

## **Supplementary data**

### **Biochemical Characterizations of the Putative Endolysin Ecd09610 Catalytic Domain from *Clostridioides difficile***

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**Figure S1. Multiple sequence alignment of the catalytic domains of Ecd09610.**

**Figure S2. Species specificity of Ecd09610CD1, Ecd09610CD3, and Ecd09610CD53 lytic activities.**

**Figure S3. Binding assay for Ecd09610CD1, Ecd09610CD3, and Ecd09610CD53 with various bacteria.**

**Figure S4. Genes around ecd09610 (CD09610) in the *C. difficile* 630 genome.**

**Figure S5. Binding ability of the purified N-terminal uncharacterized region of Ecd09610 to *C. difficile* 630 cells.**

**Figure S6. Effect of NaCl on the binding ability of purified proteins to *C. difficile* 630.**

**Figure S7. Effect of the two catalytic domains.**

**Table S1. Primers used in this study.**

**a**

		Accession no.	
Ecd09610	371	CAJ67802.1	KVFKNKLSNTGNIFVKYSAYKYNPAALMAAISIHETGNGSSSSCK-----NK
CWH	371	YP_009206142.1	KLLKGKLSNTGNIFVKYSAYKYNPALMAAISMHESARGTSNTAN-----TK
AcpCD	69	5WQW	LSNKGVLGTGGQAFVNAAKAFNDPIYILVAQCLHETGNGTSKLAKGVTITEIADESKPIYNGNGQLVGHYHMIKLSKPVTV
AtlE	104	SIN26952.1	LKGGKILNENRGKVFLEAQKYEUNVIYILVSHALVETGNGKSELAKGIKDGG-----KRY
AtlA-gl	91	6FXP	LVDRPTLLKHTDDFLKAAKDKHYNEVYLISSHALLETGAVKSELANGEVIDG-----KKY
StFlgJ	1	5DN4	MDSKDFLARLSLPARLASQSGVPHHLILAQAALSGWGQRQTLRENG-----EPS
LytB	530	AAK75086.1	NINNSLLENKGATFKEAEAPHYHNALYLILAHSALESNWGRSKLAK-----DK

Ecd09610	419	CAJ67802.1	NNFEGMKG-----MSFGSVDEGKRGISNLSRNYIHTGRKTLESIRD-KYAPL--
CWH	419	YP_009206142.1	NNFEGMKKNGDY-----MSFGSVDEGKRGISNLSRNYIHIGRKTLESIRN-KYS----
AcpCD	150	5WQW	YNLFEGGAKDNSSVFPNRAILIGTTTAYN-----RGMTSIEAATKGAEEFVSLNYHSSRYSQNTLYKMRYNQNV
AtlE	159	SIN26952.1	YNFEGGGAFDSSA-----VRSGKSYAEK-----EQWTSFPAKATIGGAKFIRNEYFEN---NQLNLYQMRWNPENP
AtlA-gl	146	6FXP	YNFEGGALDKDP-----IKTGAEYAKK-----HGMDFPEKATSGGADFHKHFLSS--TDQNTLYSMRWNPKNP
StFlgJ	53	5DN4	YNFEGGKATASWKGVPVT--EITTEYENGEAKVKAKFRVYSSYLEALSDYVALLTRN---PRYAAVTTAATAEQGAVA
LytB	578	AAK75086.1	NNFEGGATAYDTP-----YLSA-----KTEDDVKGILGATKWIKENYIDRGRTF-----LGNKAS

		Accession no.	
Ecd09610	466	CAJ67802.1	---YDSPLNKDVPGV---GKFYKQITGNAYSSNS
CWH_g	468	YP_009206142.1	-----SSSDKEVVKCV---GAFYKQITGSTYNSNS
AcpCD	221	5WQW	NIWHQYATTPWASSIADIMRS-YQDIYLEN----
AtlE	221	SIN26952.1	A-QHQYASDIRADKIAKLMKSYKOGGIKK-----
AtlA-gl	209	6FXP	G-EHQYATDIKAESNATIIADFYKNKTEG-----
StFlgJ	127	5DN4	LQAGYATDPNARKLTSMIQQLK-AMSEKVSK---
LytB	629	AAK75086.1	GMNVEYASDPYVGEKIASVMMKINEKIGGKD-----

