

Supplementary Information

Molecular identification of hyaluronate lyase, not hyaluronidase, as an intrinsic hyaluronan-degrading enzyme in *Clostridium perfringens* strain ATCC 13124

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Supplementary Fig. 1 Bacterial GAG degradation, import, and metabolism system.

Supplementary Fig. 2 Growth profile of *C. perfringens* cells by measuring OD₆₀₀ in log₁₀ form.

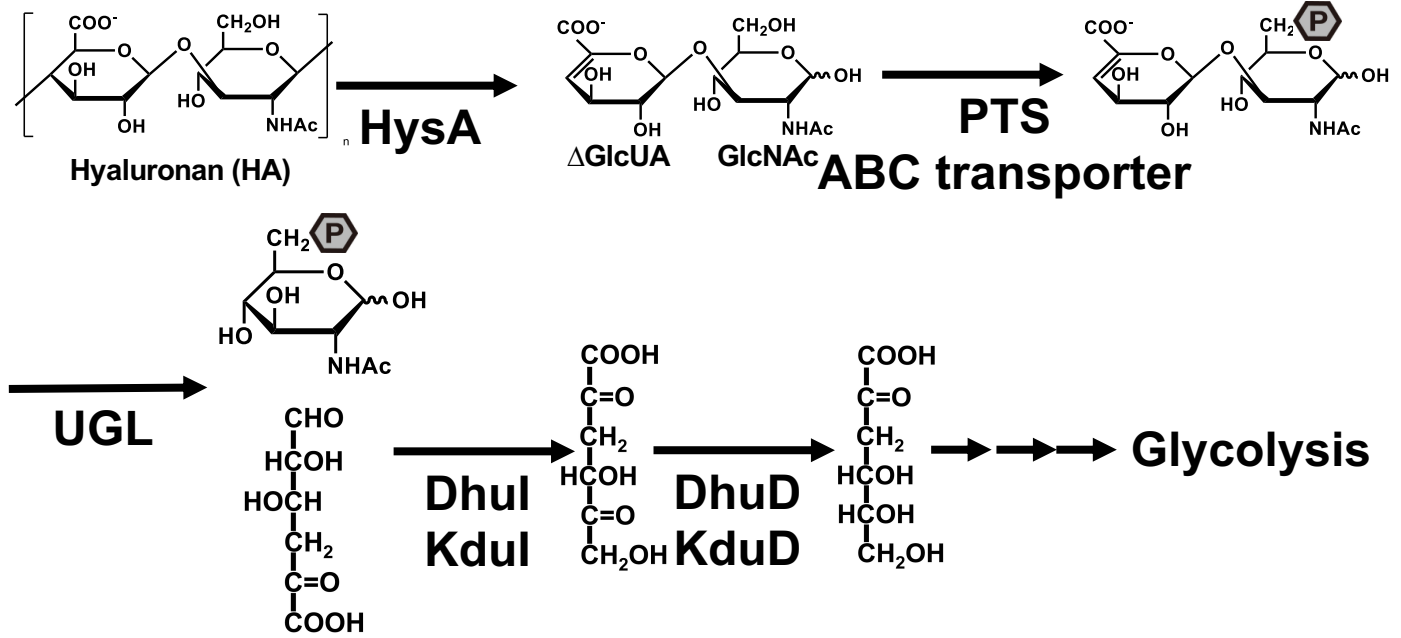
Supplementary Fig. 3 RNA-seq of *C. perfringens*.

Supplementary Fig. 4 Superimposition of CpeHysA and xanthan lyase.

Supplementary Table 1 Gene expression of *C. perfringens* through RNAseq.

