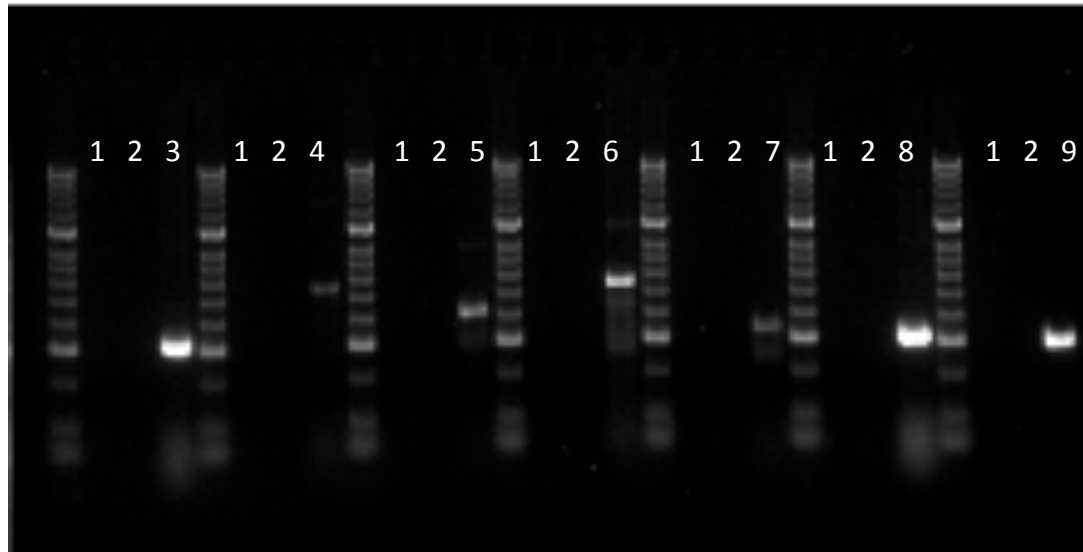
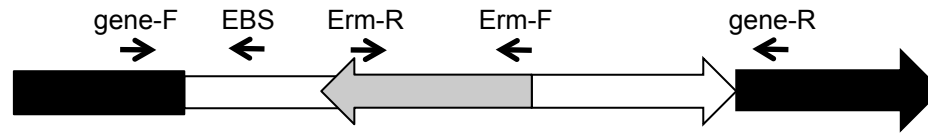
F. *cotE*::CT1203

G. *sodA*::CT394



Supplementary Figure 1B: PCR using primer gene-F and gene-R, targeting the insertion site, with no template (1) and DNA extracts from the 630 Δ *erm* (2) and *cotA*::CT555*a* (3), *cotB*::CT329*a* (4), *cotCB*::CT220*s* (5), *cotD*::CT302*s* (6), *cotE*::CT220*s* (7), *cotE*::CT1203*s* (8) and *sodA*::CT394*s* (9). Primer targeting *cotA* are shown in A, targeting *cotB* (B), *cotCB* (C), *cotD* (D), *cotE*(N) (E), *cotE*(C) (F) and *sodA* (G). (Primer sequences given on next page).

gene	primer	5'-3' sequence
<i>cotA</i>	CotA-clostron-F	TTCCTCCTACGATAGACCTTGG
	CotA-clostron-R	GCAATCATCACAATCGCAGT
<i>cotB</i>	CotB-clostron-F	CAGGGATAGAAATAAACAAAAAC
	CotB-clostron-R	GGGGTCTAGTAATCCTCTCATA
<i>cotCB</i>	CotC-clostron-F	TAACATAAGACAAGCAGACC
	CotC-clostron-R	ATCGCCAAAGACATTA
<i>cotD</i>	CotD-clostron-F	CCCGTAACCGATTTACATGA
	CotD-clostron-R	CCCGTAACCGATTTACATGA
<i>cotN</i>	CotE(N)-clostron-F	CACATCCTGGTGATTTTACACC
	CotE(N)-clostron-R	TGCCGTATAACTTGGCAATTC
<i>cotEC</i>	CotE(C)-clostron-F	AACAGACCCATACAATCCAATAGT
	CotE(C)-clostron-R	CTCCCCAACCTCCAATAGC
<i>sodA</i>	SodA-clostron-F	CCCAACCAGAACCAAAGACA
	SodA-clostron-R	GAAATAATGCAGGTGGAGCTT