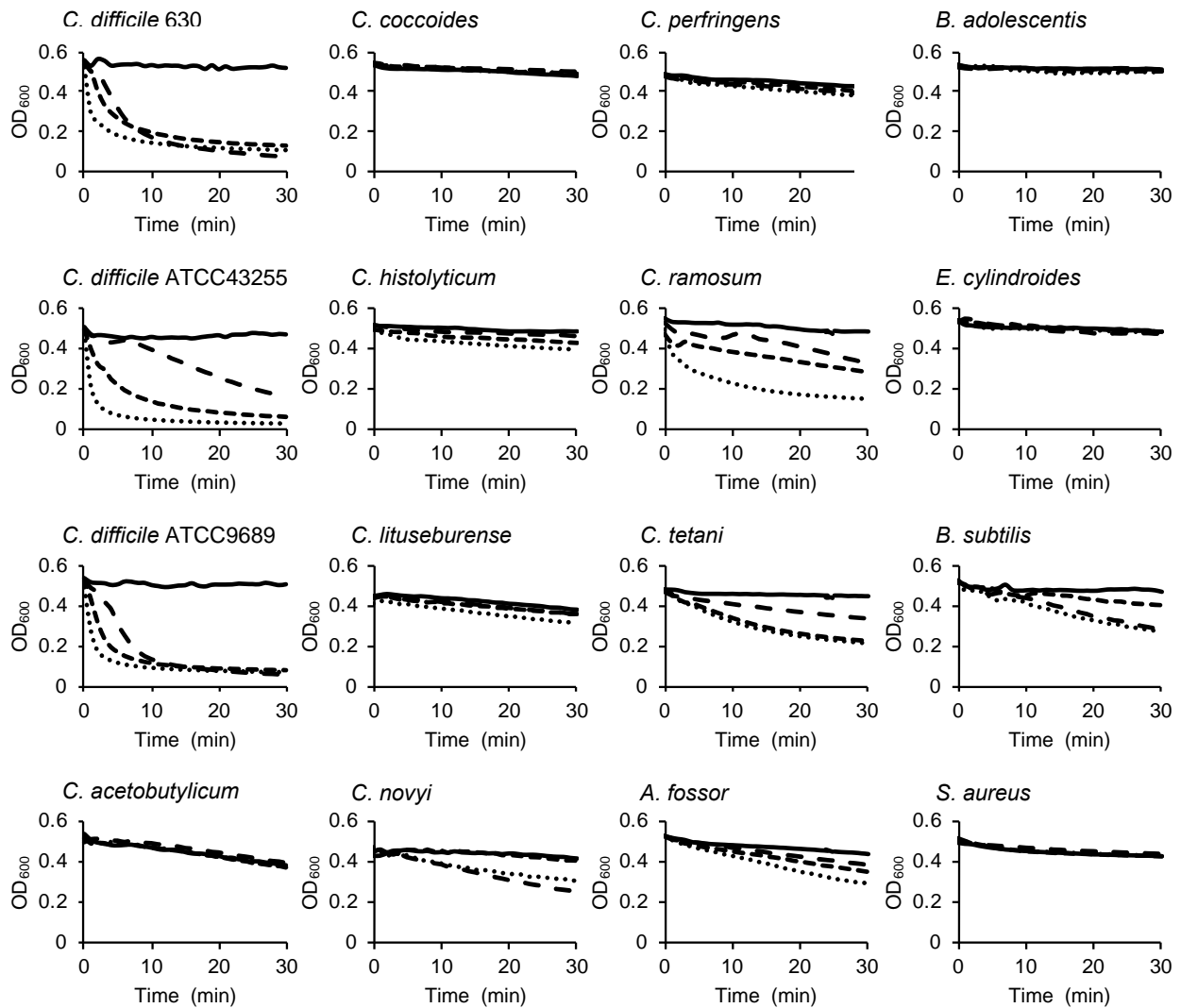
**b**

		Accession no.	
Ecd09610	529	CAJ67802.1	KVSKVIQEAKNQL-GKPYAWGGN-----GPKSFDGSGLMVWAFKRGAGINLKRVSADQSKDSRGKLLCNIND
CWH	529	YP_009206142.1	KVSKVIQEAKNQL-GKPYKWGGN-----GPKSFDGSGLMVWAFKRGAGINLKRVSADQSKDSRGKLLCNIND
Acd24020	271	CAJ69287.1	GADSVISFAKTL-LGKPYVWGAE-----GPNFSDGSGFTQYVMKKSVMGVSIPRTAQAYDAT---QHLPLSQ
CD03720	213	CAJ67194.1	LAQKVMNEALKYQGWKYVYGGSN-----PNTSFDGSGLTQWCY-GKAGSLPVSRLDQSKYG---TYVNRGD
NpPCP	97	ZP_00105875.2	LAEVIAFTQKAMQSSNYLWGGT-----VGPNYDGSGLMQAAF-ASVGWLPRDAYQOEGFT---QPITIAE
RipA	342	4Q4G	ASEYVIRRGMSQI-GVPYSWGGGNAAGPSKIDSGAGTVGFDASGLVLYSF-AGVGKLPVYSGSQYNLG---RKIPSSQ
P60_tth	127	BAD70089.1	PESPLRAVLRYL-GVPYKYGAN-----SPLALDCSAFVAQVY-AELGVALPRTTKEQYQ-----AFPPVEA
BcYkfc	188	3H41	AADDINTGKMFL-GLPYIWAGT-----SGFGFDGSGTHTTIY-KSHGTTIPRDSGFSRNG---VAVDKEH

