

Supplementary material for

Characterization of multiple alginate lyases in a highly efficient alginate-degrading *Vibrio* strain and its degradation strategy

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Running title: Alginate degradation in a *Vibrio* strain

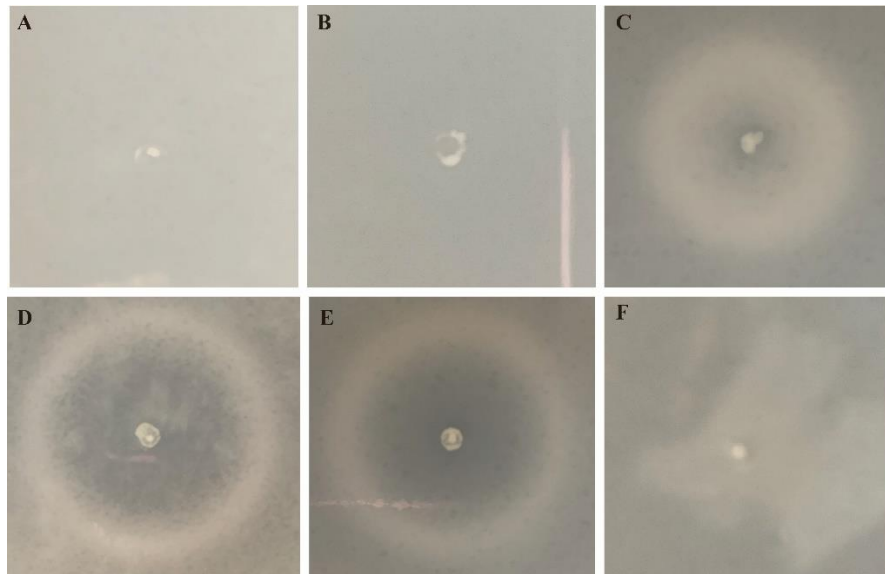


Fig. S1 Alginate degradation of strain *V. pelagius* WXL662 under different temperature. A, 0 °C; B, 4 °C; C, 16 °C; D, 28 °C; E, 37 °C; F, 45 °C.

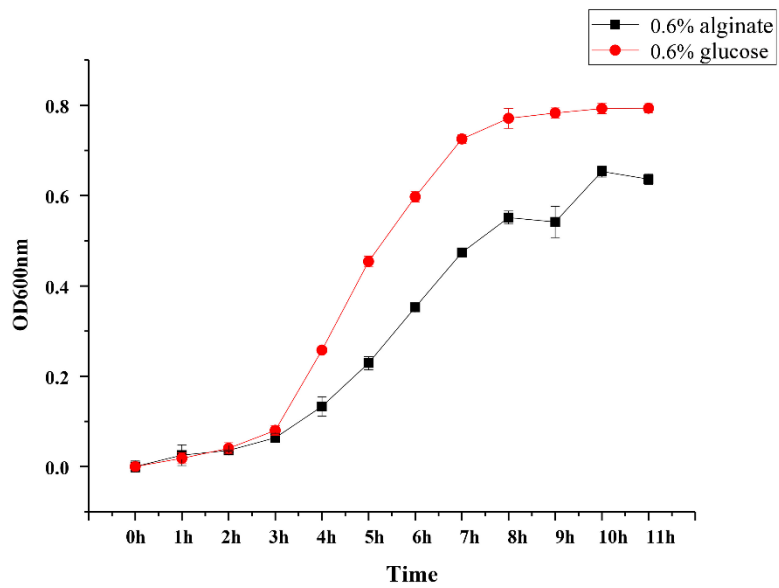


Fig. S2 The growth curves of strain WXL662 cultured in mineral medium containing 0.6 % (w/v) glucose and alginate as the sole carbon source, respectively.

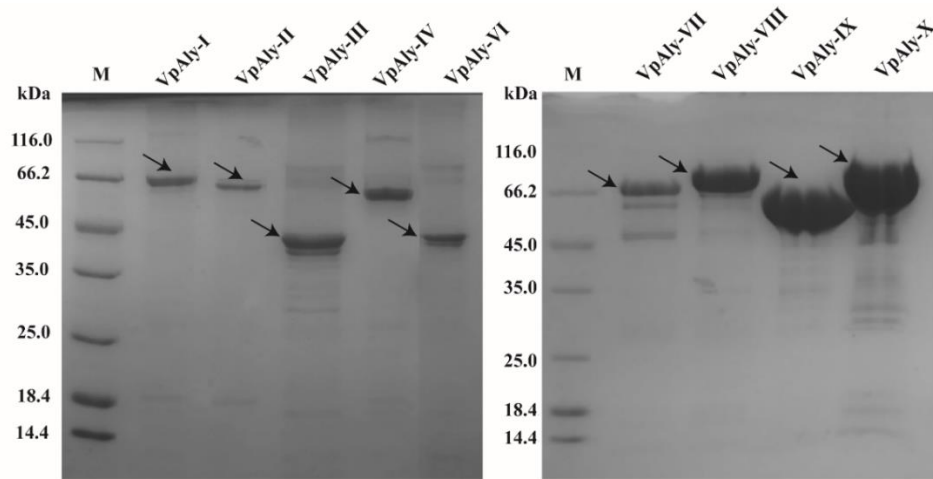


Fig. S3 SDS-PAGE of nine purified recombinant alginate lyases (VpAly-V was studied by Zhang et al., 2022). M: marker.

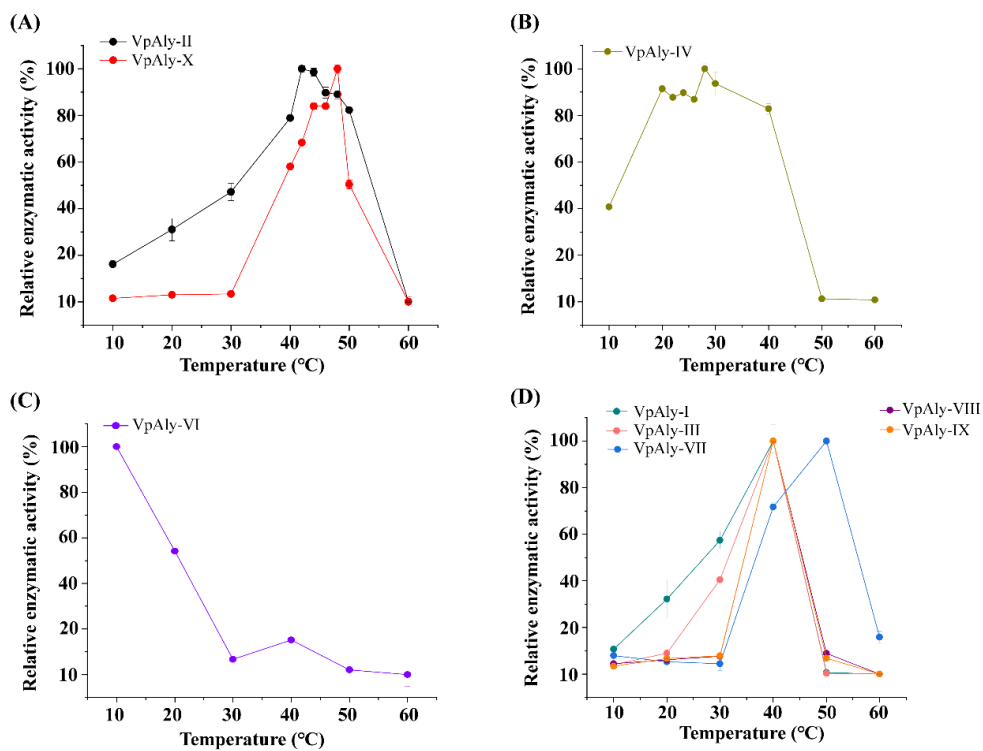


Fig. S4 The activity of nine recombinant alginate lyases in strain WXL662 under different temperature. (A), The optimum temperature of VpAly-I and VpAly-X; (B), The optimum temperature of VpAly-IV; (C), The optimum temperature of VpAly-VI; (D), The optimum temperature of VpAly-I, -III, -VII, -VIII and -IX. The optimum temperature of VpAly-V was detected at 40 °C by Zhang et al. (2022).