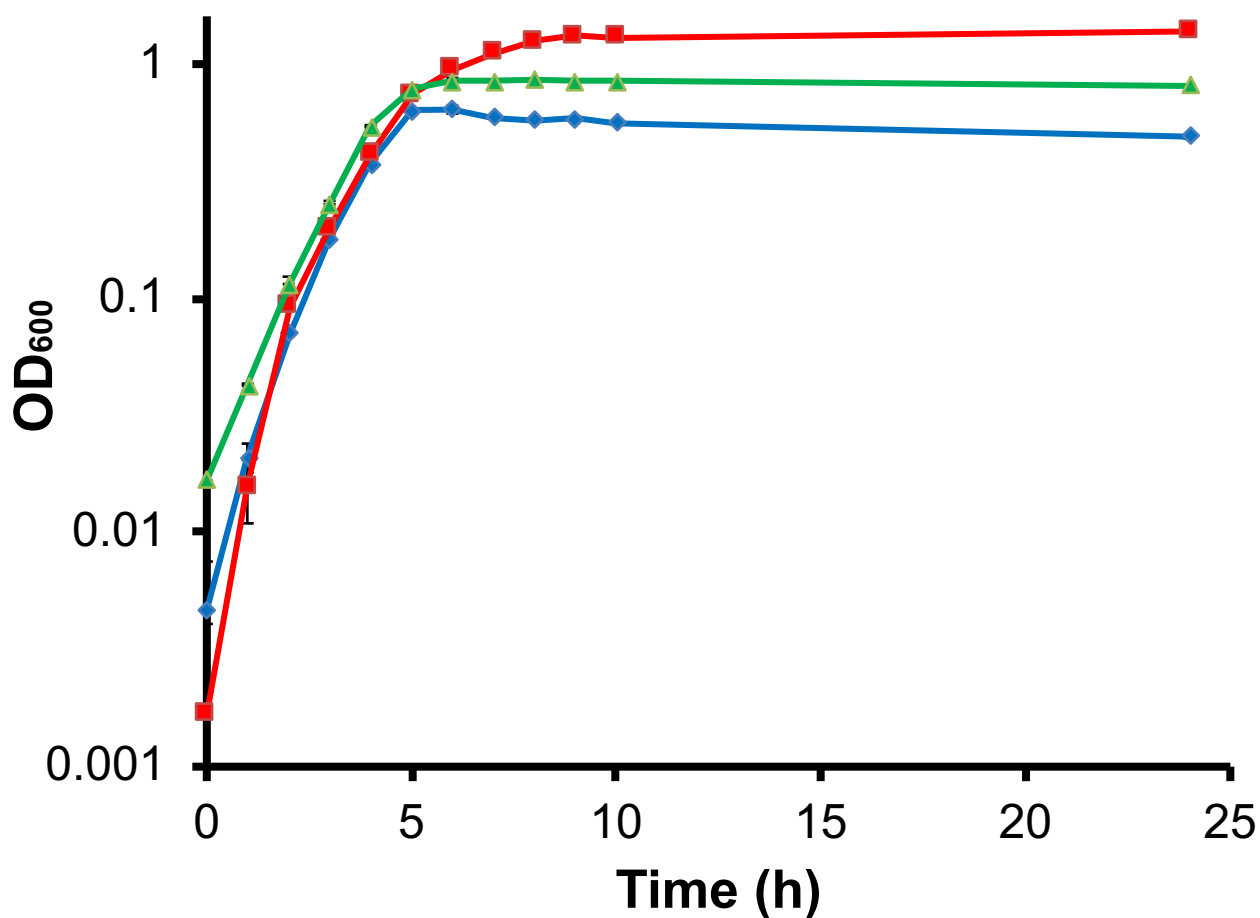
Supplementary Table 2 Primers.

Supplementary Fig. 1



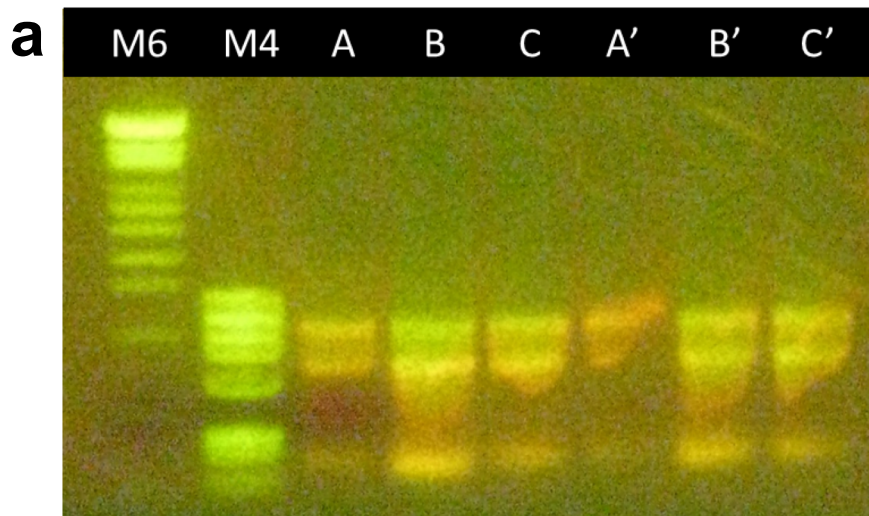
Supplementary Fig. 1 Bacterial GAG degradation, import, and metabolism system. Example of degradation enzymes, HysA and UGL; transporter, PTS and ABC transporter (non-phosphorylation of the substrate); metabolizing enzyme, Dhul/Kdul and DhuD/KduD.

