**Supplementary Materials:** 

A putative amidase endolysin encoded by *Clostridium perfringens* st13 exhibits specific lytic activity and synergizes with the muramidase endolysin Psm

Hiroshi Sekiya, Maho Okada, Eiji Tamai, Toshi Shimamoto, Tadashi Shimamoto, Hirofumi Nariya

Figure S1 Alignment of the catalytic domain T7 (PF01510: Amidase\_2) family amidase.
Figure S2 Genes flanking *psa* (CPE1138) in the *C. perfringens* st13 genome.
Figure S3 Lytic activities of Psa-his, PsaCD-his, PsaBD-his, and PsaY51F-his.
Figure S4 Zymography analysis of Psa-his, PsaCD-his, PsaBD-his, and PsaY51F-his.
Figure S5 Inhibition of Psa-his by Zn<sup>2+</sup>.
Figure S6 Binding of Psa-his to *C. perfringens* SM101 cells and spores.

Table S1 Strains and culture conditions used in this study

			z	GM					KE E
<u>PF01510</u>	Accession no.	1	PVRY <b>IVIH</b>	T2GPNFSGA	LAA CDPYF	-AIYHGVVS <mark>Y</mark>	MLDRD	-CII <u>XO</u> LV	'PP-NGRE <mark>WHAG</mark> VG
Psa(CPE1138)	BAB80844.1	19	KENKIII <b>H</b>	PEYNGS	IEGUNDIMR	SMG YM G <mark>Y</mark> I	NFY <mark>W</mark> RK <mark>D</mark>	GTVYEGF	EV-WAT <mark>GAN</mark> CYG-
T7 lysozyme	WP 015979355.1	11	STDANFVH	CSATKPSQNVG	VREIRQWHK	EQ <mark>GW</mark> LDVG <mark>Y</mark>	FIIKRD	CTVEAGE	NDE-MANGS <mark>H2</mark> KG-
3.5 lysozyme	ADX87614.1	9	QTKALLVH	C <b>TA</b> TKPHQDIG	VREVRQWHT	-RDNGWFDVG <mark>Y</mark>	FIIRRN	GVIENGE	N V-DV CAHPSG-
CBC 0677	EDS78167.1	19	NENKIII <mark>H</mark>	PEFYGT	VQSINDVMR	-NMG SM G <mark>Y</mark>	NY YNRKO	- GSVWQGF	V-SATCANCYN-
CPE0565	BAB80271.1	88	SEKRLII <mark>H</mark>	HATDSPET	PEDIHKFHL	-DNGWSGIG <mark>Y</mark>	FYIRE	-CTIYKGI	d - NV GAHAKN-
CBDKU1 36710	EMU52239.1	18	REKEKIVH	IEAEGSNWT	VEFIENMHRTE	) PNFK SAIG <mark>Y</mark>	HYYIRLD	- CSIYKGI	T - NATGAHCQG-
CLC 2409	ABS36154.1	18	NENEIDLH	AEASSCS	VYDVHSWHK	-GN <b>GY</b> AGIG <mark>Y</mark>	<b>HY</b> N KN	-CEIWKGI	ED-NAIG <mark>AH</mark> V G-
CTC p55	AA037449.1	18	NERKIII	AEATSCS	IEDVETWHU	-HNGWSGCC	YFIRKN	-estyke	-SSUCAHCIN-
CLOSPO 01453	EDU38591.1	18	NEKMUTT	AEASGCS		-NNGWSGCGY	MALIKK	-estykg	T -NA CAHCLS-
