

Supplementary Table S1. Primer sequences used in this experiments

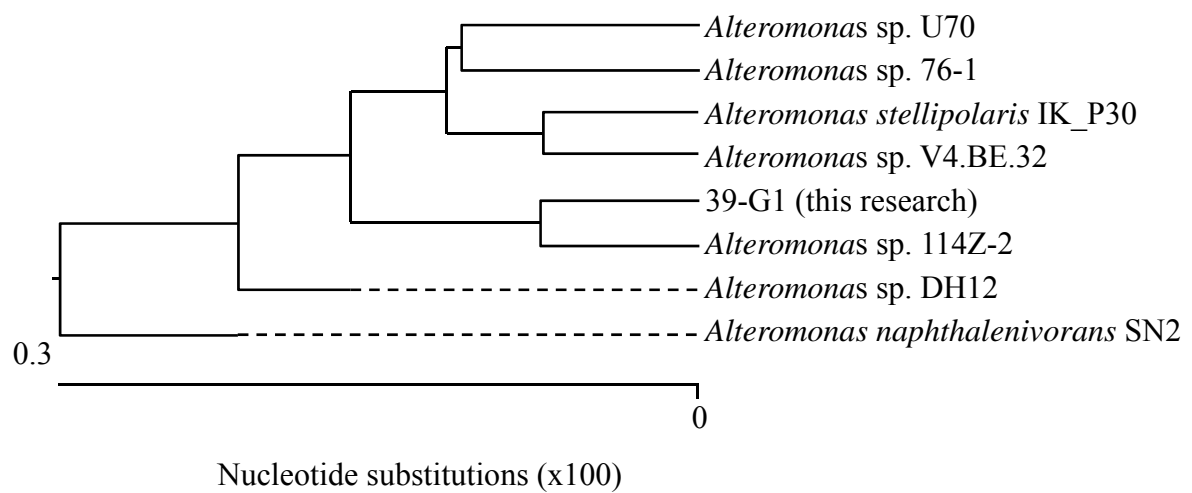
Cloning gene	Primer sequence
16s rRNA	27F 5'-AGA GTT TGA TCM TGG CTC AG-3'
	1492R 5'-GGT TAC CTT GTT ACG ACT T-3'
WT EstA1	Forward primer: 5'-CATATGAACCTACTTACCTTTTCC-3'
	Reverse primer: 5'-CTCGAGGTCGCTACTTTTAGTACACA-3'
mutant ($\Delta 20$)	Forward primer: 5'-CATATGTCTACATCTGGGGCTTCT-3'
	Reverse primer: 5'-CTCGAGGTCGCTACTTTTAGTACACA-3'
mutant ($\Delta 54$)	Forward primer: 5'-CATATGGCTAAAACGTTACCGCTG-3'
	Reverse primer: 5'-CTCGAGGTCGCTACTTTTAGTACACA-3'

Supplementary Table S2. Purification of EstA1

	Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Recovery (%)
$\Delta 20$	Cell-free extract	40100	117	343	1.00	100
	Ni-NTA agarose	14300	23.9	598	1.74	35.7
	Superose 12	8180	9.36	874	2.55	20.4
$\Delta 54$	Cell-free extract	12100	51.5	235	1.00	100
	Ni-NTA agarose	9640	16.5	584	2.49	79.7
	Superose 12	5220	7.34	711	3.03	43.1