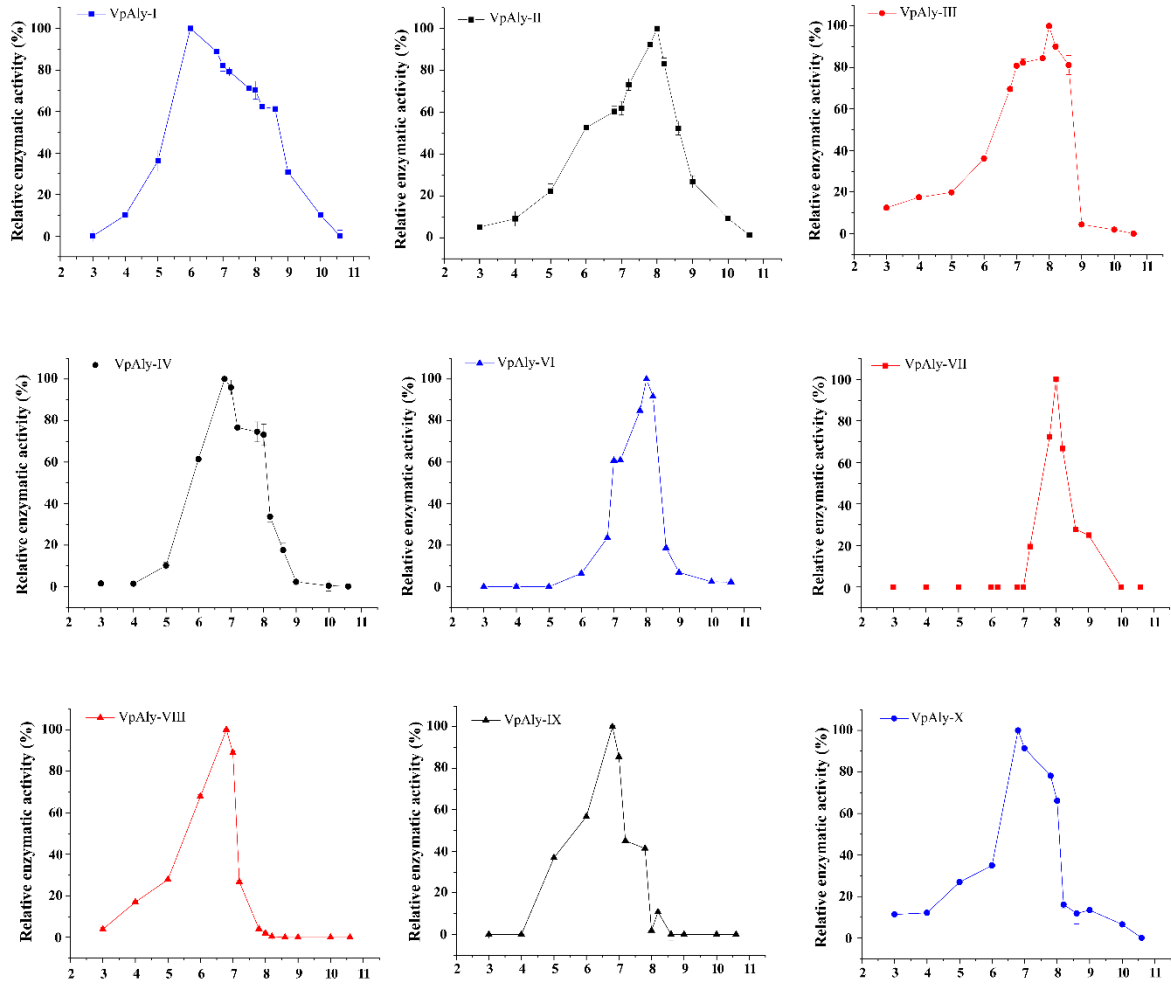


Fig. S5 The activity of nine recombinant alginate lyases in strain WXL662 under different pH.

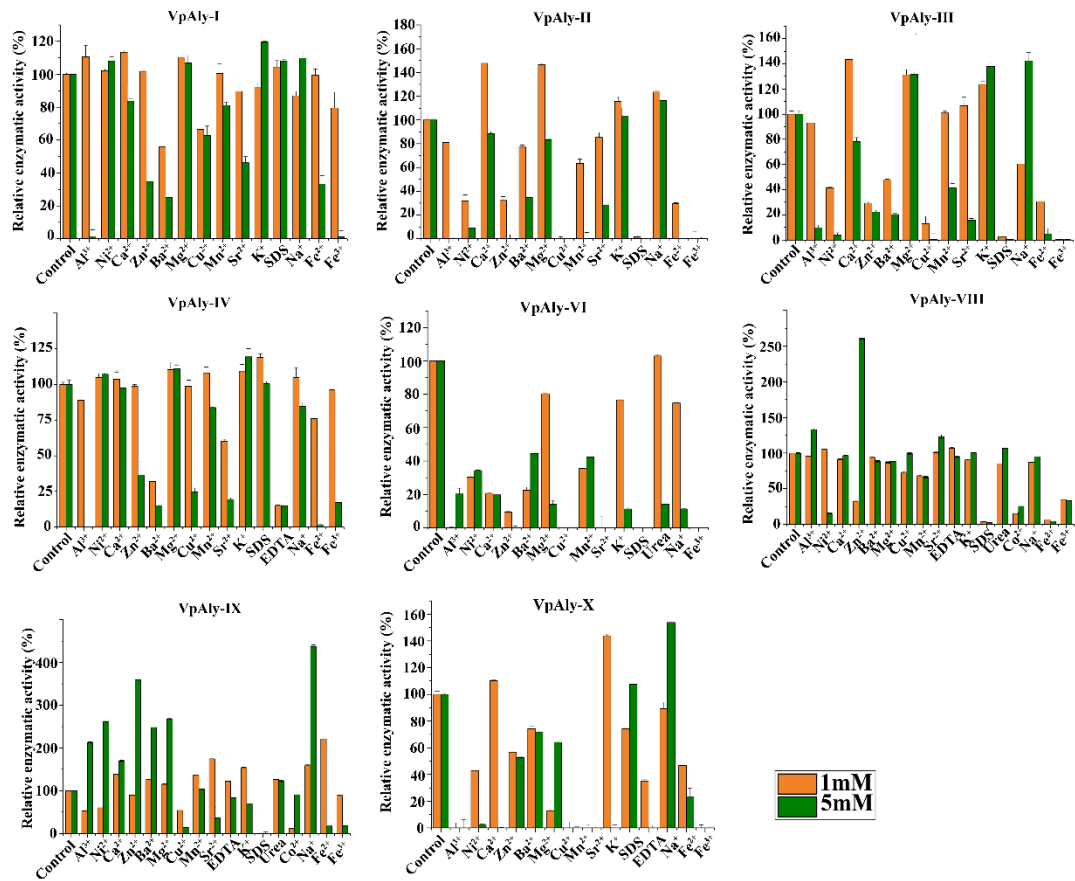


Fig. S6 Responses of recombinant alginate lyase to different metal ions and reducing agents.