		Accession no.	
Ecd09610	596	CAJ67802.1	VKAGDLVFFAYN-KGKGNVHHVGLYIGNDQYIHAPQTGD---VKISSLSG---R--QKKKHDFARARRFF----
CWH	596	YP_009206142.1	VKAGDLVFFAYN-KGKGNVHHVGLYIGNDQYIHAPQTGD---VKISSLSG---R--QKKKHDFARARRFF----
Acd24020	337	CAJ69287.1	LRSGDLVFFDTQGSNNGSVSHVGYTGNGDMIHASSGSSK-DPIGYAD-----LSSSYWQOHLIGAGRVKQ---
CD03720	276	CAJ67194.1	AKAGDLVFFHSTYNAGSYVTHVGYVGNQMYHAG-----KVTISNINS-----SYSSRYVNARRVL----
NpPCP	161	ZP_00105875.2	LVAGDLVFFGTSQK---ATHVGLYLDAGYYIHSSGKDQGRDGLGIDILSEQGDVSLSYQQLRGAGRNVFKS--
RipA	418	4Q4G	MRRGDLVFFGPNQ---SCHVTIYLGNGQMLEAPDVGLK---VRVAPVRT---AGMTPYRVRYIEY-----
P60_tth	188	BAD70089.1	PRFGDLVFFSFGG---KEVDHVGGLYLRGVFAHASSYGSR---VVIESLEA-----PFYRKVYRGARRVMASPE
BcYkfc	251	3H41	LQKGDILFQAHD-QGKGSVHHVAMYIGDGNMHSAPRAE-R--SWEIIPLNT-----PGYIEEYAGARRYLP---