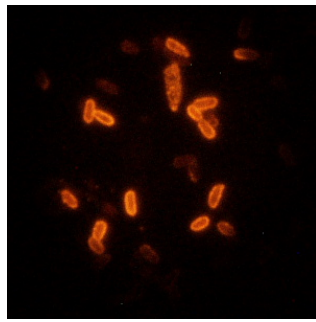


Supplementary Figure 1C: PCR using primer gene-F and the EBS-universal (EBS) with no template (1) and DNA extracts from *630Δerm* (2) and *cotA::CT555a* (3), *cotB::CT329a* (4), *cotCB::CT220s* (5), *cotD::CT302s* (6), *cotE::CT220s* (7), *cotE::CT1203s* (8) and *sodA::CT394s* (9).

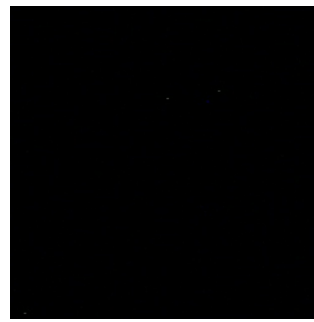
EBS

5'-CGAAATTAGAACTTGCGTTCAGTAAAC-3'

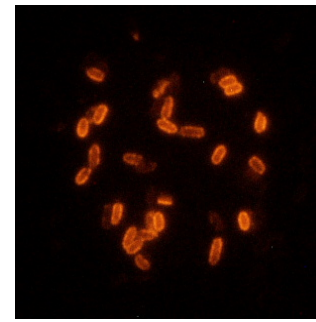
α - CotA



630Δerm

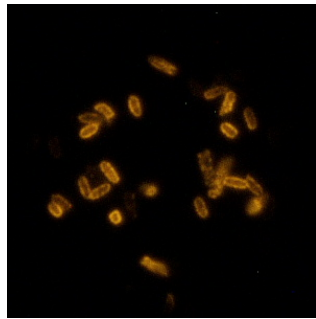


cotA::CT555a

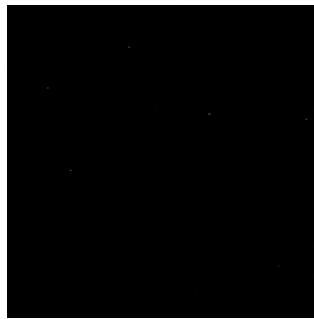


cotA::CT555a
complemented

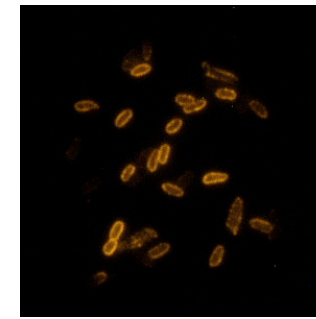
α - CotB



630Δerm

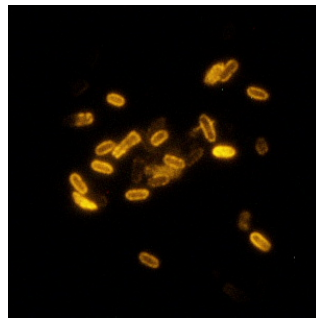


cotB::CT329a

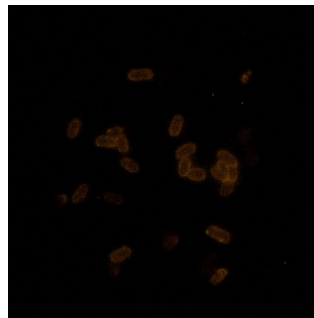


cotB::CT329a
complemented

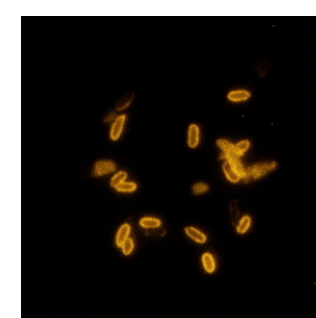
α - CotCB



630Δerm



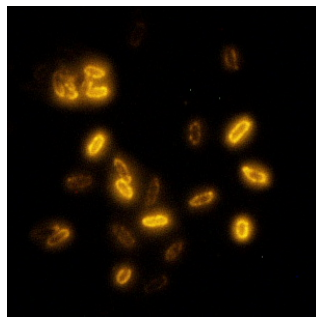
cotCB::CT220s



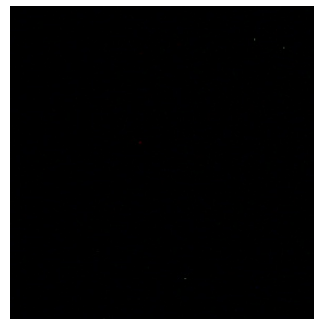