1	ATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGAAACATGTCTAGCTTGCT	60
61	AGATGATGTCGAGTGGCGGACGGGTGAGTAATGCTTGGGAACTTGCCTTTGCGAGGGGGA	120
121	TAACAGTTGGAAACGACTGCTAATACCGCATAATGTCTTCGGACCAAACGGGGCTTAGGC	180
181	TCCGGCGCAAAGAGAGGCCCAAGTGAGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAG	240
241	GCAACGATCTCTAGCTGTTCTGAGAGGAAGATCAGCCACACTGGGACTGAGACACGGCCC	300
301	AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGC	360
361	CATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGTTGTGAGGAAAAG	420
421	TTAGTAGTTAATACCTGCTAGCCGTGACGTTAACAACAGAAGAAGCACCGGCTAACTCCG	480
481	TGCCAGCAGCCGCGTAATACGGAGGGTGCAGCGTTAATCGGAATTACTGGGCGTAAGC	540
541	GCACGCAGGCGGTTTGTAAAGCTAGATGTGAAAGCCCCGGGCTCAACCTGGGACGGTCAT	600
601	TTAGAACTGGCAGACTAGAGTCTTGAGAGGGGAGTGGAAATTCAGGTGTAGCGGTGAAA	660
661	TGCGTAGATATCTGGAGGAACATCAGTGGCGAAGGCGACTCCCTGGCCAAAGACTGACGA	720
721	CTCATGTGCGAAAGTGTGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCACACCGTAA	780
781	ACGCTGTCTACTAGCTGTTTGTGGCTTTAAGCCGTGAGTAGCGAAGCTAACGCGATAAGT	840
841	AGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACCTCAAATGAATTGACGGGGGCCGCA	900
901	CAAGCGGTGGAGCATGTGGTTTAATTTCGATGCAACGCGAAGAACCTTACCTACACTTGAC	1060
1061	ATGTTGAGAAGTTACTAGAGATAGTTTCGTGCCTTCGGGAACTCAAACACAGGTGCTGCA	1120
1121	TGGCTGTCGTCAGCTCGTGTGCTGAGATGTTGGGTAAAGTCCCACGAGCGCAACCCCT	1180
1181	TGTCCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGA	1240
1241	GGAAGGTGGGGACGACGTCAAGTCATCATGGCCCTTACGTGTAGGGCTACACACGTGCTA	1300
1301	CAATGGCATATACAGAGGGATGCGAGACAGTGATGTGGAGCGGACCCCTTAAAGTATGTC	1360
1361	GTAGTCCGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAG	1420
1421	GTCAGAATACTGCGGTGAATACGTTCCCGGGCCTTGACACACCGCCCGTCACACCATGG	1480
1481	GAGTGGGATGCAAAAGAAGTAGTTA	1505

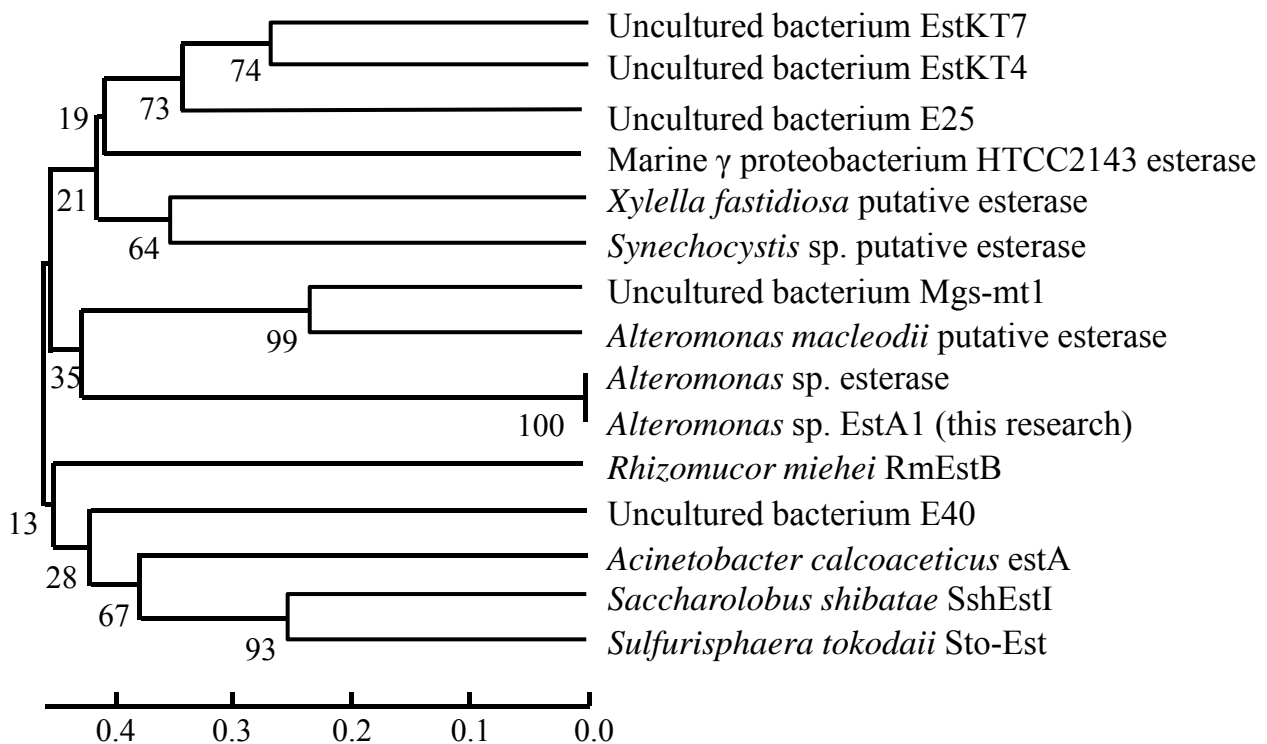
Supplementary Fig. S1. 16S rRNA sequence of strain 39-G1