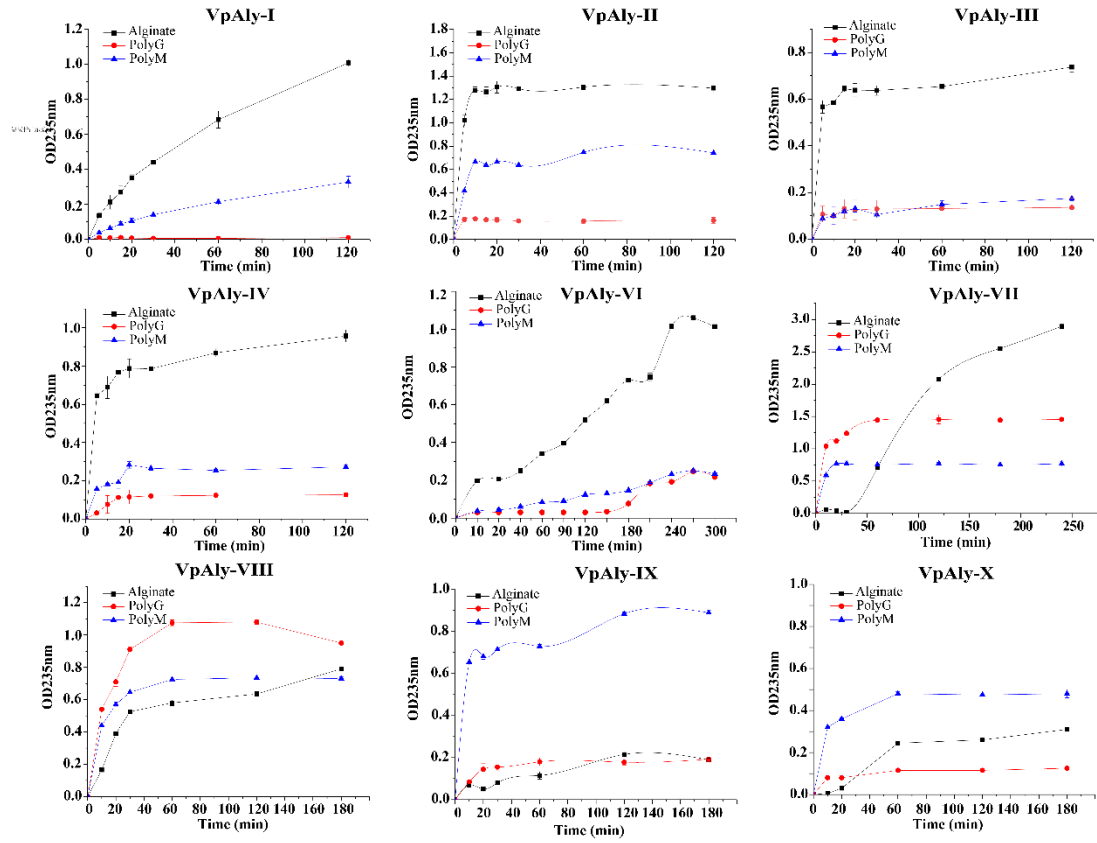


Fig. S7 Substrate preferences of nine recombinant alginate lyases. The black squares represent the degradation of alginate, the red dots represent the degradation of polyG, and the blue triangles represent the degradation of polyM.

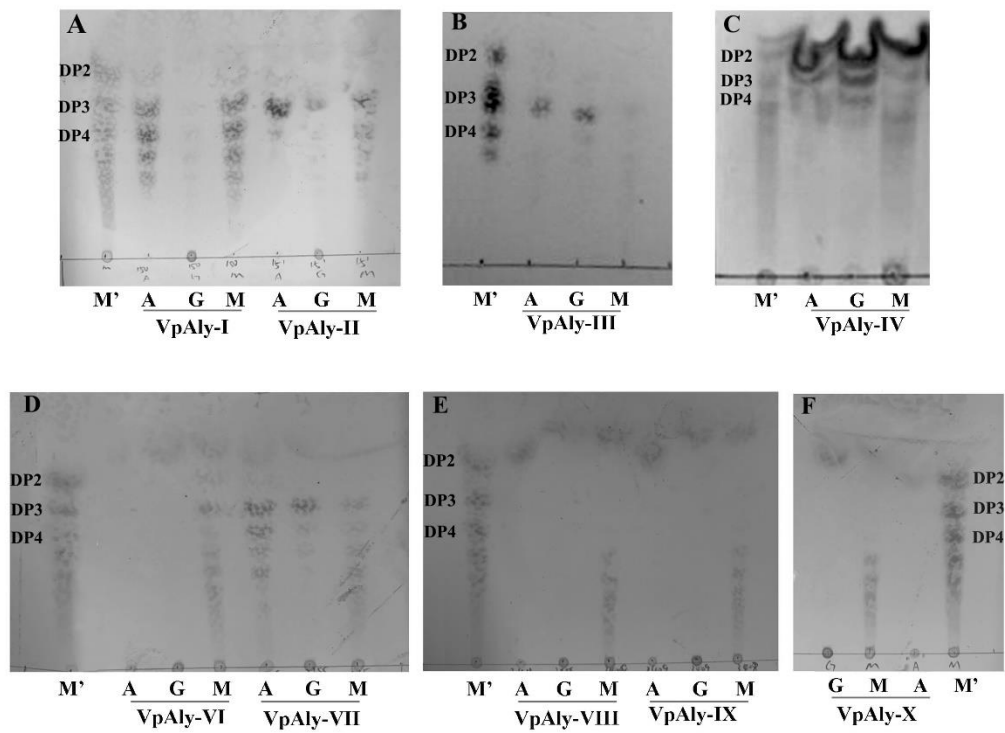


Fig. S8 Thin layer chromatography (TLC) analysis of the alginate oligosaccharides. Std: alginate oligosaccharide standards, DP2-DP4: the mannuronic acid sodium salt dimer, trimer and tetramer (M'). A, alginate; G, polyG; M, polyM.