Ph2119	AHF20915.1	23	PRIVIN	TAGPVDOA	PEVIBDEHE	KGREWPHICY	NLAYOF	-GRVYKTI	IN-NA PICVRE-
HuPGRP-S	AAH96155.1	53	PLRYVV	TAGSSCNTPASCOODA	RNVO YHMK	-TLENCDVE <mark>Y</mark>	N L GE		GW-NETCAHSCHL
and D	33073054 1	4.2	D.T. IZ		DOGT	TT TDYOUG			
AmilD	AAC/3934.1	43		NICLODCERCOMIDALES		-TLTDKQVSS	CI DE	RPR DLV	TE DE NUNCUS
AmpD 3	A14500555.1	10	TRITE A	MISLPPGEFGGFWIDALF.	A CUL	- LIGHLANAA	CL RR.	COD	IN EDD MUNCUS
Ampons	AAG04196.1	10	RVR V		AASVA	TITUCO	T PAPHDPSIKAAGP	GOR	
Amponz	AAG08870.1	100	RVQ IV		PHSLG-				D -NRR WHAGVS
Lysh5/LytA	ACE///96.1	199	NEN LV H	NDEGSKGAT	COOVENEL AND	APLSRLEAGIA	SINSG		JD -SQ <b>GWH</b> T NQ
CDEOGOG	IF_009700770.1	207	NEEG V	DUDGRSS	GQQIENSLANA	IGIAN ANGIA	U GSE		
CPEUGUO	BABOUSI2.1	09		DI DIRQAGA	NAPIANEN1FA-	-NHPNAN SA			THE TWICHING DG
XIYA DI OI	KIX82519.1	22	THE	NTAVGA	DAAA ARI	-KNPDTTTSW	II 1 DD	TETYCHI	LE LENGWHAGDG
PIYZI	CAA/226/.1	22	TETE T H	NTYNDA	PAINERNN	-ANNSQGTSE		-KENIGLI	EF-NRN WHAGDG
PIYG	ABB55421.1	22	KERTITVH	NTYNDA	PAPNEVSY0	-ISNNNEVSE	IAWDD	-KK IQGI	L-ERN WACGDG
PlyI	1YB0_A	22	KEKTUTVH	NTYNDA	PABNEVNY0	-ITNNNEVSE	VANDD	-KOMIOGI	W-ERNA <b>WA</b> C <mark>G</mark> DG
LysZ5	AEP68521.1	21	GANEVIA	ETANSKS	TINEVSYN	TRN <b>R</b> QN/#FV	THERGGG	GRUVOVA	4NV-NYVSWGAGQY
Ply-1	AA081086.1	43	SNELIVI <mark>H</mark>	ESCNERNLGPH	SLINEVAYM	KRN <b>A</b> SNAYV:	SYBOGSG	GROKOLA	4EA-GQIIQ <b>Y</b> GA <mark>G</mark> SL
PIY-2	AA081277.1	44	TNEL	ESCNGNNVGLN	SLENETAYO	-KRNATSAYV	SM BWGSG	-GR KOLA	4 V-GQIIQ <mark>W</mark> G <b>MG</b> AT
									<b>n</b> * <b>r</b>
DE01610		63	~ d		DDDD	a a di la di cel	WCTDDDD		B'Z DO <mark>DVTDE</mark>
PF01510		0.5	G=====G	DRSIGI <mark>L</mark> EGNIGG	FFIDagi L	ARE ALCO	RIGIPPDR	(IV <mark>GR</mark> KDIA	PG <mark>KK</mark> IDBC
Psa(CPE1138)	BAB80844.1	77	H	NEDSIGVCFEGNYDKE	TDMPQECENA	GVELIKYLKS	YGINF	IVN <mark>CHKHYY</mark>	(NTA <mark>CPG</mark> Q
T7 lysozyme	WP_015979355.1	73	Y	N N <mark>SIG</mark> VC V <mark>G</mark> GIDDK	-GKFDANFTPA <mark>C</mark> MOS	IRSL VTL A	YEGAV	/LR <mark>A</mark> hh <b>d</b> væ	––––––P <mark>k</mark> a <mark>ce</mark> sf
