

Table S1 Bacterial strains and plasmids used in this study.

Strains or plasmids	Genotypes	Sources
Strains		
<i>E. coli</i>		
S17-1	[C600::RP4-2 (Tc::Mu)(Km::Tn7) <i>thi pro</i> <i>hsdRM</i> ⁺ <i>recA</i> , Tc ^r	(1)
S17-1 λ <i>pir</i>	S17-1 with λ <i>pir</i> lysogen	(1)
BL21(DE3)	F ⁻ <i>ompT hsdSB</i> (r _B ⁻ , m _B ⁻) <i>gal dcm</i> (DE3)	Novagen
<i>V. vulnificus</i>		
MO6-24/O	Pathogenic clinical isolate	(2)
KPR101	Derivative of MO6-24/O with a deletion in <i>rpoS</i>	(3)
Δ <i>leuO</i>	Derivative of MO6-24/O with a deletion in <i>leuO</i>	(4)
Δ <i>vhuAB</i>	Derivative of MO6-24/O with double in frame deletions in <i>vhuAB</i>	This study
Δ <i>leuO</i> Δ <i>vhuAB</i>	Derivative of MO6-24/O with triple in frame deletions in <i>vhuAB</i> and <i>leuO</i>	This study
Plasmids		
pDM4	Suicide vector for allelic exchange, <i>sacB</i> , Cm ^r	(5)
pDM- <i>vhuA</i> KO	pDM4 with the in frame 79 amino acid deletion (bp +19 to +255 from the start codon) of <i>vhuA</i> of <i>V. vulnificus</i>	This study
pDM- <i>vhuB</i> KO	pDM4 with the in frame 79 amino acid deletion (bp +19 to +255 from the start codon) of <i>vhuB</i> of <i>V. vulnificus</i>	This study
pRK415	IncP <i>ori</i> , broad-host-range vector; <i>oriT</i> of RP4, Tc ^r	(6)

pRK-vhuAB	pRK415 with <i>V. vulnificus</i> <i>vhuAB</i> operon with the promoter region	This study
pMZtc	pDM4 with the promoter-less <i>lacZ</i> gene for transcriptional fusion	This study
pRE1-leuO	<i>leuO</i> expression vector	(4)
pMZtc-katG	pMZtc with the promoter region of <i>katG</i>	This study
pMZtc-vhuA	pMZtc with the promoter region of <i>vhuA</i>	This study
pMZtc-vhuB	pMZtc with the promoter region of <i>vhuB</i>	This study
pMZtc-leuO	pMZtc with the promoter region of <i>leuO</i>	This study
pMZtc-rpoS	pMZtc with the promoter region of <i>rpoS</i>	This study
pRZtl-rpoS	pRK415 vector with <i>rpoS-lacZ</i> fusion in-frame	This study
pET14b	Expression vector, N-terminal His-tag, Ap ^r	Novagen
pET-rpoS	pET14b vector with <i>V. vulnificus</i> <i>rpoS</i> gene	This study
pBBR1-MCS2	Broad host range expression vector, Km ^r	(7)
pBBR12-vhuAB	pBBR1-MCS2 with <i>V. vulnificus</i> <i>vhuAB</i> operon with the promoter region	This study
pBBR12-leuO	pBBR1-MCS2 with the <i>leuO</i> operon of <i>V. vulnificus</i>	This study
pBBR12-leuO-ara	pBBR-MCS2 vector with the <i>V. vulnificus</i> <i>leuO</i> under the <i>araC</i> promoter	This study

References

1. **Simon R, Priefer, U., Pühler, A.** 1983. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram negative bacteria. *Nature Biotechnol* **1**:784-791.
2. **Reddy GP, Hayat U, Abeygunawardana C, Fox C, Wright AC, Maneval DR, Jr., Bush CA, Morris JG, Jr.** 1992. Purification and determination of the structure of capsular polysaccharide of *Vibrio vulnificus* M06-24. *J Bacteriol* **174**:2620-2630.
3. **Park KJ, Kang MJ, Kim SH, Lee HJ, Lim JK, Choi SH, Park SJ, Lee KH.** 2004. Isolation and characterization of *rpoS* from a pathogenic bacterium, *Vibrio vulnificus*: role of sigma^S in survival of

exponential-phase cells under oxidative stress. *J Bacteriol* **186**:3304-3312.