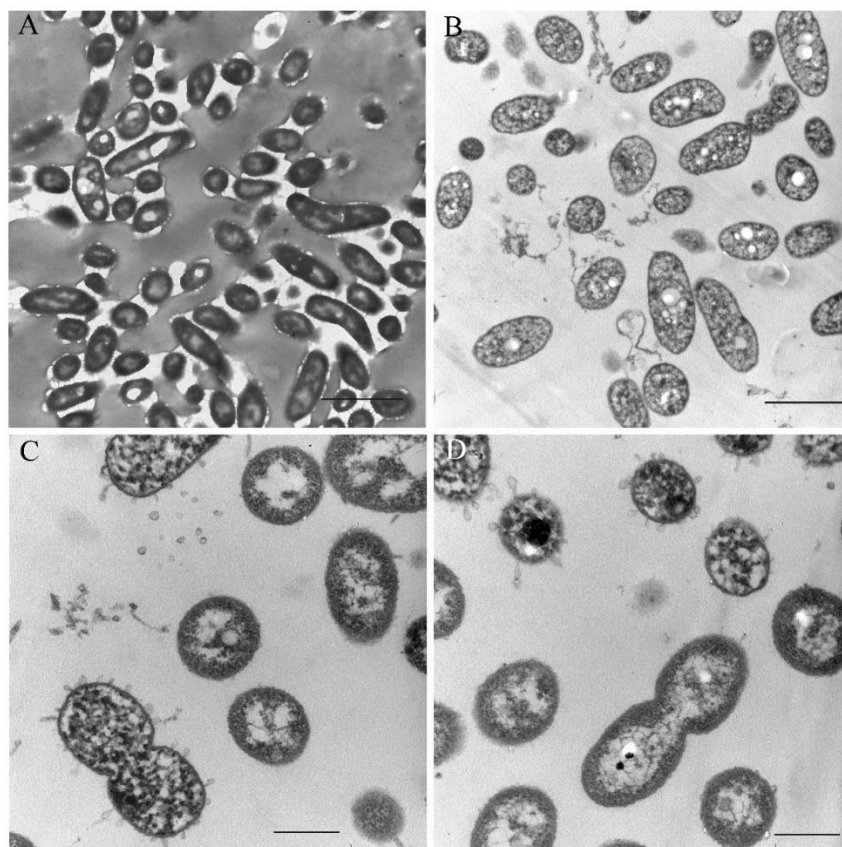


Fig. S9 Scanning electron microscopy (SEM) of the morphology and internal structure of the strain WXL662 in different medium. A: Morphology and internal structure of strain WXL662 cultured on MA, (Bar, 2 μm); B: Morphology and internal structure of strain WXL662 cultured with glucose as the sole carbon source, (Bar, 2 μm); C, D: Morphology and internal structure of strain WXL662 cultured with alginate as the sole carbon source, (Bar, 500 nm).

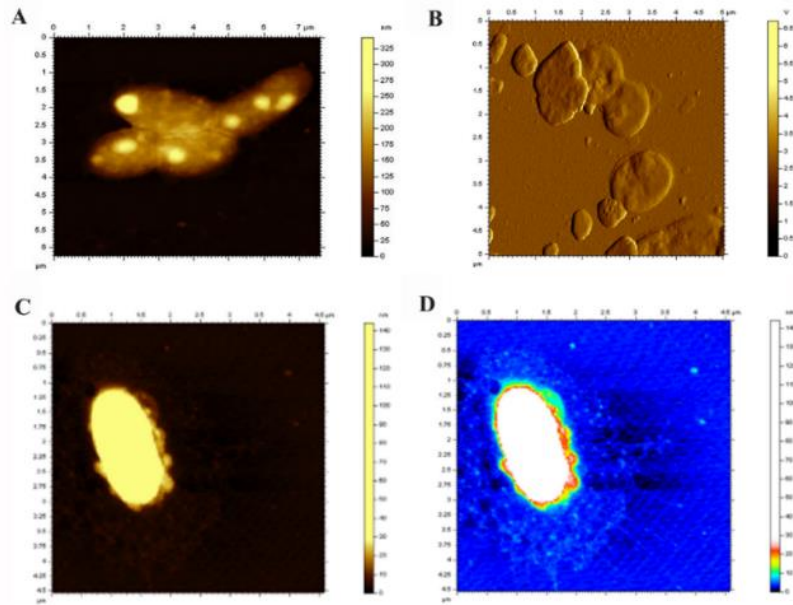


Fig. S10 Atomic force electron micrograph (AFM) of strain WXL662 under different culture conditions. A: Morphology of strain WXL662 cultured with glucose as the sole carbon source; B, C, D: Morphology of strain WXL662 cultured with alginate as sole carbon source.

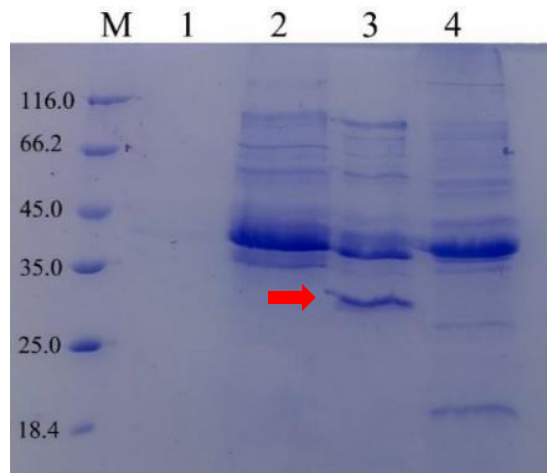


Fig. S11 SDS-PAGE of crude vesicles under different culture conditions.

M: marker; 1, Fermentation supernatant of glucose sole carbon source mineral medium; 2, Crude out membrane vesicles (OMVs) component cultured with glucose as the sole carbon source; 3, Crude OMVs component cultured with alginate as sole carbon source; 4, Crude OMVs component cultured in MB medium.



Fig. S12 The AUL encoded in the 21 alginate-degrading *Vibrio* strains. A-type DehR, 3-hydroxyisobutyrate dehydrogenase; B-type DehR, 2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase; Aly, alginate lyase; SSF, sodium-solute symporter (alginate transport system, termed ToaA); polyM lyases, poly(beta-D-mannuronate) lyase; KdgF, Pectin degradation protein KdgF; KdgK, (KdgK-like protein, 2-dehydro-3-deoxygluconokinase); Eda, 2-dehydro-3-deoxyphosphogluconate aldolase; hyp, hypothetical protein; SDR, Short-chain dehydrogenase/reductase SDR, KdgM, oligogalacturonate-specific porinin KdgM; NanC, N-acetylneuraminic acid outer membrane channel protein, NanC.

Table S1 The general information of *V. pelagius* WXL662 genome

<i>V. pelagius</i> WXL662	
Genome size	4.64 Mbp
Contigs	
macro-chromosome (Chr-I)	3.06 Mbp
micro-chromosome (Chr-II)	1.31 Mbp
large-plasmid (Plas-I)	0.24 Mbp
small-plasmid (Plas-II)	0.03 Mbp
GC Content	45.1%
N50	3057849
Number of coding proteins	4178
Number of RNAs	175 (13 16S rRNA)

Table S2 Genes related to alginate degradation in the genome of *V. pelagius* WXL662