cotCB::CT220s
complemented

Supplementary Figure 2: Complementation of *cot* mutants using immunofluorescence microscopy

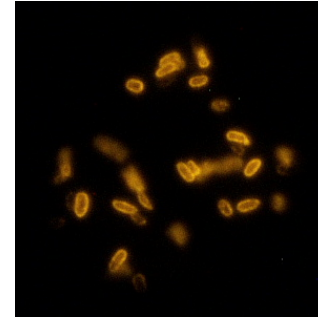
α - CotD



630 Δ erm

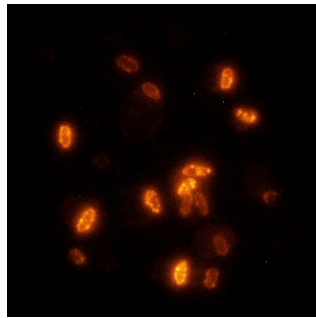


cotD::CT302s

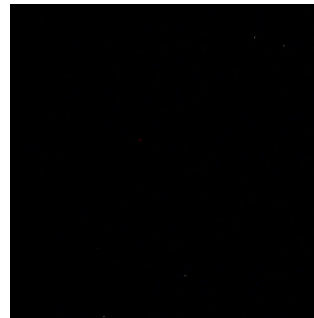


cotD::CT302s
complemented

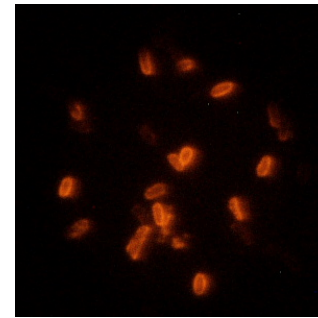
α - CotEN



630 Δ erm

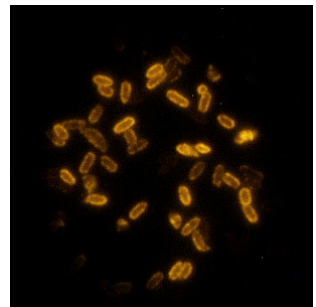


cotE::CT220s

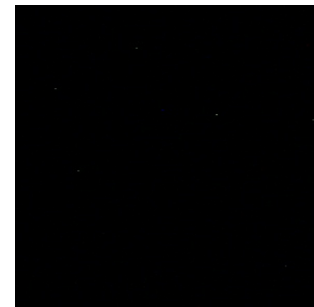


cotE::CT220s
complemented

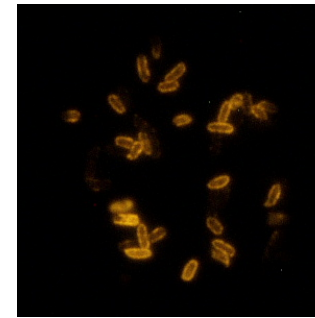
α - SodA



630 Δ erm



sodA::CT394s



sodA::CT394s
complemented

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SodA      MKKKILIPVIMSLFIIISQCITSFAPTPENNKFKVKPLPYAYDALEPYIDKETMKLHHDKH 60
C.a.      -----MTASGGRCLQ--LLRRFHMAFEVPLPYDYNALEPYIDEATMHYHHDNH 48
C.p.      MKNNFLKHKKSPMPAYCGS---SSTKGEFGFKLPLDYPYDALEPSIDEETVKIHHDKH 57
          .          *          *:: ** * *::***** *:: *:: **::*

