

Supplementary Information

Fast, simple and highly specific molecular detection of *Vibrio alginolyticus* pathogenic strains using a visualized isothermal amplification method

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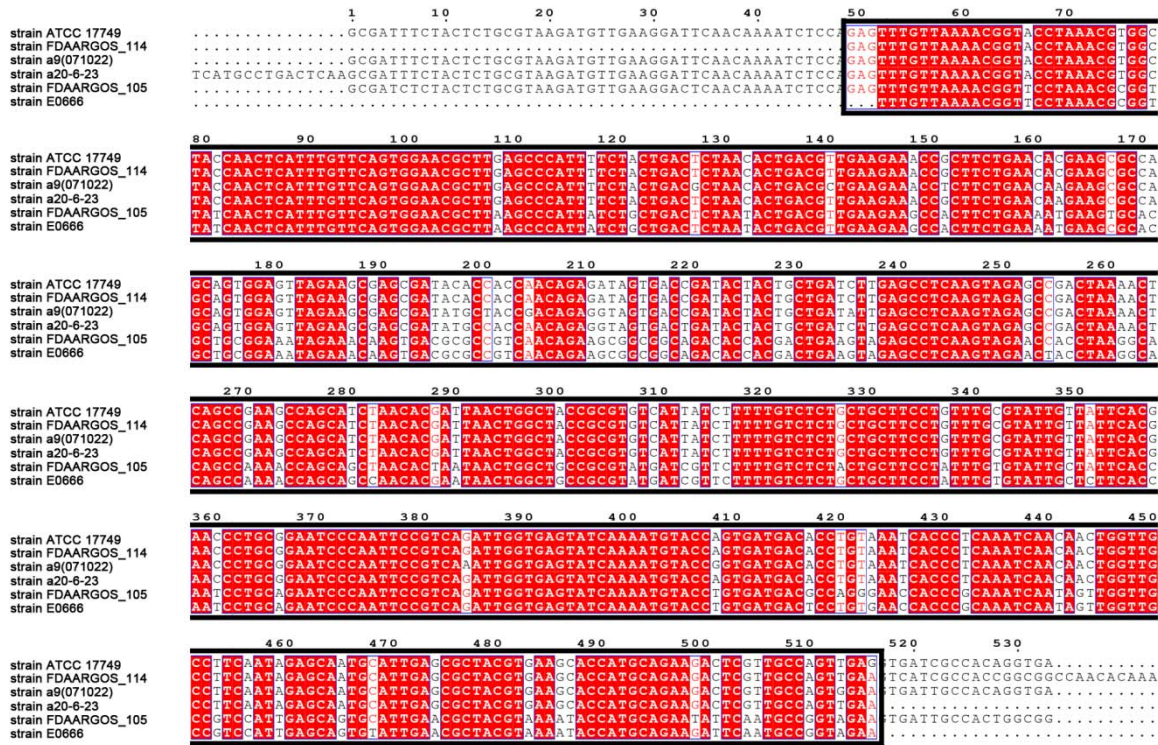


Fig. S2 Sequence alignment of the *toxR* genes of *V. alginolyticus* pathogenic strains. Information of the corresponding strain is indicated on the left of each sequence. GenBank numbers of the *toxR* genes of the strains are EU155543.1, CP014036.1, AB372531.1, AB372526.1, CP014036.1, and JN188451.1 (from top to bottom). The conserved region is indicated by the black box.