Gene ID	Gene locus	Gene function
peg.150	tig00000000_188109_189860	Alginate lyase
peg.151	tig00000000_191636_190068	Alginate lyase
peg.153	tig00000000_194879_194013	Outer membrane porin (KdgM)
peg.154	tig00000000_195934_195617	Pectin degradation protein KdgF
peg.155	tig00000000_196935_196156	Short-chain dehydrogenase/reductase (SDR)
peg.718	tig00000000_877423_876386	Alginate lyase
peg.2434	tig00000000_2809217_2810566	Alginate lyase
peg.2547	tig00000000_2942395_2943258	Alginate lyase
peg.2812	tig00000003_204206_203487	SDR
peg.2942	tig00000003_350099_349050	Alginate lyase
peg.3358	tig00000003_828676_827060	Alginate lyase
peg.3399	tig00000003_886197_886871	N-acetylneuraminic acid outer membrane channel protein NanC (NanC)
peg.3408	tig00000003_898818_896668	poly(beta-D-mannuronate) lyase (polyM lyase)
peg.3409	tig00000003_901004_898821	polyM lyase
peg.3410	tig00000003_901337_901672	Pectin degradation protein KdgF
peg.3411	tig00000003_901707_902645	2-dehydro-3-deoxygluconate kinase (KdgK-like protein)
peg.3412	tig00000003_902776_903402	2-dehydro-3-deoxyphosphogluconate aldolase (Eda)
peg.3679	tig00000003_1214759_1214130	Eda
peg.3680	tig00000003_1215727_1214780	KdgK-like protein
peg.3962	tig00000004_207008_204972	Alginate lyase
peg.3968	tig00000004_216126_216800	NanC
peg.3967	tig00000004_215052_216110	Alginate lyases
peg.3968	tig00000004_216126_216800	NanC
peg.3972	tig00000004_221933_221151	Transcriptional regulator KdgR, KDG operon repressor
peg.3976	tig00000004_223537_223857	Pectin degradation protein KdgF
peg.3978	tig00000004_225489_224866	Eda
peg.3979	tig00000004_226432_225503	KdgK-like protein

Table S3 Primers used in cloning 11 alginate lyases in *V. Pelagius* WXL662. Primers were designed using the Primer-primer 5 design tool. The cleavage sites are represented in lowercase.

Primer	Sequence (5'-3') b	Restriction Site and Mutant Amino Acid
VpAly-I-F	5'- CAGGAATTCATGTCATCAAGCGAGC -3'	EcoR I
VpAly-I-R	5'- CCGCTCGAGTTACTGTGTATGTTTCG -3'	Xho I
VpAly-II-F	5'- CCGGAATTCATGGCAAGTACCTCAG -3'	EcoR I
VpAly-II-R	5'- CCGCTCGAGTTAGCCTTGGA ACTCA -3'	Xho I
VpAly-III-F	5'- CGCGGATCCATGGCAGATACAGATA -3'	BamH I
VpAly-III-R	5'- CCGCTCGAGTTACTGGTTTAAATCC -3'	Xho I
VpAly-IV-F	5'-CACGGATCCATGGAACAGCTAAATA -3'	BamH I
VpAly-IV-R	5'-CCGGAATTCGTTATGACTATTATCG -3'	EcoR I
VpAly-V-F	5'- CACGGATCCATGGCAAATGCTTCAG -3'	BamH I
VpAly-V-R	5'- CCGCTCGAGTTAACGAATTACTGGC -3'	Xho I
Aly2942-F	5'- CGCGGATCCATGGAAGTAGTTAACA -3'	BamH I
Aly2942-R	5'- CCGCTCGAGTTACTTTCCGTTTAAAC -3'	Xho I
VpAly-VI-F	5'- CGCGGATCCATGCAAGAGAACACAT-3'	BamH I
VpAly-VI-R	5'- CCGCTCGAGCTACTTGGTCGTATTA -3'	Xho I
VpAly-VII-F	5'- CGCGGATCCATGACTATCAACCAC -3'	BamH I
VpAly-VII-R	5'- CCGCTCGAGAAGCTTAGCGAATGCA -3'	Xho I
VpAly-VIII-F	5'- CGCGGATCCATGACTACACAACCAA -3'	BamH I
VpAly-VIII-R	5'- CTGCTCGAGTTTCTCCTGACCAGCT -3'	Xho I
VpAly-X-F	5'- CACGGATCCATGATCCAGTTTAGCG -3'	BamH I
VpAly-X-R	5'- CCGCTCGAGACTGAATGTCAGTTTC -3'	Xho I
VpAly-XI-F	5'-CGGAATTCCTGAAGCAAGATTCAATT-3'	EcoR I
VpAly-XI-R	5'-CCGCTCGAGTTATTTAAACAATAGA-3'	Xho I

Table S4 The primers used in RT-qPCR. Primers were designed using the Primer-primer 5 design tool. The abbreviation of each gene in the primer: R5A, Ribose 5-phosphate isomerase A; KdgK, KdgK-like protein (2-dehydro-3-deoxygluconate kinase); Eda, 2-dehydro-3-deoxyphosphogluconate aldolase; PGK, Phosphoglycerate kinase; TonB, TonB-dependent heme receptor A; PGAM-i, 2,3-bisphosphoglycerate-independent phosphoglycerate mutase.

Primer	Sequence (5'-3') b
recA-2-F	5'-AGTTCTACGCATCTGTTCTGCTTTGA -3'
recA-2-R	5'- TGACCAATCTTATCGCCGTTGTAGC-3'
ftsZ-F	5'-GCAACAGGTATTGGCACTGAGAGAA -3'
ftsZ-R	5'- GCCTGACGTCGTAAGAATGCTGGAA-3'
YG-R5A-F	5'-CAGTAGAAGTTATCCCAATGGCTCG -3'
YG-R5A-R	5'-GACCAACAGTTACAACACCAGCAAT -3'
YG-KdgM1-F	5'- CAAGTTCGTGTTCAATACTCGTTTCG-3'
YG-KdgM1-R	5'- TACCGAATTCTACATACGGACGCCA-3'
YG-KdgM2-F	5'- TCTTTTGATTTCAGGGTTGTTACTA-3'
YG-KdgM2-R	5'- GCCGTTATCAAAGAATACTTGGTCA-3'
YG-TonB-F	5'-CTTTTCAGTGGCTTACGTTCTGTTGC -3'
YG-TonB-R	5'- GCACCGAACGAGAGCCCTTTGACTT-3'
YG-KdgK -F	5'-GCATTATGTTGCTCCTAACCCAGTA -3'
YG-KdgK-R	5'-TTCTTTTGGGAATAATCGCACCTTGG -3'
YG- Eda -F	5'-TAGTTGAACAAGCAATGGAAATGGG -3'
YG- Eda A-R	5'-GGGATAGATAGGTATTTCGTCAACGT -3'
YG-PGK-F	5'- CGCTAAAACCTATCCTTTGGAACGGT-3'
YG-PGK-R	5'- TTCAACGAACTCAAGGAAAGCACCG-3'
YG-PGAM-i-F	5'-AGAGAACACATACGGTGAGTGGCTA -3'
YG-PGAM-i-R	5'-ATTTCTGGTTGTAGGTCGTAAGTCG -3'
YG-VpAly-I-F	5'- TGGTGGATGGTCAGATGCACTTTTCG -3'
YG-VpAly-I-R	5'- GAGTATGATTTTTTGCCTTCGTTTCG -3'
YG-VpAly-II-F	5'- GCGTGAAGGCAAAGAAGATGCTGTT -3'
YG-VpAly-II-R	5'- TTGGAACTCACCGTATGTTGCGTCT -3'
YG-VpAly-III-F	5'- CAGAATGCGAAAGATGAGAACGGTA -3'
YG-VpAly-III-R	5'- TTACCTTTTCGCCAAATCCACTTCAT -3'
YG-VpAly-IV-F	5'- ATCTGAATAGTCTAAGTAACCCGAG -3'
YG-VpAly-IV-R	5'- TCTGATAGAAGGTCACCTTGGGCATA -3'
YG-VpAly-V-F	5'- TGCCTGGTTACAAAAATCGTAGTGA -3'
YG-VpAly-V-R	5'- ACCACAGTTCACAGCATTGTTCTTA -3'
YG-VpAly-VI-F	5'- CAAACCGTGAGGATATTGCTTACCC -3'
YG-VpAly-VI-R	5'- ACTGACCATACGCACCTGCTTTGAA -3'
YG-VpAly-VII-F	5'- AAATCGGGTGACAACACAATCGCAG -3'
YG-VpAly-VII-R	5'- ATCACACCATCACGCCACGAGTAT -3'
YG-VpAly-VIII-F	5'- ACGACTCAGTAATCAAGACAACCTC-3'
YG-VpAly-VIII-R	5'- GTTGAATGGTTTGATTTCTGCTGCT-3'
YG-VpAly-IX-F	5'-AGTTATCTTTACTCGTTCTGGCGCG -3'
YG-VpAly-IX-R	5'-TCAGTAGTGTGAGTCGCACCCAGTT -3'
YG-VpAly-X-F	5'-TATTCTGTGTTGGCGAAGCCTATGT -3'
YG-VpAly-X-R	5'-TAGCGATGGGAGTGTGCTGAAACCG -3'