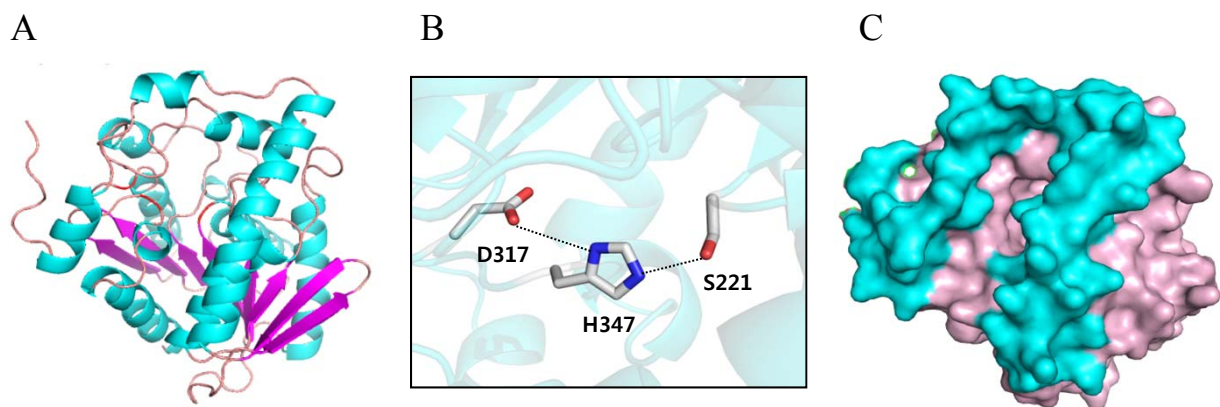
Supplementary Fig. S2. Phylogenetic tree of strain 39-G1 using 16S rRNA sequence. The 16S rRNA sequence of this strain had 99.86% homology with that of *Alteromonas* sp. 76-1 (GenBank: KY697829.1), 99.86% with *Alteromonas naphthalenivorans* SN2 (GenBank: GU166736.2), and 99.64% with *Alteromonas stellipolaris* IK_P30 (GenBank: LR218081.1).