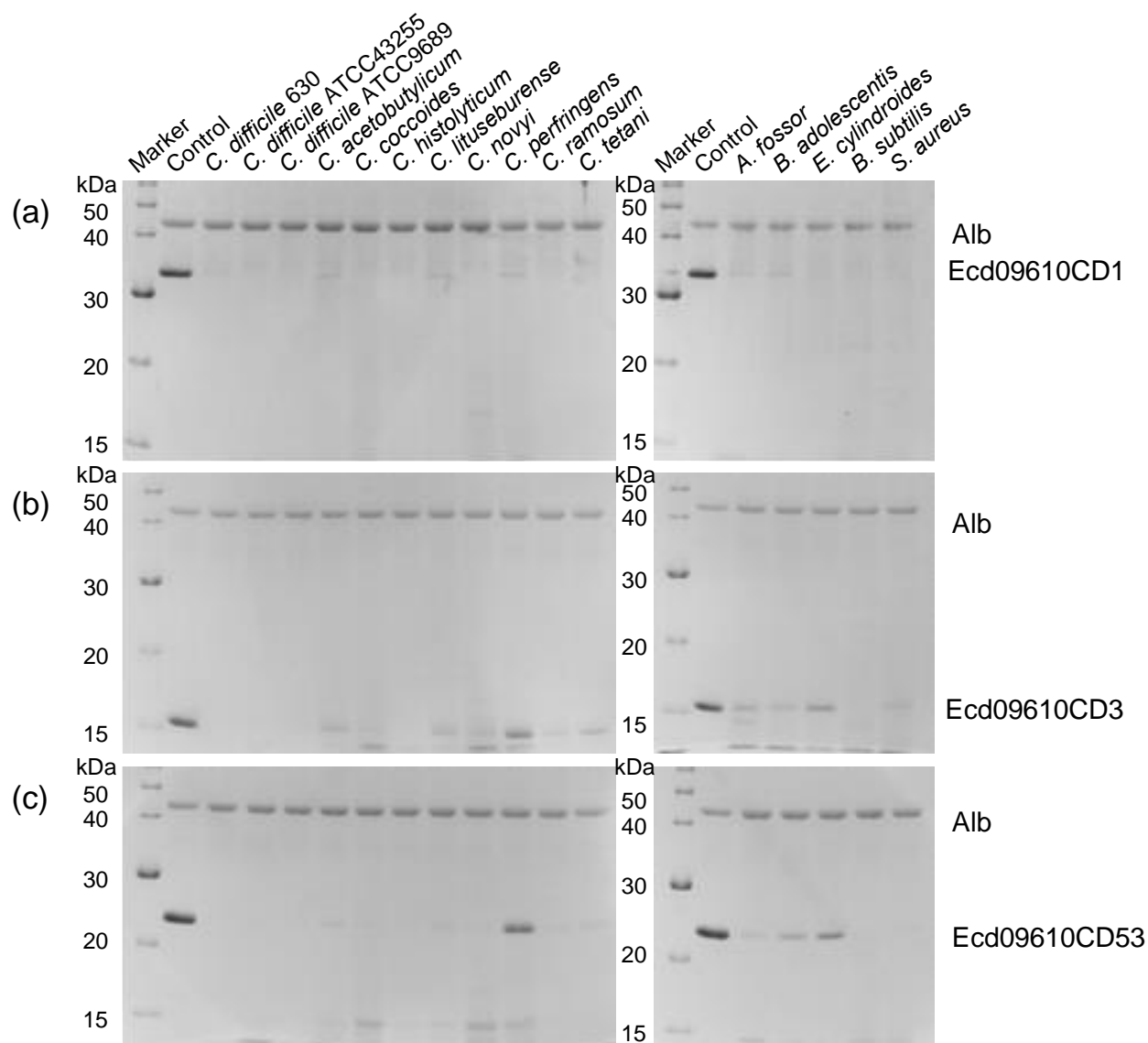
**Figure S1. Multiple sequence alignment of the catalytic domains of Ecd09610.**

(a) N-acetylglucosaminidase (PF01832: GH73 family) and (b) endopeptidase (PF00877: NlpC/P60 family) are shown. The multiple sequence alignment was created using Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). Accession no. indicates the NCBI GenBank accession number. Conserved and identical residues (more than half) are shaded in gray and black, respectively.