Table S5 The information of 95 *Vibrio* genomes.

Genome	Accession	Numbers of chromosome	Numbers Plasmid
<i>Vibrio metschnikovii</i> 07-2421	GCA_009763925.1	2	0
<i>V. metschnikovii</i> 2012V-1020	GCA_009665235.1	2	0
<i>V. metschnikovii</i> 9502-00	GCA_009763765.1	2	0
<i>V. cincinnatiensis</i> 1398-82	GCA_009763485.1	2	0
<i>V. cincinnatiensis</i> 2070-81	GCA_009763705.1	2	0
<i>V. cincinnatiensis</i> F8054	GCA_009763885.1	2	0
<i>V. fluvialis</i> 12605	GCA_001952955.1	2	0
<i>V. fluvialis</i> FDAARGOS_100	GCA_002953375.1	2	0
<i>V. fluvialis</i> F8658	GCA_009764195.1	2	1
<i>V. furnissii</i> 2419-04	GCA_009665335.1	2	1
<i>V. furnissii</i> 2012V-1225	GCA_009764175.1	2	0
<i>V. furnissii</i> 2013V-1001	GCA_009763225.1	2	0
<i>V. metoecus</i> 06-2478	GCA_009763865.1	2	0
<i>V. metoecus</i> 08-2459	GCA_009665275.1	2	0
<i>V. metoecus</i> 2011V-1015	GCA_009763905.1	2	0
<i>V. mimicus</i> 2011V-1073	GCA_009665195.1	2	0
<i>V. mimicus</i> SCCF01	GCA_001767355.1	2	0
<i>V. mimicus</i> FDAARGOS_112	GCA_001558475.2	2	0
<i>V. cholerae</i> E4	GCA_003719755.1	2	1
<i>V. cholerae</i> O395	GCA_000021625.1	2	0
<i>V. paracholerae</i> NCTC 30	GCA_900538065.1	2	0
<i>V. gazogenes</i> ATCC 43942	GCA_002196515.1	2	1
<i>V. spartinae</i> 3.6	GCA_014083925.1	2	0
<i>V. tritonius</i> JCM 16456	GCA_001547935.1	2	0
<i>V. anguillarum</i> CNEVA NB11008	GCA_002212025.1	2	0
<i>V. anguillarum</i> J360	GCA_003399575.2	2	1
<i>V. anguillarum</i> 90-11-286	GCA_001660505.2	202 contigs	
<i>V. qinghaiensis</i> Q67	GCA_002257545.1	2	0
<i>V. alginovor</i> SA2	GCA_002218005.2	2	0
<i>V. rumoiensis</i> FERM P-14531	GCA_002218045.2	2	2
<i>V. aphrogenes</i> CA-1004	GCA_002157735.2	2	0
<i>V. casei</i> DSM 22364	GCA_002218025.2	2	3
<i>V. breoganii</i> FF50	GCA_001677275.1	2	1

<i>V. haliotocoli</i> IAM 14596	GCA_003568965.1	6 scaffolds	
<i>V. nigripulchritudo</i> SFn1	GCA_000801275.1	2	0
<i>V. nigripulchritudo</i> TUMSAT-TG-2018	GCA_015097735.1	2	4
<i>V. tapetis</i> CECT4600	GCA_900233005.1	2	1
<i>V. atlanticus</i> LGP32	GCA_000091465.1	2	0
<i>V. splendidus</i> 12B01	GCA_000152765.1	26 scaffolds	
<i>V. splendidus</i> BST398	GCA_003345295.1	2	0
<i>V. cyclitrophicus</i> ECSMB14105	GCA_005144905.1	2	0
<i>V. chagasii</i> ECSMB14107	GCA_004022545.1	2	0
<i>V. splendidus</i> OU02	GCA_003408655.1	3 scaffolds	
<i>Vibrio</i> sp. THAF190c	GCA_009363355.1	1	5
<i>V. mediterranei</i> 117-T6	GCA_003726935.1	2	1
<i>V. mediterranei</i> QT6D1	GCA_002214345.1	2	1
<i>V. astriarenae</i> HN897	GCA_010587385.1	2	0
<i>V. coralliilyticus</i> RE22	GCA_003391375.1	2	1
<i>V. coralliilyticus</i> RE98	GCA_000772065.1	2	2
<i>V. coralliilyticus</i> SNUTY-1	GCA_002073995.1	2	2
<i>V. aquimaris</i> THAF100	GCA_009363415.1	1	3
<i>V. europaeus</i> 07/118 T2	GCA_015654285.1	2	1
<i>V. europaeus</i> NPI-1	GCA_013154935.1	2	1
<i>V. tubiashii</i> ATCC 19109	GCA_000772105.1	2	3
<i>V. taketomensis</i> C4III282	GCA_009938165.1	2	0
<i>V. taketomensis</i> C4III291	GCA_009938185.1	2	1
<i>V. panuliri</i> JCM 19500	GCA_009938205.1	2	1
<i>V. ponticus</i> DSM 16217	GCA_009938225.1	2	1
<i>V. scophthalmi</i> VS-05	GCA_001687805.1	2	2
<i>V. scophthalmi</i> VS-12	GCA_001685465.1	2	3
<i>V. cidicii</i> 2756-81	GCA_009763805.1	2	0
<i>V. navarrensis</i> 08-2462	GCA_009665215.1	2	0
<i>V. navarrensis</i> 2462-79	GCA_009763725.1	2	0
<i>V. vulnificus</i> FORC_017	GCA_001675245.1	2	1
<i>V. vulnificus</i> FORC_053	GCA_003522555.1	3	0
<i>V. vulnificus</i> FORC_036	GCA_002117205.1	2	1
<i>V. alginolyticus</i> K04M3	GCA_002149105.1	2	1
<i>V. alginolyticus</i> K06K5	GCA_002119565.1	2	2
<i>V. neocaledonicus</i> CGJ02-2	GCA_005239365.1	2	0