3.5 lysozyme	ADX87614.1	72	F	NSDS GVC VGGINE	GKADANFTLECVV	KTL DILKA	SYPEAF	LIK <mark>ch</mark> rdvi	1NG <mark>K</mark> E <mark>CE</mark> SF
CBC0677	EDS78167.1	77	Q	NSSIGVCFECNYDND	KTMPD <b>C</b> K	GELQYINN	IYGITE	IVN <mark>CHKYYF</mark>	<−−−−− <b>N</b> TD <mark>C</mark> PGR
CPE0565	BAB80271.1	148	A	NYNTLGICIECNFEKD	GLKEAGKNS	KLGTYLSL	YPIKI	DILP <mark>H</mark> R <b>D</b> VV	/DTL <mark>C</mark> PGK
CBDKU136710	EMU52239.1	82	A	NTNTLGIAFEGNYDKR	TVMPDAQYN <b>A</b>	WRELSDYLDS	I <mark>YGI</mark> II	PTY <mark>ch</mark> rdro	SSE <mark>C</mark> PGK
CLC2409	ABS36154.1	77	H	NTN GICAEG YMR	TMPQ QK	LIELGRYLCN	YGIKP	KVY <mark>Ch</mark> rdva	SSN <mark>C</mark> PGT
CTCP55	AAO37449.1	77	Y	NSVSIGICVEGNYMVD	HMPLSCKS	TIELIKYDCG	YGIKF	LIY <mark>ch</mark> gpin	1STN <mark>C</mark> PGR
CLOSPO01453	EDU38591.1	77	Y	NGVSIGICECRLNVD	EMRNSOYNS	LKELVE YLQN	YNNN	KIYA <mark>h</mark> r <b>d</b> lı	1QTD <mark>C</mark> PGN
Ph2119	AHF20915.1	84	F	NPVSICIADVGDFSQG	PAWPDNAPGWK	L ELKDAL K.	A <u>Y</u> PKA\	/LVL <mark>H</mark> K <b>D</b> LI	l'QTT <mark>C</mark> PGV
HuPGRP-S	AAH96155.1	122	W	NPMSIGISFMGNYMDR	VPTPQAIR	QGLI ACG A	QGALRSNYV	LK <mark>CH</mark> RDV	2 <mark>R</mark> TL <mark>S</mark> PGN
									_
AmiD	AAC73954.1	104	AWRGATRL	NDTSIGI <mark>E</mark> BNRGWQKSAC	GVKYFAPFEP <b>s</b> QI0 <mark>6</mark>	an i phardi ta	MHUKPE	IVVA <mark>H</mark> ADI <i>I</i>	PQ <mark>RK</mark> DDPGP
AmpD	AIZ50835.1	101	QYQGRERC	NDFSIGI <mark>E</mark> EGDTL	AYTD CYOC	DAATTAL	OYPDIAKP	IMT <mark>CH</mark> CDI <i>I</i>	PD <mark>RK</mark> T <mark>D</mark> PGP
AmpDh3	AAG04196.1	84	GWARRDNL	NDTSIGI <mark>E</mark> IVNLAR-DDDO	GVFTFPDYERSCING	ALKQLAKNILQ	MPDMTPKP	IVV <mark>CH</mark> SDI <i>I</i>	VG <mark>RK</mark> S <mark>D</mark> PGP
AmpDh2	AAG08870.1	91	EWQGRTWL	NAT <mark>SIGIE</mark> VNQGYRDTP(	)GRVWYPFSE CIO	TIPLLKDIAK	RH <b>GI</b> TPDP	RII <mark>CH</mark> SDI <i>i</i>	PG <mark>RK</mark> V <mark>DPG</mark> P
LysH5/LytA	ACE77796.1	262	IG	NYGYGI <mark>E</mark> VCQSMGAD	NATFLKNEO	TFQECAR	KMGI PANRN7	TIRL <mark>H</mark> NDF1	2STS <mark>C</mark> EHR
LysK	YP_009780776.1	269	TGANSG	NFRF GIE CQ MSAS	DAQFLKNE	FQFTAEKFK	E <b>GU</b> TPNRK	WRL <mark>H</mark> M <b>B</b> FU	/PTA <mark>CE</mark> HR