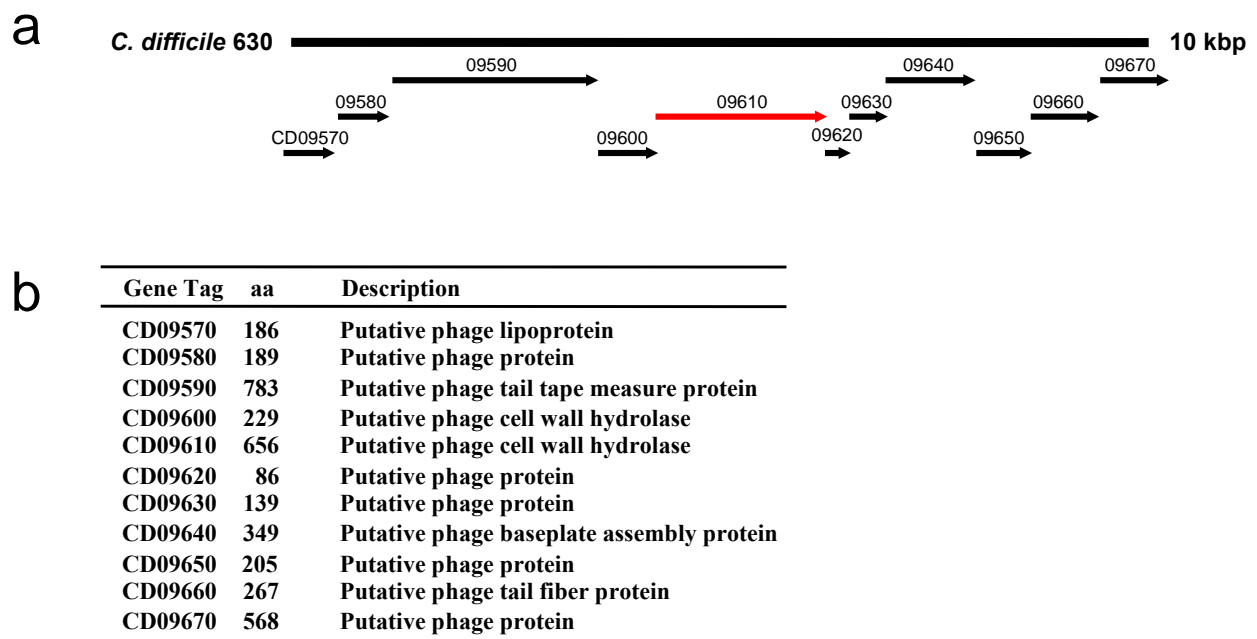


**Figure S2. Species specificity of Ecd09610CD1, Ecd09610CD3, and Ecd09610CD53 lytic activities.**

Experiments were repeated three times with similar results. Ecd09610CD1 (0.14  $\mu$ M, dotted line), Ecd09610CD3 (0.2  $\mu$ M, dashed line), or Ecd09610CD53 (0.3  $\mu$ M, long dashed line) or buffer (solid line) was added to the cells, and OD<sub>600</sub> was measured at 1-min intervals for 30 min.



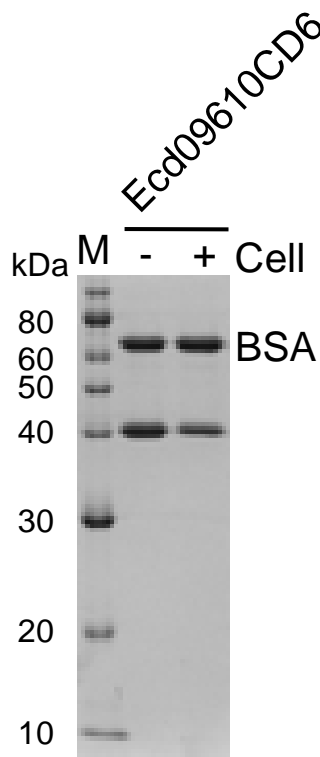
**Figure S3. Binding assay for Ecd09610CD1, Ecd09610CD3, and Ecd09610CD53 with various bacteria.** Experiments were repeated three times with similar results. One microgram of purified Ecd09610CD1 (a), Ecd09610CD3 (b), Ecd09610CD53 (c), or ovalbumin (Alb) as a nonbinding internal standard were incubated on ice with or without heat-inactivated cells as indicated at the top of the gel. After centrifugation, the supernatants were analyzed by 13.5% SDS-PAGE.



**Figure S4. Genes around ecd09610 (CD09610) in the *C. difficile* 630 genome.**

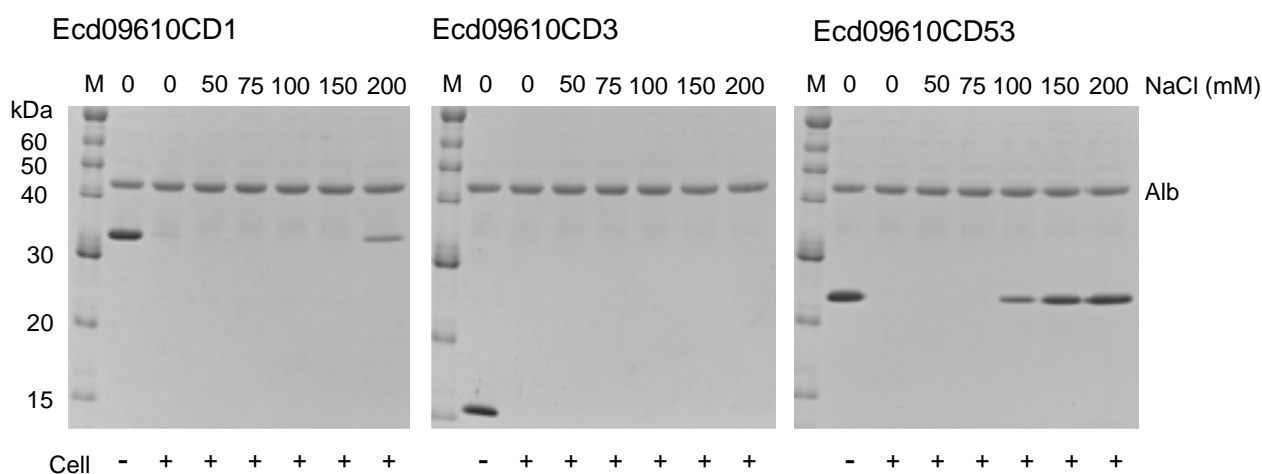
(a) *C. difficile* 630 has a group of phage-related genes of about 60 kbp that constitute a phage remnant region.