a

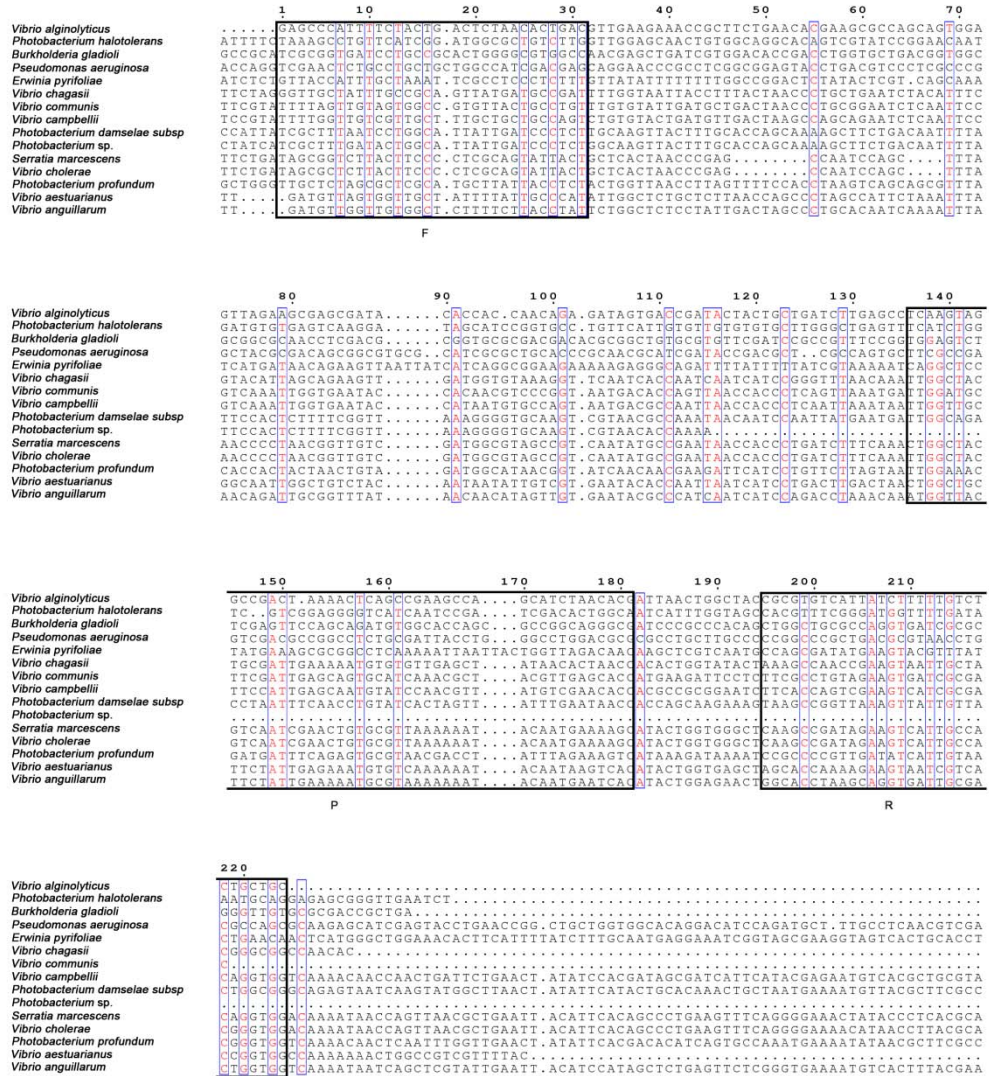


Fig. S3 Sequences targeted by primer-probe set F2/Probe 2/R in the *toxR* genes of *Vibrio* species and other bacteria. The name of the corresponding species is indicated on the left of each sequence. GenBank numbers of the *toxR* genes of the species are EU155543.1, HQ452616.1, LT797832.1, NC_002516.2, NC_012214.1, AY751345.1, JX401922.1, HQ318823.1, KU760757.1, KX280762.1, AF414370.1, MF100077.1, NC_006370.1, AM183574.1, and AB042547.1 (from top to bottom). The targeted regions by the forward primer (F), the probe (P) and the reverse primer (R) are indicated by the black boxes.

C

Vibrio alginolyticus
Vibrio parahaemolyticus
Vibrio sp.
Vibrio natrigiens
Vibrio splendidus
Vibrio pomeroiy
Vibrio tasmaniensis
Vibrio mimicus
Vibrio shilonii
Vibrio vulnificus
Vibrio neptunius