SodA      YQAYVDKLNAALEKYPELYNYSLCELLQNLDSLPKDIATTVRNNAGGAYNHKFFFDIMTP 120
C.a.      HQTYVTNLNNALANYPELQSKTIEELLGNLDAIPEAIRTAVRNNGGGHWNHTFFWEIMAP 108
C.p.      QQAYVDKLNKALEKHPELYGKSLYDILSNLDDMPEDIMADLVNQGQGGVYNHEFYWSILGK 117
          *::** *::** ** *::*** . :: *::* *** *:: * : : *::.** *::* *::.*:

SodA      EKT-IPSESLKEAIDRDFGSFEKFKQEFQKSALDVFGSGWAWLVATKDGKLSIMTTPNQD 179
C.a.      NAGGAPTGDLAALAAIDAAFSGSFDFAFKEKFKAAALGRFGSGWAWLVAAKDGSLSIMSTPNQD 168
C.p.      GCN-RPVAEIADAIDRDFGSFEFKEKFKQCGISTFGSGWAWLVSDKDGKLEIMSTKDQS 176
          * .: *** ****: **::*: ..:. *****: ***.*.*:*:*.



SodA      SPVSKNLTPIIGLDVWEHAYYLKYQNRNEYIDNWFNVVNWNGALENYKNLKSQD 234
C.a.      NPLMEGKTAILGLDVWEHAYYLKYQYKRAAYVDAWVNVVNWAKVADHYAAAKG-- 221
C.       SPISLGLIPILTMDVWEHAYYLKYQNRPEYIDYFFDIINWKKCEEYNNR---- 227
          .*: . .*: :***** * *:* *:::*** : *


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SodA: from *C. difficile*

C.a.: *Chloroflexus aurantiacus J-10-fl*

C.p.: *Clostridium perfringens*

 Homologous with the Sod FeN super-family
 Homologous with the Sod FeC super-family

Alignment Score

SodA- C.p. 51%

SodA - C.a. 52%

"*" residues or nucleotides in that column are identical in all sequences in the alignment.
 ":" conserved substitutions have been observed
 "." semi-conserved substitutions are observed

Supplementary Figure 3: Alignment of SodA (234 amino acids; 27kDa) from *C. difficile* with superoxide dismutase from other bacteria