4. **Kim JA, Park JH, Lee MA, Lee HJ, Park SJ, Kim KS, Choi SH, Lee KH.** 2015. Stationary-phase induction of *vvpS* expression by three transcription factors: repression by *LeuO* and activation by *SmcR* and *CRP*. *Mol Microbiol* **97**:330-346.
5. **Milton DL, O'Toole R, Horstedt P, Wolf-Watz H.** 1996. Flagellin A is essential for the virulence of *Vibrio anguillarum*. *J Bacteriol* **178**:1310-1319.
6. **Keen NT, Tamaki S, Kobayashi D, Trollinger D.** 1988. Improved broad-host-range plasmids for DNA cloning in gram-negative bacteria. *Gene* **70**:191-197.
7. **Kovach ME, Elzer PH, Hill DS, Robertson GT, Farris MA, Roop RM, 2nd, Peterson KM.** 1995. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* **166**:175-176.

Table S2 Primers used in this study.

Name	Nucleotide sequence (5' to 3') ^a
Construction of a <i>vhuAB</i> in-frame deletion mutant	
<i>vhu</i> α -koF1	GTACTCGAGGGGTCGACCAGACAAAGGGTGTTAGG
<i>vhu</i> α -koB1	CAATTAGTTTACTGAGGTCTTGTTCATTAGGTTTC
<i>vhu</i> α -koF2	CTAATGAACAAGACCTCAGTAAACTAATTGCTCTG
<i>vhu</i> α -koB2	TATCGATACCGTCGACAGTAACGTTTCTACTTCC
<i>vhu</i> β -koF1	GTACTCGAGGGGTCGACACGGAAGTCGGTGGCGATT
<i>vhu</i> β -koB1	GATTAGTTACAAGCAGTTTTTGTTCACTGTGATT
<i>vhu</i> β -koF2	ACAGTGAACAAAAGTCTTGTAATAATCGCGATA
<i>vhu</i> β -koB2	TATCGATACCGTCGACGAATCAGTTCACGACGCA
Construction of pMZtc	
pDM4-lacZF	CGGGTAACCTGAGCTCATCGAGCAACATATTAATGAG
pDM4-lacZB	AATCCCGGGAGAGCTCACATAATGGATTCCTTACG
Cloning of <i>vhuAB</i>	
pRK- <i>vhu</i> α -comF	GACGGCCAGTGAATTCCTCAATCACATCTGCCG
pRK- <i>vhu</i> α -comB	TACCGAGCTCGAATTCGGTGCAGAGCAATTAGT
pRK- <i>vhu</i> β -comF	TAGAGTCGACCTGCAGCAAAAATAAGTAAAA
pRK- <i>vhu</i> β -comB	GCTTGCATGCCTGCAGTTCGGCTTTCACATCGCA
pBBR12- <i>vhu</i> comF	TAGAACTAGTGGATCCCTCAATCACATCTGCCGTG
pBBR12- <i>vhu</i> comB	GCTTGATATCGAATTCAAACGGGTTTGATTGAAC
Construction of <i>lacZ</i> fusion	
<i>katG</i> -tcF	CCTTCTAGATAGATCTCGAGTTTTAGTCACGAC
<i>katG</i> -tcB	CCCGCATGCAAGATCTGCATTACTGGGCATTGGCC
<i>vhu</i> α -scF	CCTTCTAGATAGATCTTGTGCACCCGCTGTTCGAG
<i>vhu</i> α -scB	CCCGCA TGCAAGATCTTCAAGAGCAGCTTTCGCT
<i>vhu</i> β -scF	CCTTCTAGATAGATCTAGCCTAACGGGTAACCC
<i>vhu</i> β -scB	CCCGCATGCAAGATCTAGAGCACGACCTGCTG

RpoS-scF	CCTTCTAGA <u>AGATCT</u> AGCCAAGTGGCAGTGGCCA
RpoS-sctcB	CCCGCATGCA <u>AGATCT</u> GATTAACATCGAAATCT
leuO-scF	<u>CTCGAGTTCGGCGTGGGCTG</u>
leuO-scB	<u>TCTAGACTGTGACATGCCTA</u>
pRZtl-rpoSF	CTAGAGTCGAC <u>CTGCAGACT</u> CCCAAACCAACACCGAC
pRZtl-rpoSB	CGTTGTAAAACGACGGGCGTTGAAACCTCTGGGTCAG
rpoS-lacZtlF	CCAGAGGTTTCAACGCCCGTCGTTTTACAACGTCGTG
rpoS-lacZtlB	AGCTTGCATGC <u>CTGCAGTACATAATGGATTTCCTTAC</u>