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1           10           20
..          ..          ..          ..          ..          ..          ..          ..          ..          ..
..AACTCAATGGTACTGTTGAACGCCCTAA.....GCCCACATTTCTACTACTCTAAAC...
..AGTTAACTGTGTTCTAGAACGATTA.....ATCGCTTTCCTCTTTGTTAAC...T
..AATTTATGCAACCGTTGACGCCTGCACCAC..TTTCATCAAATGATCAGC..ACGAGCGGCTAAATTCGAGAAA
..AATTTATGCAACCGTTGACGCCTGCACCAC..TTTCATCAAATGATCAGC..ACGAGCGGCTAAATTCGAGAAA
..AATTTATGCGAAGGTTGAACGTTGACGCCAC..TGTCATCAAATGATCAGC..AGTACAGGTGAAATTCGAGAAA
..AATTTATGCGAAGGTTGAACGTTGACGCCAC..TGTCATCAAATGATCAGC..AGTACAGGTGAAATTCGAGAAA
..AGCTCTTAAAGCTTGAATGGCCCGGT.....TGTTTACCAATAGCCAAAGAAATGAGGTAGCAGAAATGCCACTT
..AGGGGTTAAAGCTTGAATGGCCCGGT..TAG..TGAGCAGTAATACTGCCAGGGAAAGTAAAGCCGCTATCAGAAA
TTGCGAAATTTGATTCAGCTGGATTGCTAAAATAGAATGACGCACAAGGCATCAATAAGTGTAGAGGCGATGATAC

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F

Vibrio alginolyticus
Vibrio parahaemolyticus
Vibrio sp.
Vibrio natrigiens
Vibrio splendidus
Vibrio pomeroiy
Vibrio tasmaniensis
Vibrio mimicus
Vibrio shilonii
Vibrio vulnificus
Vibrio neptunius

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30           40           50           60           70           80           90
CTGAACTTCTAG...AAACCGCTTTGAACAC...GAAGCGCCACAGTGGAGTTAAGGAGCCATACACCACAA
TTGAACTTGAAG...AGCCAGCTTTGATAACCAATGACGCCTCTCTAATGAGGTAAGCAAGATCAGAGCGCTTT
CAGAACTCTGAG...AGCTAGCTTTAGACGTTGAGCCAGACAAAACAAAAGCCAGGATATTTCCTGACGACCA
CAGAACTCTGAG...AGCTAGCTTTAGACGTTGAGCCAGACAAAACAAAAGCCAGGATATTTCCTGACGACCA
ATGACTTAGAACCTATCTTAGCGTTTGAACACCTGACGCGACTGAAAGAACGATCACCTGAGAGGCTT
GTGACTTAGAGCCGATCTTAACGTTTGAATACCACTTTAGCTGAAAGAACCATCACTTAAACTATGAGCCAAAGCC
ACGACTTGA...TGAGGT...AACTCA...TCAGAAACTTGAATCTCTTTCCAAATC...AACCA
CGACTTTGCC...AAACGGTTCGGTGTCTTTCTTTTGGACTGGTGGGATCACTGGCTCTAGTGGAGAA
TAGCGAGTCGATTTCCCAAGTTGGAGCGAATTTATCCGCACGACATGGCTGGCTGGCTGGTGTGTACACAGGGT
GCTTACCAATTTGATTTACCTGTTGAGGCGCGGTTGGCTCTCTGACTGCCCTTTTCACTGCCGTTTATTCGCCGG

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Vibrio alginolyticus
Vibrio parahaemolyticus
Vibrio sp.
Vibrio natrigiens
Vibrio splendidus
Vibrio pomeroiy
Vibrio tasmaniensis
Vibrio mimicus
Vibrio shilonii
Vibrio vulnificus
Vibrio neptunius

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100          110          120          130          140          150          160          170
ACA...GATAGTACCCTACTGCTATCTTATGCTCAAGTAGAGCGAATAAACCCAGCCGAAGCCAGCATC
TTG...GACGCTTCTGACCAATCGTTGAACCAAGGCGCCAGTAGTACC..TGAAAAGACACTGTGGCT...TC
GCA...GCCGATCTCCGAACAAAAGTAAAGCACAGTITTCGTAGTGAAAGAGCTCAGGTTACGCCGCAAAA...
GCA...GCCGATCTCCGAACAAAAGTAAAGCACAGTITTCGTAGTGAAAGAGCTCAGGTTACGCCGCAAAA...
ATTAC...AAGTTTCAAGAACCAAGTTAAGTGAACAGCAGCAACAAAGTCGGGCCGGGTTAAG...
GTA...AAAGTTCAAAGAACCAATGCAAGCTGACAGCAGCAGGCTCTGCTCAGACTCCAGTTAAAG...
ATT...AAAGTCTCTAAACTCATATCAAGTAGGCCAATAACCAATAGGCTAAGCTTAAACTGAAAG...
ATTG...ACGCTTATCGAAC...TCGAC...AGAACAGCGACTCTCAAGAGTACCACTAAGC...
GTTAA...CCGAGGTTTGGCTTTCGAGTTCTTGGAAATCAGACAGAGGCTTCGTTATGGCTCTGA...
GATTAAGGCACCTTCTTATTCGCGTATTCATGACAGATTCCTGGCTGAGAGATGTATGACGACTTCGCTT...TC
TGTAA...TTCCGGTTCATTTCTTGTAGCTTCTCTTCAGATTCCTCTGAAACGCTTTTITTTGAAAGTGTCTC...