CPE0606	BAB80312.1	150	NNPNIN	NSTITAT <mark>E</mark> CVNKEND	FDKTLEN	IGHE TAYLAN	YNTPAEN	IV <b>VM<mark>H</mark>RDA</b> S	−−−−−G <mark>K</mark> T <mark>C</mark> SRM
XlyA			NCCC	A REAL CENTRE		THE OLD THE PROPERTY AND A	CUMPCT 2 D	IWVP <b>H</b> EVWS	
	KIX82519.1	81	NGSG	ASIGIE CENADG	FAKATAN	ABQW LINT OA.			aG <mark>v</mark> r <mark>c</mark> ikw
Ply21	KIX82519.1 CAA72267.1	81 78	GSGRG	NRHSIGVE CYSKSGG	FAKATAN PRYEQAVRN	JAQWILIKTIMA JAIIVIRQIMD	QUNIPIDF	R KT <mark>h</mark> Q <b>B</b> RI	1G <mark>K</mark> Y <mark>CE</mark> H
Ply21 PlyG	KIX82519.1 CAA72267.1 ABB55421.1	81 78 78	GSGRG	N HSIGIE CENADG N HSIGVE CY KSGG N QSI VE CY KSGG	FAKATAN PRYEQAVRN DRYYKAEDN	IAQWULKTLIJA JATIVIRQIMD JAVDVVRQIMSJ	YNIPIEN	RVKT <b>HQD</b> RM IVRT <mark>H</mark> QSW	G <mark>KYC</mark> HR G <mark>KYC</mark> HR
Ply21 PlyG PlyI	KIX82519.1 CAA72267.1 ABB55421.1 1YB0_A	81 78 78 78	NGSG NGSG NGSG	NRASIGIE CENADG NRHSIGVEICYSKSGG NRQSISVEICYSKSGG NRESISVEICYSKSGG	FAKATAN PRYEQAVRN DRYYKAEDN DRYYKAE	IAQWILLKIIIMA JAIIVIRQIMD JAVDVVRQIMSJ JAVDVVRQIMSJ	YNIPIEN YNIPIEN YNIPIEN	R KTHQ <b>D</b> RM I RTHQSW I RT <mark>H</mark> QSW	IGKYCEHR IGKYCEHR IGKYCEHR
Ply21 PlyG PlyI LysZ5	KIX82519.1 CAA72267.1 ABB55421.1 1YB0_A AEP68521.1	81 78 78 78 80	NGSG NGSG NGSG ANGPG	N ASIGIE CENADO N HSIGVEICYSKSGG N ROSISVEICYSKSGG N RESISVEICYSKSGG SYSYAQVEICRISNAT	FAKATAN PRYEQAVRN DRYYKAEDN DRYYKAE	IAQMUI KILMA JATIVI RÇLMD JAVDVVRÇLMSI JAVDVVRÇLMSI VYCQ <mark>L</mark> I VDLAK	ANISLA NIPID NNIPIE	KTHQER KRTHQSW KRTHQSW GIKSHKW	VGKYCHR VGKYCHR VGKYCHR VCHR VCHR
Ply21 PlyG PlyI LysZ5 Ply1	KIX82519.1 CAA72267.1 ABB55421.1 1YB0_A AEP68521.1 AA081086.1	81 78 78 78 80 106	NGSG NGSG NGPG ANGPG AN	N ABIGIELCENADG N HSIGVELCY KSGG N CSIVELCY KSGG N ESIVELCY KSGG SYSYAQVELCK KSGG SYSYAQVELCK SNAT OKAYAQIELAR NNAA	FAKATAN PRYEQAVRN DRYYKAEDN DRYYKAE FFKKDIEV FFKKDIEV	AQWLATIMA ATIVIRQIMD AVDVVRQIMSI AVDVVRQIMSI VYCQITVLAR YVNIARLAQI	NIPID YNIPIE NIPIE AGIPITLDSGSKTSDKO NIG DFSLDDGTGY	RVKTHQERM IVRTHQSW IVRTHQSW GIKSHIW GIVTHDW 1	JGKYCFHR GKYCFHR GKYCFHR DKLGGTTHQDFYA ?-KNWWGDHTDFYG