<i>V. alginolyticus</i> ATCC 33787	GCA_001469735.1	2	3
<i>V. antiquarius</i> EX25	GCA_000024825.1	2	0
<i>V. diabolicus</i> LMG 3418	GCA_002953355.1	2	0
<i>V. diabolicus</i> FDAARGOS_96	GCA_002953335.1	2	1
<i>V. diabolicus</i> FDAARGOS_105	GCA_002953395.1	2	0
<i>V. natriegens</i> CCUG 16371	GCA_001680045.1	2	0
<i>V. natriegens</i> CCUG 16373	GCA_001680065.1	2	0
<i>V. natriegens</i> CCUG 16374	GCA_001680085.1	2	0
<i>V. parahaemolyticus</i> 20160303005-1	GCA_009883875.1	2	5
<i>V. parahaemolyticus</i> PB1937	GCA_003351885.1	2	2
<i>V. parahaemolyticus</i> 19-021- D1	GCA_009734325.1	2	2
<i>V. alfacsensis</i> CAIM 1831	GCA_003544875.1	2	1
<i>V. rotiferianus</i> AM7	GCA_007182835.1	2	1
<i>V. rotiferianus</i> B64D1	GCA_002214395.1	2	0
<i>V. hyugaensis</i> 090810a	GCA_002906655.1	2	0
<i>V. jasicida</i> 090810c	GCA_002887615.1	2	0
<i>V. owensii</i> 1700302	GCA_003691505.1	2	2
<i>V. owensii</i> SH14	GCA_001310575.2	2	2
<i>V. owensii</i> V180403	GCA_003691545.1	2	2
<i>V. harveyi</i> 345	GCA_002850295.1	2	2
<i>V. harveyi</i> QT520	GCA_001908435.2	2	3
<i>V. harveyi</i> FDAARGOS_107	GCA_001558435.2	2	0
<i>V. campbellii</i> LA16-V1	GCA_002142655.1	2	4
<i>V. campbellii</i> 170502	GCA_003691485.1	2	1
<i>V. campbellii</i> LMB29	GCA_001969325.1	2	4

Table S6 The predicted subcellular localization of key proteins in the alginate metabolism pathway of strain WXL662.

CP, cytoplasm; EC, extracellular space; IM, inner membrane; OM, outer membrane; PP, periplasm; UN, Unknown; Y, Yes (coding peptide); N, No, (No codingpeptide); The numbers in the Bomp category column indicate the number of β barrels in the outer membrane protein; SP I, signal peptide Type I; SP II, signal peptide Type II; CYT, No signal peptide in the cytoplasm.

Cello gives the predicted score at different positions based on the amino acid composition. The location with the score marked * is the more likely position of the target protein. PsortB provide the location of the protein. Phobius TM and TMHMM predict the number of transmembrane spirals (TMs) in the inner membrane component. Bomp determines the number of β barrels in the outer membrane protein. Phobius SP, SMART and Lipop can predict whether proteins have signal peptides.

Gene No.	Protein name	Predicted localization	Cello	PsortB	Phobius TM	TMHMM	Phobius SP	SMART (Signal p)	LipoP class	Bomp category
peg.150	VpAly-I	EC	EC: 1.090 OM: 0.236 PP: 1.063 IM: 0.335 CP:2.276 *	UN	0	0	Y	Y	SP II	0
peg.151	VpAly-II	EC	EC: 0.855 OM: 0.169 PP: 1.402 IM: 0.336 CP:2.239 *	EC	0	0	Y	Y	SP II	0
peg.718	VpAly-III	PP	EC: 0.832 OM: 0.160 PP: 1.499* IM: 0.315 CP:2.194 *	EC	0	0	Y	Y	SP I	0
peg.2434	VpAly-IV	EC	EC: 1.388 OM:0.195 PP: 0.925 IM: 0.319 CP:2.173 *	EC	0	0	Y	Y	SP I	0
peg.2547	VpAly-V	EC	EC: 0.782 OM:0.193 PP: 1.461 IM: 0.326 CP:2.239 *	UN	0	0	Y	Y	SP I	0
peg.2942	VpAly-VI	EC	EC: 0.863 OM:0.160	EC	0	0	Y	Y	SP I	0

Table S7 Comparison of qRT-qPCR and transcriptome results of different genes.

Number	Gene ID	Protein	RT-qPCR (log ₂)	transcript (log ₂ FC)
1	peg.150	VpAly-I	2.44	2.09
2	peg.151	VpAly-II	4.8	2.46
3	peg.718	VpAly-III	2.43	2.68
4	peg.2434	VpAly-IV	3.75	4.96
5	peg.2547	VpAly-V	2.26	1.94
6	peg.2942	VpAly-VI	1.37	1.97
7	peg.3358	VpAly-VII	0.35	NC
8	peg.3408	VpAly-VIII	0.38	0.51
9	peg.3409	VpAly-IX	0.6	1.18
10	peg.3962	VpAly-X	0.3	0.56
11	peg.153	KdgM-1	4.5	2.28
12	peg.3399	KdgM-2	2.95	3.49
13	DHCAACBI_018 22	Ribose 5-phosphate isomerase A	2.78	0.65
14	DHCAACBI_037 61	2-dehydro-3- deoxygluconate kinase (kdgk-like)	1.57	2.38
15	DHCAACBI_029 65	2-dehydro-3- deoxyphosphogluconate aldolase (Eda)	1.98	0.44
16	DHCAACBI_018 13	Phosphoglycerate kinase	2.89	3.32
17	DHCAACBI_032 74	TonB-dependent heme receptor A	3.25	4.34
18	DHCAACBI_011 71	2,3-bisphosphoglycerate- independent phosphoglycerate mutase (EC 5.4.2.1)	2.74	3.67

Table S8 Fold changes of alginate-degrading genes revealed by the transcriptome sequencing. Chr-I, Chromosome-I; Chr-II, Chromosome-II; Plas-I, Plasmid-I.

Number	Gene ID	Products	log2FC (Alg/Glu)	Position in the genome
1	peg.148	Nuclease SbcCD subunit C	NC	Chr-I.-AUL1
2	peg.149	Nuclease SbcCD subunit D	NC	Chr-I.-AUL1
3	peg.150	VpAly-I	2.09	Chr-I.-AUL1
4	peg.151	VpAly-II	2.46	Chr-I.-AUL1
5	peg.152	putative biofilm-associated surface protein	1.67	Chr-I.-AUL1
6	peg.153	KdgM	2.28	Chr-I.-AUL1
7	peg.154	Pectin degradation protein KdgF	1.66	Chr-I.-AUL1
8	peg.155	Short-chain dehydrogenase/reductase (SDR)	1.19	Chr-I.-AUL1
9	peg.156	Predicted signal-transduction protein containing cAMP-binding and CBS domains	4.78	Chr-I.-AUL1
10	peg.157	DNA polymerase III epsilon subunit	1.52	Chr-I.-AUL1
11	peg.158	sodium-solute symporter, putative	1.32	Chr-I.-AUL1
12	peg.159	Methyl-accepting chemotaxis protein, homolog 13	NC	Chr-I.-AUL1
13	peg.718	VpAly-III	2.68	Chr-I
14	peg.719	Proton/glutamate-aspartate symporter	2.74	Chr-I
15	peg.723	Outer membrane lipoprotein SmpA	1.39	Chr-I
16	peg.2434	VpAly-IV	4.96	Chr-I
17	peg.2547	VpAly-V	1.94	Chr-I
18	peg.2942	VpAly-VI	NC	Chr-II
19	peg.3212	TonB-dependent receptor	4.73	Chr-II.
20	peg.3358	VpAly-VII	1.97	Chr-II.
21	peg.3399	NanC	3.49	Chr-II-AUL2
22	peg.3406	putative ABC transporter solute-binding protein YclQ	NC	Chr-II-AUL2
23	peg.3408	VpAly-VIII	NC	Chr-II-AUL2
24	peg.3409	VpAly-IX	NC	Chr-II-AUL2
25	peg.3410	Pectin degradation protein KdgF	NC	Chr-II-AUL2
26	peg.3411	2-dehydro-3-deoxygluconate kinase (kdgK-like protein)	3.19	Chr-II-AUL2
27	peg.3412	2-dehydro-3-deoxyphosphogluconate aldolase (Eda)	2.02	Chr-II-AUL2
28	peg.3413	sodium-solute symporter, putative	3.31	Chr-II-AUL2
28	peg.3414	hypothetical protein	1.26	Chr-II-AUL2
30	peg.3415	hypothetical protein	NC	Chr-II-AUL2