1 ATGAACCTACTTACCTTTTCCTTCGCCTTTTTCATTTTGGTGGTATCAATGACCTTTTCGGCTTCTACATCTGGGGCTTCTACCCACAAC 90
1 M N L L T F S F A F F I L V V S M T F S A S T S G A S T H N 30
91 ACTGATACGGCCAGTGCAGTAAACAAAAGGCCGATACAGTCAAAATCTCTAATGAATCTCCGTTAACCGTGCCAGCTAAAACGTTACCG 180
31 T D T A S A S K Q K A D T V K I S N E S P L T V P A K T L P 60
181 CTGCCAAGTGCTTCAAGCGATGAACCTAAAAGCGGATTCTCAATACCCATGCCGTCAGTTGATGAAGTCATTAACAACACGCCTCAA 270
61 L P S A S S D E L K S A I S Q Y P M P S V D E V I N N T P Q 90
271 TCTATAGAGCAGTGGCGTGAATTGATTCAAATAAGAAATGCAGATCAGAAAAAGAAAAATCAAAAAATGCGCAAGCAGTTTGATGTTGAT 360
91 S I E Q W R E L I Q I R N A D Q K K K I K K M R K Q F D V D 120
361 GTGTCTTTGAAAAAATTAATGGCGTACCGGTCAGGCGACTTACGCCAAAGACCATTGCCCTGAATTTAAAAACAAGGTATTTATTGAC 450
121 V S L E K I N G V P V R R L T P K T I A P E F K N K V F I D 150
451 GTACACGGTGGCGTTACGTGTTTTTTCCGGGCTGCCCAGCATAGAAGAAAGCTTGTTAATTGCCCATAGAGTGGGTATTACAGTGATT 540
151 V H G G A Y V F F S G L P S I E E S L L I A H R V G I T V I 180
541 AGTATCGATTATAGCATGCCACCTCATGCGCAATTTCCAGCAGCACTAACGATGTAGTGTCCGTTTATAGCAGCGTGGCTGCAGAACAT 630
181 S I D Y S M P P H A P F P A A L N D V V S V Y S S V A A E H 210
631 GGCGCACAGAATCTTTTATAGGTGGCACATCTGCAGGAGCAGGTTTAGTGCTTGCTGCCGTGCAAACGCTTATTGCTGACAAGCAACCG 720
211 G A Q N L F I G G T S A G A G L V L A A V Q T L I A D K Q P 240
721 TTGCCAGCAGCAGTGTATGCGGGCACCCCTTGGGCTGATTTGACGAAAACCGGCGATACCCTTTATACCAATGAAGGTGTTGATCGTATT 810
241 L P A A V Y A G T P W A D L T K T G D T L Y T N E G V D R I 270
811 TTGGTGACATACCAAGGGTTTTTGAAGCAGCGGCTAATCTATATGCTGGAAGCGAAAGCTTAACCCACTCTCTATTTCCCTCTTTAT 900
271 L V T Y Q G F L E A A A N L Y A G S E S L T H S S I S P L Y 300
901 GGCAATTTTGATGGCTTCCCGCTACGTTTTTAATATCTGGCAGCGAGATATGTTTCTAAGCGATACGGTGGGGTGAATCGTAAGCTA 990
301 G N F D G F P P T F L I S G T R D M F L S D T V R V N R K L 330
991 CGTGATGCACAAGTACGCACCCAGTTAGAGTTTTTGAAGGTTATCCCATGCCGATTACGTTGTTGCTTATGAAACCCAGAATCTCAC 1080
331 R D A Q V R T Q L E V F E G L S H A D Y V V A Y E T P E S H 360
1081 TCTGTATACCAAGAGCTTAAGCAATTTCTACTAAGCGTGTGTACTAAAAGTAGCGACTAA 1140
361 S V Y Q E L K Q F L L S V C T K S S D *** 379

Supplementary Fig. S3. Nucleotide and protein sequence of esterase EstA1



Supplementary Fig. S4. Phylogenetic analysis of esterase EstA1 with closely related family IV proteins. Esterase EstA1 sequence was compared with similar proteins including uncultured bacterium EstKT7 (GenBank ADH59413.1), uncultured bacterium EstKT4 (GenBank ADH59412.1), uncultured bacterium E25 (PDB 4Q05), marine gamma proteobacterium HTCC2143 putative esterase (ZP_01615954.1), *Xylella fastidiosa* putative esterase (UniProtKB/Swiss-Prot Q9PCN5), *Synechocystis* sp. putative esterase (UniProtKB/Swiss-Prot P72953), uncultured bacterium Mgs-mt1 (PDB 4Q30), *Alteromonas macleodii* esterase (YP_002127854.1), *Alteromonas* sp. esterase (GenBank PHS53692.1), *Rhizomucor miehei* RmEstB (PDB 4WY8), uncultured bacterium E40 (PDB 4XVC), *Acinetobacter calcoaceticus* estA (GenBank CAA50601.1), *Saccharolobus shibatae* SshEstI (PDB 3WJ1), *Sulfurisphaera tokodaii* Sto-Est (PDB 3AIK). The phylogenetic tree was generated with the neighbor-joining method (MEGA 5.2). Bootstrap values (n = 1,000 replicates) are reported as percentages.



Supplementary Fig. S5. Structure homology model of esterase EstA1 A, Homology model of esterase EstA1 constructed by SWISS-MODEL with MGS-MT1 (PDB 4Q3O) used as the template. The α -helix, β -sheet, loop and catalytic triad are shown cartoon in sky blue, purple, pink, red, respectively. B, An enlarged view of the esterase EstA1 putative catalytic triad. Three amino acids (S221, D317, H347) that were expected to affect enzyme activity were located close to each other. C, Cap domain and catalytic domain are shown purple and red, respectively.