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P

Vibrio alginolyticus
Vibrio parahaemolyticus
Vibrio sp.
Vibrio natrigiens
Vibrio splendidus
Vibrio pomeroiy
Vibrio tasmaniensis
Vibrio mimicus
Vibrio shilonii
Vibrio vulnificus
Vibrio neptunius

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180          190          200          210          220
TAACATCTTACTGGCTACGCGTGTCTATCTTTTGTTCCTGGCG...
TGCTGGTAATCATGGATTCACGGCTTATTTATTTTGGACATTACTAAGATTTGCTACTCTGTATGAAAC
GAAACCAATATGGTTGACACGGCTGATTTATTTGGTAGTATATGTTTCAGTTGGCTACTACTATTCAGGAA
GAAACCAATATGGTTGACACGGCTGATTTATTTGGTAGTATATGTTTCAGTTGGCTACTACTATTCAGGAA
...CAATATAGTGGTTAGGTTTCTGTTATGCTTGGGTTTCACTGCAATCTGTTTACATTTAATAAC
...CAATATAGTGGTTAGGTTTCTGTTATGCTTGGGTTTCACTGCAATCTGTTTACATTTAATAAC
...CTGGATTAATGGTTAAAGTTTGGAAAGTGGTTGGTTGGCTTTCCCTGCAATTTCAATTTAAGCGTAAAGAGCC
...GATTAATAGTGGGGATCTGGCTGATTTTGTGATCGGATGTTGATTCGTTAGCGTATGTTTAACTAACT
...TGAGCGCAGGCAAGAGCGGCTCAACTAGATATTAAGCTGATAGCC...GCITAGCAGCCCTCTTTAAGAA
CGGAGTTCTTCTTCAAGCGTTTCCAGTGGGCTGATTTGGTAAAG...GTTTCCAGCCTTTTGAAGTAT
ACCACCTCATATGGCTTTCGGCTCAACATACAGAGAGGCTGATAGCC...GTTTTCAGCCTTTTGAATAT

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R

Fig. S3 (continued) Sequences targeted by primer-probe set F2/Probe 2/R in the *toxR* genes of *Vibrio* species and other bacteria. GenBank numbers of the *toxR* genes of the species are EU155543.1, EU155587.1, NC_016613.1, FM999823.1, AY751344.1, AY751342.1, AY751343.1, AF170881.1, EU727208.1, KF322110.1, and HQ452617.1 (from top to bottom).