32	peg.3962	VpAly-X	NC	Plas-I-AUL3
32	peg.3963	Uronate isomerase (EC 5.3.1.12)	NC	Plas-I-AUL3
33	peg.3964	sodium-solute symporter, putative	1.45	Plas-I-AUL3
34	peg.3965	sodium-solute symporter, putative	3.18	Plas-I-AUL3
35	peg.3966	Streptococcal hemagglutinin protein	1.25	Plas-I-AUL3
36	peg.3967	VpAly-XI	NC	Plas-I-AUL3
37	peg.3968	NanC	2.88	Plas-I-AUL3
38	peg.3969	Methyl-accepting chemotaxis protein	NC	Plas-I-AUL3
39	peg.3970	DNA polymerase III epsilon subunit	NC	Plas-I-AUL3
40	peg.3971	Predicted signal-transduction protein containing cAMP-binding and CBS domains	NC	Plas-I-AUL3
41	peg.3972	Transcriptional regulator KdgR, KDG operon repressor	1.37	Plas-I-AUL3
42	peg.3973	hypothetical protein	NC	Plas-I-AUL3
43	peg.3974	2-deoxy-D-gluconate 3-dehydrogenase (KduD)	NC	Plas-I-AUL3
44	peg.3975	Arginine/ornithine antiporter ArcD	NC	Plas-I-AUL3
45	peg.3976	Pectin degradation protein KdgF	1.37	Plas-I-AUL3
46	peg.3977	DEH ketol-isomerase	1.71	Plas-I-AUL3
47	peg.3978	2-dehydro-3-deoxyphosphogluconate aldolase (Eda)	NC	Plas-I-AUL3
48	peg.3979	2-dehydro-3-deoxygluconate kinase (KdgK-like protein)	NC	Plas-I-AUL3

Table S9 Key proteins involved in the alginate degradation of 25 *Vibrio* strains.

AUL, alginate utilization loci; A-type DehR, 3-hydroxyisobutyrate dehydrogenase; B-type DehR, 2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase; SSF, sodium-solute symporter; polyM lyases, poly(beta-D-mannuronate) lyase; KdgF, Pectin degradation protein KdgF; KdgK, 2-dehydro-3-deoxygluconokinase; Eda, 2-dehydro-3-deoxyphosphogluconate aldolase; hyp, hypothetical protein; SDR, Short-chain dehydrogenase/reductase SDR, KdgM, oligogalacturonate-specific porinin KdgM; NanC, N-acetylneuraminic acid outer membrane channel protein, NanC.

Strains	Type	Location	Alginate lyases	polyM lyases	DehR (3types)	KdgF	KdgK	Eda	KdgM	NanC
<i>V. alginivorus</i> SA2	One AUL	Chromosome II	5	2	2	1	2	2	1	1
<i>V. haliotocoli</i> IAM 14596T	Scattered system	Chromosome I, II	9	1	3	1	1	1	0	0
<i>V. splendidus</i> 12B01	One AUL + one alginate lyase	Contig CH724170.1 (one alginate lyase) and Contig CH724171.1 (One AUL) plasmid	5	2	3	1	3	3	1	2
<i>V. splendidus</i> OU02	One AUL		6	1	3	1	5	5	1	1
<i>V. alfacensis</i> CAIM 1831	One AUL	Chromosome I	2	1	3	1	3	4	1	0
<i>V. alginolyticus</i> ATCC 33787	One AUL	Chromosome II	5	1	5	1	1	2	1	0
<i>V. aphrogenes</i> CA-1004	One AUL	Chromosome I	5	2	2	1	1	2	1	0

<i>V. astriarenae</i> HN897	Scattered system	Chromosome I, II	4	0	5	1	3	4	1	3
<i>V. breoganii</i> FF50	Scattered system	Chromosome I, II	6	2	4	1	1	2	0	1
<i>V. chagasii</i> ECSMB 14107	One AUL	Chromosome I	2	2	1	1	1	3	1	0
<i>V. cyclitrophicus</i> ECSMB14105	One AUL+ one alginate	Chromosome I + II	5	1	3	1	1	1	2	0
<i>V. europaeus</i> 07- 118T2	One AUL	Chromosome I	1	1	4	1	3	3	2	1
<i>V. europaeus</i> NPI-1	One AUL	Chromosome I	1	2	4	1	3	3	1	1
<i>V. harveyi</i> FDAARGOS 107	One AUL	Chromosome I	2	1	4	1	4	5	1	1
<i>V. harveyi</i> QT520	One AUL	Chromosome I	2	1	3	1	4	5	1	0
<i>V. hyugaensis</i> 090810a	One AUL+ Two alginate lyases	Chromosome I + one alginate lyases + Chromosome II one alginate lyases	8	1	4	1	2	3	2	0
<i>V. jasicida</i> 090810c	One AUL+ one alginate lyases	Chromosome I	6	1	4	1	4	5	1	0
<i>V. mediterranei</i> 117- T6	One AUL	Chromosome II	3	1	4	1	3	3	0	3
<i>V. neocaledonicus</i> CGJ02-2	One AUL	Chromosome II	4	1	4	1	1	2	1	0
<i>V. owensii</i> 1700302	One AUL	Chromosome I	4	1	4	1	4	4	0	1
<i>V. owensii</i> SH14	One AUL	Chromosome I	4	1	5	1	4	5	0	0
<i>V. owensii</i> V180403	One AUL+ Two alginate lyases	Chromosome I + one alginate lyases + Chromosome II one alginate lyases	8	0	5	1	4	5	1	0

<i>V. rumoiensis</i> FERM P-14531	One AUL	Chromosome II	3	0	4	1	2	3	1	0
<i>Vibrio</i> sp. THAF190c	Scattered system	Chromosome I, II	5	1	6	2	2	2	1	0
<i>V. splendidus</i> BST398	One AUL	Chromosome I	5	2	5	1	4	4	1	2

Table S10 The enzymatic characteristics of 91 previously studied alginate lyases from families PL6, -7, -14, -17.

See Supplementary_material_Table S10.

Table S11 LC-MS/MS-30 min sequencing for the coarse band of the crude vesicles cultured with alginate as sole carbon source (Fig. S11).

See Supplementary_material_Table S11.