Supplementary Fig. 2



Supplementary Fig. 2 Growth profile of *C. perfringens* cells by measuring OD₆₀₀ in log₁₀ form. Blue, nutrient-poor medium; red, nutrient-poor medium containing HA; and green, nutrient-poor medium containing mucin. The profile is identical to Fig. 2c.

Supplementary Fig. 3



b

No.	growth medium	quantity (μg)	ratio(Abs260/Abs280)	RIN	rRNA ratio
A	Poor+	17.019	1.89	7.2	1.1
B	Poor+HA	36.716	1.9	7.3	1
C	Poor+mucin	33.961	1.97	7.2	1

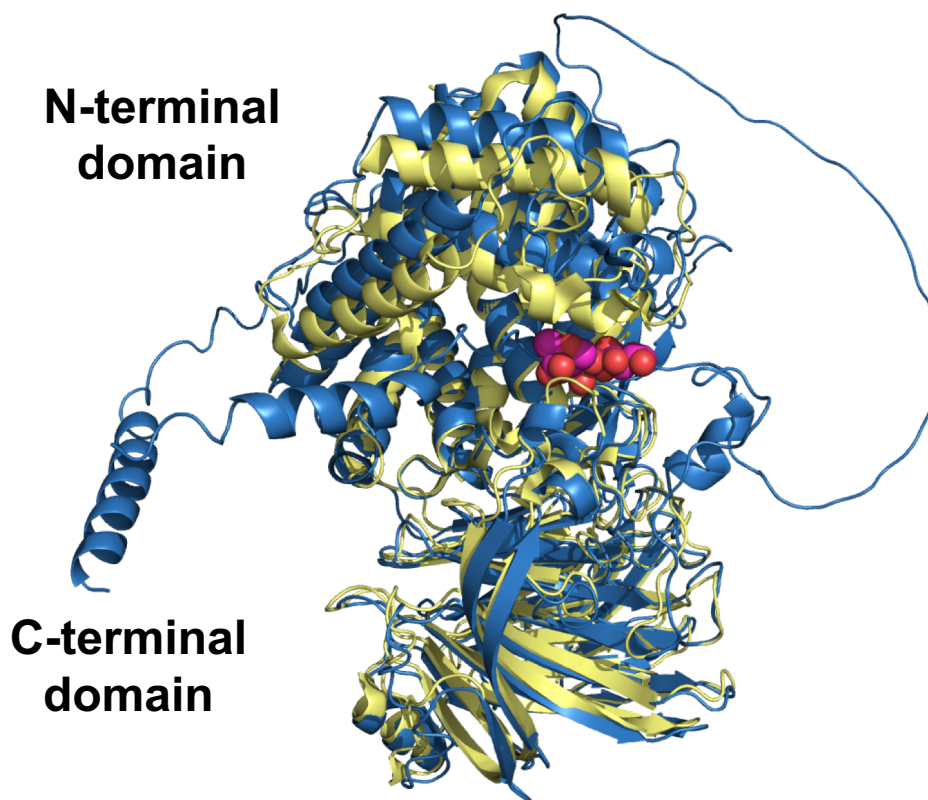
No.	growth medium	total read bases	total reads
A	Poor+	5041504082	49915882
B	Poor+HA	5041270974	49913574
C	Poor+mucin	6955687796	68868196

No.	growth medium	total read bases	total reads
A	Poor+	4955895319	49366152
B	Poor+HA	4964571195	49416680
C	Poor+mucin	6842367613	68121182

No.	growth medium	processed data	mapped read	failed to aligned	multiple aligned
A	Poor+	49366152	44776526 90.70%	2548788 5.16%	2040838 4.13%
B	Poor+HA	49416680	44736304 90.53%	2232878 4.52%	2447498 4.95%
C	Poor+mucin	68121182	60852264 89.33%	3693250 5.42%	3575668 5.25%

Supplementary Fig. 3 RNA-seq of *C. perfringens*. (a) Extracted RNA was applied to agarose gel electrophoresis. A, A ': Poor + medium B, B': Poor + HA medium C, C ': Poor + mucin medium. (b) Basic statistics of RNA-seq.

Supplementary Fig. 4



Supplementary Fig. 4 Superimposition of CpeHysA and xanthan lyase. Blue, the AlphaFold2 model of CpeHysA; yellow, crystal structure of *Bacillus* sp. GL1 xanthan lyase (PDB ID, 1X1J). Ball model shows xanthan disaccharide.

Supplementary Table 2 Primers

name	Base sequence (5'-3')	Fragment length
F-CPF_0394	5'-AAGGAGATATAC <u>CATATG</u> AAAGAATCTAGAAAAAAC-3'	3039 bp
R-CPF_0394	5'-GGTGGTGGTGCT <u>CGAGT</u> TTTTTCTTTTCTTAGTAAAC-3'	