MSLNRRELLERISEYQFACIELNLYLDNNPRDKKALDSYNRYCDKFTQAVC

DYESKYGALTNFGYESSEYPWSWISEPWPWDKSFYK

Supplementary Figure 4: CotF. (11 kDa; 88 amino acids, tyrosine -= 10.34%)


```

C.ca.      MFKHEKQLLK--DVKVERPNPQYAVLMQEQQLGGNGELKAAMQYISQSFRIKDPAIKDLF 58
C.ce.      MFKHEKQLLNQLEVKVERPNPQYAVLMQEQQLGGNGELKAAMQYLSQSFRIKQTIKDLF 60
CotG       MFKHDKALLK--EVKVERPNPQYAVLMQEQQLGGANGELKAAMQYLSQSFRIKDPQIKDLF 58
C.b.       MFKHEKPLLR--DVKVERPNPQYAVLMQEQQLGGANGELKAALQYLSQSFVRVKDPEIHDLF 58
          ****:* ** . :*****.*****:*.*****:* * :***

C.ca.      LDIGSEELSHMEMVAQTINLLNGHAVDFNTVTSGEIETHVLGGLSPVLTNSSGEPWTANY 118
C.ce.      LDIGTEELSHMEIVAETINLLNGHNVDNTSVGSGEIEETHVLTGLSPVLINSSGEPWTANY 120
CotG       LDIAAEELSHMEMVAQTINLLNGHDVDYNSVNTGEIETHVLTGLSPVLINSSGAPWTANY 118
C.b.       LDIAAEELSHMEMVAQTINLLNGHEVDYTKTPGGEIETHVLTGLAPGLINASGHPWTADY 118
          ***.:*****:*.***** ** .. ***** **:* * *:* ** ***:

C.ca.      VTVTGDVLVADLLSNIASEQRAKVVEYLYRQVNDKYVRETINFLLEEEAHNALFREARN 178
C.ce.      VTVTGDVLVADLLSNIASEQRAKVVEYLYRQINDKHVKNTIDFLLNREEAHNALFREARN 180
CotG       VTVTGDVLVADLLSNIASEQRAKVVEYLYRQIDDKYVKETIDFLLNREEAHNALFRDALN 178
C.b.       VNVTGDIAADLLSDIAAEQRAKVVEYLYRQINDKYVKETIDFLLNREEAHNALFREARN 178
          * .****: .****:*.*****:*****:*.***:*****:*****:***

C.ca.      NVKDTGSNKNFGVTEDESKLYFDLSTPG-RFYQNPNPTEPSFANPRKESELAKH 231
C.ce.      KIKDTGSNMDFGVTEDESRLYFDLSTPG-RYFENPNPTAPEFANPRR----- 225
CotG       KVKDTGSNRDFGVTEDESKLYFDLSTPGPNHDTKIDINPPSF EKPLKK----- 225
C.b.       KVQDRASNKDFGVTKDSRLYFDLSTPG-RYFDNPEPTPPSFKNPRENSRETTRS 231
          :::* .** :****:*.***** .. : : . *. * :* .

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CotG: from *Clostridium difficile*

C.ca.: *Clostridium carboxidivorans* P7

C.ce.: *Clostridium cellulovorans* 743B

C.b.: *Clostridium beijerinckii* NCIMB 8052

"*" residues or nucleotides in that column are identical in all sequences in the alignment.

":" conserved substitutions have been observed

"." semi-conserved substitutions are observed

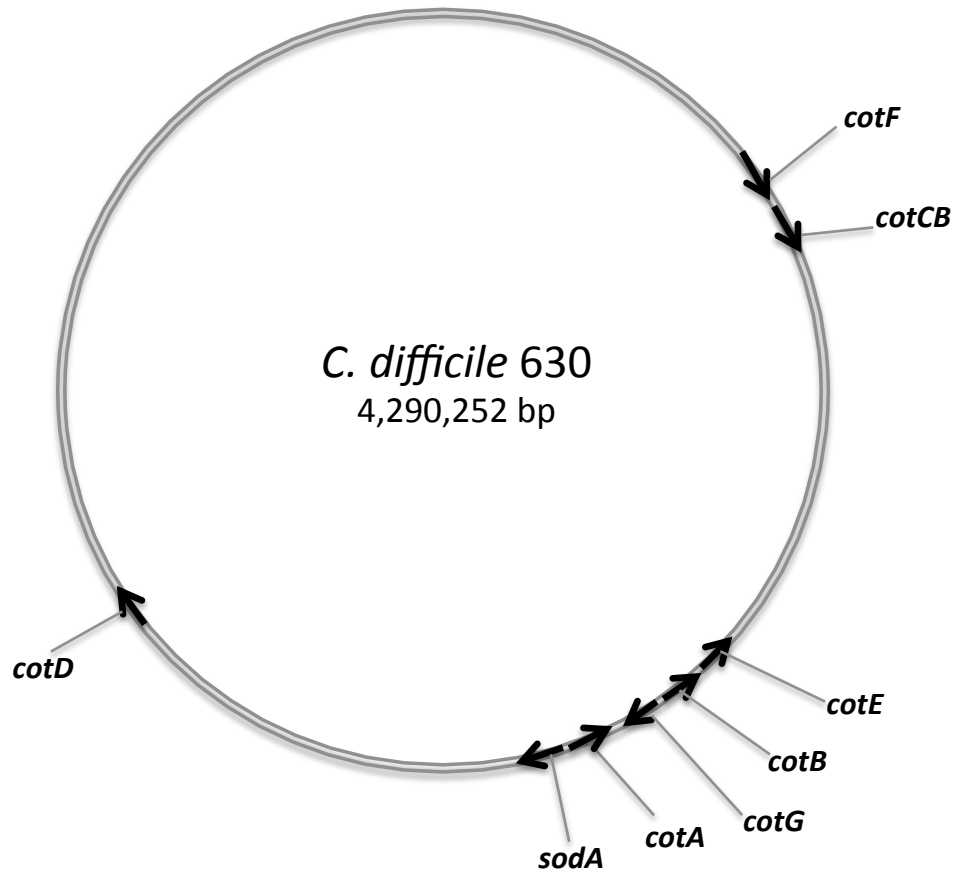
Alignment score

CotG-C.ca. 80%

CotG-C.ce. 81%

CotG-C.b. 79%

Supplementary Figure 5: Alignment of CotG (266 amino acids; 28 kDa) from *C. difficile* with manganese catalases from other bacteria



Supplementary Figure 6: Chromosomal location of spore coat genes