(b) Genes in the genome region from CD09570 to CD09670 in *C. difficile* 630 are shown.



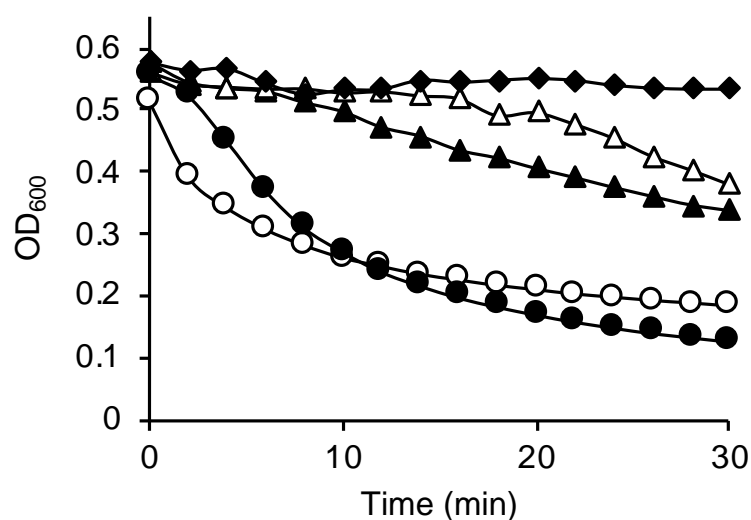
**Figure S5. Binding ability of the purified N-terminal uncharacterized region of Ecd09610 to *C. difficile* 630 cells.**

One microgram of purified Ecd09610CD6, which has only the N-terminal uncharacterized region of Ecd09610, and nonbinding internal standard (bovine serum albumin: BSA) were incubated with or without heat-inactivated cells on ice. After centrifuging the samples, the supernatants were analyzed by 13.5% SDS-PAGE.



**Figure S6. Effect of NaCl on the binding ability of purified proteins to *C. difficile* 630.**

The binding abilities of Ecd09610CD1, Ecd09610CD3, and Ecd09610CD53 to *C. difficile* 630 are shown. One microgram of purified Ecd09610CD1, Ecd09610CD3, Ecd09610CD53, and nonbinding internal standard (ovalbumin: Alb) were incubated with or without heat-inactivated cells on ice. After centrifuging the samples, the supernatants were analyzed by 13.5% SDS-PAGE.



**Figure S7. Effect of the two catalytic domains.**

The lytic activities of proteins (0.08 μM) were determined by the turbidity reduction assay against *C. difficile* 630 cells. Both Ecd09610CD3 and Ecd09610CD53 together (filled circles), Ecd09610CD1 (open circles), Ecd09610CD3 (filled triangles), Ecd09610CD53 (open triangles), and control (filled diamonds) are shown.

Table S1 Primers used in this study.

Primer name	Primer sequence (5' - 3')
CD09610-N	CGCcatatgGTTGATGAATTAGTGTTAG
CD09610-m1	CCCCAAGCATACGGCTTACCAAG
CD09610-m2	CTTGGTAAGCCGTATGCTTGGGG
CD09610-C	CGCggatccTTAAAAGAATCTTCTAGCTCTTG
CD09610-CD1	CCCcatatgACCACAAGTAAAGAAGACAATG
CD09610-CD3	CCCcatatgAAAGTAAGTAAAGTTATTCAAGAAGC
CD09610-CD5 R	CCCggatccTTAATACGGCTTACCAAGTTG
pCold F	ACGCCATATCGCCGAAAGG
pCold R	TGGCAGGGATCTTAGATTCTG