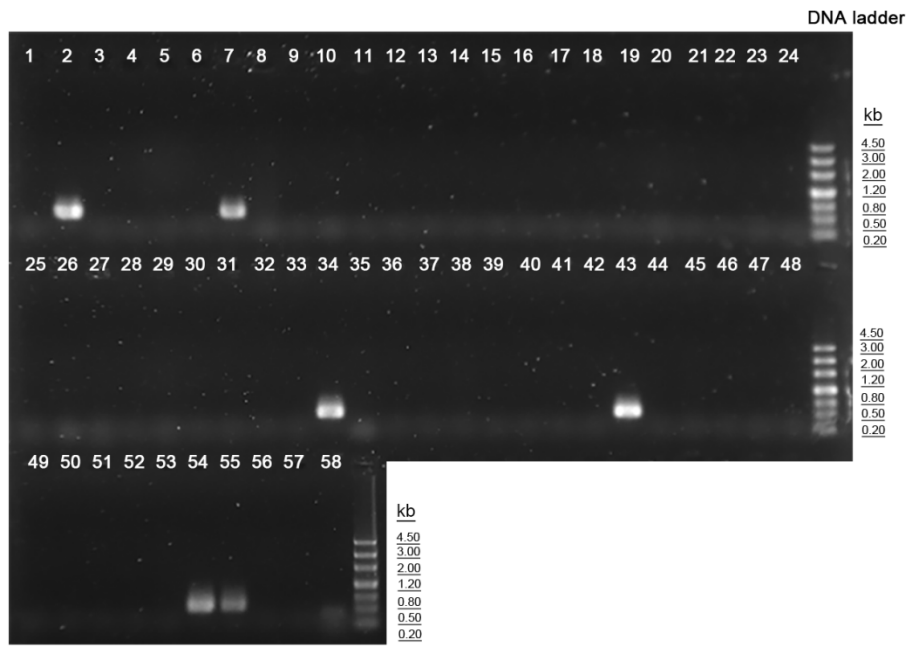


Fig. S4 Agarose gel image of the PCR detection of *V. alginolyticus* in shrimp samples. Sample numbers are indicated on the wells of the gel. The DNA ladder was run in the last lane of each row. The band sizes of the DNA ladder are shown on the right of each row.

Table S1 Strain information of bacteria used in this study and RPA-LFD detection results

Species	Source	strain	RPA-LFD detection results
<i>Vibrio alginolyticus</i>	Reference strain	ATCC 17749	+ (positive)
<i>V. alginolyticus</i>	Environmental strain	Nantong, China	+
<i>V. alginolyticus</i>	Environmental strain	Nantong, China	+
<i>V. alginolyticus</i>	Environmental strain	Nantong, China	+
<i>V. alginolyticus</i>	Environmental strain	Nantong, China	+
<i>V. alginolyticus</i>	Environmental strain	Nantong, China	+
<i>V. alginolyticus</i>	Environmental strain	Lianyungang, China	+
<i>V. parahaemolyticus</i>	Reference strain	ATCC 17802	- (negative)
<i>V. anguillarum</i>	Reference strain	ATCC 19264	-
<i>V. vulnificus</i>	Reference strain	ATCC 27562	-
<i>V. harveyi</i>	Reference strain	ATCC 43516	-
<i>V. mediterranei</i>	Reference strain	ATCC 43341	-
<i>V. shilonii</i>	Reference strain	ATCC BAA-91	-
<i>V. cholera</i>	Reference strain	ATCC 14100	-
<i>V. fischeri</i>	Reference strain	ATCC 700601D	-
<i>V. azureus</i>	Reference strain	MCCC 1A06651	-
<i>V. ichthyenteri</i>	Reference strain	MCCC 1A00057	-
<i>V. mimicus</i>	Reference strain	MCCC 1A02602	-
<i>V. splendidus</i>	Reference strain	MCCC 1A04096	-
<i>V. campbellii</i>	Reference strain	MCCC 1A02605	-
<i>V. chagasii</i>	Reference strain	MCCC 1B00386	-
<i>V. fluvialis</i>	Reference strain	MCCC 1A02761	-
<i>V. natriegens</i>	Reference strain	MCCC 1D00129	-
<i>Vibrio</i> sp.	Reference strain	MCCC 1A00047	-
<i>V. ponticus</i>	Reference strain	MCCC 1H00061	-
<i>V. rotiferianus</i>	Reference strain	MCCC 1B00068	-
<i>V. diabolicus</i>	Reference strain	MCCC 1D00126	-
<i>Listeria monocytogenes</i>	Reference strain	ATCC 19115	-
<i>Staphylococcus aureus</i>	Reference strain	ATCC 6538	-
<i>Escherichia coli</i> O157:H7	Reference strain	ATCC 43888	-
<i>Bacillus cereus</i>	Reference strain	ATCC 14579	-
<i>S. typhimurium</i>	Reference strain	ATCC 14028	-
<i>B. subtilis</i>	Reference strain	ATCC 6051	-
<i>Aeromonas veronii</i>	Reference strain	MCCC 1A00180	-
<i>Citrobacter freundii</i>	Reference strain	MCCC 1A00198	-
<i>Leuconostoc lactis</i>	Reference strain	MCCC 1A07814	-
<i>B. amyloliquefaciens</i>	Reference strain	ATCC 23842	-
<i>A. hydrophila</i>	Reference strain	MCCC 1A00007	-
<i>A. allosaccharophila</i>	Reference strain	ATCC 51208	-
<i>Bacillus</i> sp.	Reference strain	MCCC 1A00006	-
<i>Clostridium butyricum</i>	Reference strain	ATCC 3627	-