**Figure S1**. Alignment of the catalytic domain T7 (PF01510: Amidase\_2) family amidase. Sequence alignment was created using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Accession no.: NCBI GenBank accession number. Conserved and identical residues (more than half) are shaded in gray and black, respectively. \*: catalytic residue, Z : zinc-binding residue, G: GlcNAc-binding residue, M: MurNAc-binding residue, E: γ-D-Glu (Gln)-binding residue, and K: L-Lys-binding residue.

Amidase catalytic site

Cheng X, Zhang X, Pflugrath JW, Studier FW. (1994)The structure of bacteriophage T7 lysozyme, a zinc amidase and an inhibitor of T7 RNA polymerase. Proc Natl Acad Sci U S A. 91(9):4034-4038.

Zn binding residues

Low LY, Yang C, Perego M, Osterman A, Liddington RC. (2005) Structure and lytic activity of a Bacillus anthracis prophage endolysin. J Biol Chem. 280(42):35433-35439.

Substrate binding site

Guan R, Roychowdhury A, Ember B, Kumar S, Boons GJ, Mariuzza RA. (2004) Structural basis for peptidoglycan binding by peptidoglycan recognition proteins. Proc Natl Acad Sci U S A. 101(49):17168-17173



## b

Gene Tag	aa	Description	Gene Tag	aa	Description
CPE1095	338	Phage_integrase/Site-specific	CPE1117	194	Phage terminase, small subunit, TerS
		recombinase, Xer	<b>CPE1118</b>	558	Phage terminase, large subunit, TerL
CPE1096	99	Нуро.	CPE1119	57	Hvpo.
CPE1097	70	HTH_XRE	CPE1120	407	Phage portal HK97 family
CPE1098	154	Нуро.	CPE1121	200	Phage prohead protease HK97 family
CPE1099	100	Нуро.	CPE1122	378	Phage major capsid protein, HK97 family
CPE1100	230	HTH_XRE	CPE1123	97	Head-Tail Connector Protein, HK97 family
CPE1101	77	Нуро.	CPE1124	105	Phage head-tail joining protein
CPE1102	276	Нуро.	CPE1125	147	Phage protein, HK97 family
CPE1103	1064	Cons., Phage-related	CPE1126	116	Phage protein
CPE1104	143	Cons.	CPE1127	199	Phage major tail protein, phi13 family
CPE1105	106	Нуро.	CPE1128	101	Phage protein
CPE1106	56	Нуро.	CPE1129	111	Cons.
CPE1107	306	Cons.	CPE1130	933	Phage tail tape measure protein
CPE1108	60	Нуро.	CPE1131	231	Phage tail protein
CPE1109	151	Нуро.	CPE1132	983	Phage endopeptidase/lysozyme tail
CPE1110	365	Bro-N, Prophage antirepressor	CPE1133	624	Phage minor structural protein,
CPE1111	52	Нуро.			choline-binding
CPE1112	88	Toxin YafO, type II toxin-	CPE1134	648	Phage protein
		antitoxin system	CPE1135	80	Hypo.
CPE1113	127	Нуро.	CPE1136	59	Hemolysin XhlA family
CPE1114	59	Нуро.	CPE1137	67	Cons. Phage exported protein, Holin
CPE1115	80	Нуро.	CPE1138	304	Amidase 2. Endolvsin
CPE1116	136	HNH endonuclease			·

**Figure S2.** Genes flanking psa (CPE1138) in the *C. perfringens* st13 genome. (a) Straight lines represent the genomes of *C. perfringens* st13 (black), ATCC13124 (dark gray), and SM101 (light gray). The arrows indicate the genes (open reading frames), and the Gene Tags and names of the putative functions or protein products are indicated above the arrows. The region between duplication 1 and 2 (white square) of the *C. perfringens* st13 genome is considered a phage remnant region (light blue), given the existence of many phage-related genes. The other *C. perfringens* strains lack the phage remnant region upstream of psa. Genes related to citrate fermentation are indicated by red arrows. (b) The genes in the genome from region CPE1095 to CPE1138 in *C. perfringens* st13 are listed. Cons.: conserved protein, Hypo.: hypothetical protein. Lipo.: lipoprotein.

а



**Figure S3.** Lytic activities of Psa-his, PsaCD-his, PsaBD-his, and PsaY51F-his. The lytic activities of the purified enzymes were determined by the turbidity reduction assay using *C. perfringens* HN1314 cells suspended in PBS(-). Purified Psa-his (black circles), PsaCD-his (white circles), PsaBD-his (black triangles), and PsaY51F-his (white triangles) were added to pre-incubated cells to a final concentration of 10  $\mu$ g/ml, and then OD<sub>600</sub> was measured at 2-min intervals for 35min. A parallel no-enzyme control experiment was performed in the same manner using buffer control (black diamonds).



**Figure S4.** Zymography analysis of Psa-his, PsaCD-his, PsaBD-his, and PsaY51F-his. Purified Psa-his, PsaCD-his, PsaBD-his, and PsaY51F-his (each 2 µg) were electrophoresed in a 12.5% SDS-polyacrylamide gel containing 20 OD<sub>600</sub> of heat-inactivated and SDS-treated *C. perfringens* HN1314 cells. Zymography analysis was carried out as described previously [31]. The arrowheads indicate cell lysis.



**Figure S5.** Inhibition of Psa-his by Zn<sup>2+</sup>. Psa-his was tested at various concentrations of ZnCl<sub>2</sub> against *C. perfringens* HN1314 in 25 mM Tris-HCl pH 7.0, 250 mM NaCl, and 1 mM dithiothreitol.



**Figure S6.** Binding of Psa-his to *C. perfringens* SM101 cells and its spores. The binding activity was measured according to the Materials and Methods. Purified PsaY51F-his and BSA were mixed with mid-logarithmic growth phase *C. perfringens* cells (3 h cell) or mid-stationary growth phase *C. perfringens* cells (8 h cell) or its spores (spore) or buffer (No cell), centrifuged, and the supernatants were analyzed by 12.5% SDS-PAGE. The spores of *C. perfringens* SM101 were prepared by culturing in mDS for 48 h, sonicating, washing three times with PBS(-), and counting in a Thoma cell counter.

Ta	ble	<b>S1</b>	Strains	and	culture	conditions	used	in	this	study	v.

Organism	Strain	Culture conditions
Gram(+)	Stram	Culture conditions
Clostridium perfringens	HN1314	AN Static 37°C 8 h TY-G1
Clostridium perfringens	st13	AN Static $37^{\circ}$ C 8 h TY-G1
Clostridium perfringens	ATCC13124	AN Static $37^{\circ}$ C 8 h TY-G1
Clostridium perfringens	SM101	AN Static 37°C 4h and 8 h TV-G1
Clostridium perfringens	SM101(spore)	AN Static $37^{\circ}$ C 48 h mDS
Clostridium acetohutylicum	ATCC824	AN Static $37^{\circ}$ C $24 \text{ h}$ TV-G1
Clostridium cellulohticum	ATCC35319	AN Static 37°C 24 h TV/2-CB0 5
Clostridium coccoides	ATCC29236	AN Static 37°C 10 h TV-G1
Clostridium histolyticum	ATCC19401	AN Static $37^{\circ}$ C 12 h TV-G1
Clostridium litusehurense	ATCC25759	AN Static $37^{\circ}$ C 8 h TY-G1
Clostridium novvi	ATCC17861	AN Static $37^{\circ}$ C 14 h TV-G1
Clostridium ramosum	ATCC25582	AN Static $37^{\circ}$ C 12 h TV-G1
Clostridium sporogenes	ATCC3584	AN Static $37^{\circ}$ C 8 h TY-G1
Clostridium tetani	KZ1113	AN Static 37°C 8 h TY-G1
Clostridioides difficile	ATCC9689	AN Static 37°C 8 h TY-G1
Atopohium fossor	ATCC43386	AN Static 37°C 24 h GAM
Racillus subtilis	168	AE Shaking 37°C 8 h LB
Bifidobacterium adolescentis	ATCC15703	AN Static 37°C 10 h TY-G1
Enterococcus faecalis	IID682	AE, Shaking, 37°C, 6 h, BHI
Euhacterium cylindroides	ATCC27805	AN. Static, $37^{\circ}$ C, 8 h. TY-G1
Lactobacillus reuteri	ATCC23272	AN. Static, 37°C, 8 h. MRS
Listeria monocytogenes	HCIPH A5-1	AE, Shaking, 37°C, 8 h, BHI
Micrococcus luteus	ATCC4698	AE, Shaking, 37°C, 14 h, LB
Staphylococcus aureus	ATCC6538P	AE. Shaking, $37^{\circ}$ C. 8 h. LB
Staphylococcus enidermidis	ATCC35984	AE, Shaking, 37°C, 8 h, BHI
Streptococcus pneumoniae	IID555	AE, Static, 37°C, 8 h, BHI
Streptococcus progenes	124/0207	AE, Static, 37°C, 8 h, BHI
Gram(-)		
Bacteroides fragilis	ATCC25285	AN, Static, 37°C, 16 h, TY-G1
Escherichia coli	K-12	AE, Shaking, 37°C, 8 h, LB
Pseudomonas aeruginosa	PAO1	AE, Shaking, 37°C, 8 h, LB
Vibrio cholerae	O1/P1418	AE, Shaking, 37°C, 8 h, LB
Vibrio cholerae	O139/MDO-6	AE, Shaking, 37°C, 8 h, LB
Vibrio parahaemolyticus	RIMD2210115	AE, Shaking, 37°C, 8 h, LB

AN and AE are aerobic and anaerobic conditions, respectively.

AN : Static culture under anaerobic conditions in tightly capped tubes

AE : Culture in test tube, shaking at 160/min

Media:

BHI: Brain Heart Infusion broth (BHI ; Eiken Chemical Co., Ltd., Tokyo, Japan)

GAM: Gifu Anaerobic Medium broth (Nissui Seiyaku Co., Ltd., Tokyo, Japan)

LB: Luria-Bertani broth (Wako Pure Chemical Industries Ltd., Osaka, Japan)

mDS (modified Duncan-Strong medium): 1.5% BACT-Peptone, 1% Na<sub>2</sub>HPO<sub>4</sub> 7H<sub>2</sub>O, 0.4% BACT-Yeast Extract, 0.4% raffinose, 0.1% sodium thioglycolate, pH 7.8

MRS: de Man Rogosa and Sharpe broth (Sigma Aldrich, St. Louis, MO, USA)

TY-G1: 3% Tryptone, 2% Yeast Extract, 0.1% sodium thioglycolate, and 1% glucose (30)

TY/2-CB0.5: 1.5% Tryptone, 1% Yeast Extract, 0.05% sodium thioglycolate, and 0.5% cellobiose