Construction of pBBR12-leuO and pBBR12-leuO-ara.

leuO_comP_F_xhoI	<u>CTCGAGCGCTAATATTGACGAG</u>
leuO_comP_R_kpnI	<u>GGTACCTTATACCGCAGCAACCAC</u>
BAD-leuOF	ATGTTAGATAAAA AAAGATGC
BAD-leuOB	TTATACCGCAGCAACCACTTC
BBR12-bad-leuOF	GCTTGATATCGA <u>AATTCGCAACGTTCAAATCCG</u>
BBR12-bad-leuOB	CGGGCTGCAGGA <u>AATTCGGCATGCATAATGTGCCTG</u>

Electrophoresis mobility shift assay

vh α -EMSAF	TGCCCTTGAAGAAATCCCTC
vh α -EMSAB	TGCTTTCTCTGCGATAAAGTC
vh β -EMSAF	GAGTTTGACGCTAAAAAATAG
vh β -EMSAB	ATCTGCGTTTGCAGCGATTG

Construction of RpoS

rpoS-ndeI	GCGCGGCAGCC <u>CATATGAGTATCAGCAACACAGTCACC</u>
rpoS-bamHI	GTTAGCAGCC <u>GGATCCTTTCTATTGGAGAATGCAAAT</u>

qRT-PCR

V. vulnificus

RpoS-RTF	TTTGATGCCAGCAACAAGAG
RpoS-RTB	TACTGTAACGGCGGGAAATC
clpX-qRTF	GCTGGTCCATCCGTTTACAT

clpX-qRTB	CCATCACTCGTTGTGTCACC
rssB-qRTF	GATCTTTCGCCGAATTACCA
rssB-qRTB	AACCACAATCAAAGGCAAGG
dksA-qRTF	AGGTGTGGAGCCATATCAGG
dksA-qRTB	GGTCAACTGGGTCAGGAAAA
hfq-qrtF	ATGGCTAAGGGGCAATCTCT
hfq-qrtB	GCTGTGGTGACTCACTGGAC
hns-qrtF	TGTTGAACATTCGCAGCCTA
hns-qrtB	TCTGCGATAGCCGCTAGTTT
vvpE-RTF	TGTCCAGGAGCCAGCAATTA
vvpE-RTB	ACCATCGCCAAATGTCATCG
gabD-RTF	CCCGTAGGCCAAACTGTTGAT
gabD-RTB	AACATAAAGGCGGTTTGCAC
aldB-RTF	ATTGCCAAACTGGCGTTTAC
adlB-RTB	GTACACACTTCGCCCTGGTT
gapNAD-RTF	CGTATCGGTCGTTTCGTTTT
gapNAD-RTB	TACGTCAACACCGATTGCAT
<i>V. cholerae</i>	
Vc_hu α -RTF	GCAGCTCTTGAAGCGACTCT
Vc_hu α -RTB	AGCGCTTTACCTGCTACGAA
Vc_hu β -RTF	AGAACAATTGCAGCGAACG
Vc_hu β -RTB	TTCGCCAGTTTTTGGGTTAC
Vc_rpoS-RTF	GTGCGGTTGAGAAATTCGAT
Vc_rpoS-RTB	TGGTGTAGGTTTCGTGGTCAA
Vc_leuO-RTF	GGCGCGTTTAAAAGTGATGT
Vc_leuO-RTB	GAGGAGCAAAGCGTAAATCG
Vc_gap-RTF	TTTAAGAGCTTCGTTTGATTGGCC
Vc_gap-RTB	TTTTTCTTGTGTTGTGCGAATGCG

V. parahaemolyticus

Vp_hu α -RTF	AGCTGCTCTTGAAGCGACTC
Vp_hu α -RTB	AGCGCTTTACC GCTACGAA
Vp_hu β -RTF	AATCGCAGAAAATGCAGACA
Vp_hu β -RTB	GCGATTTGGATTTCTTCACC
Vp_rpoS-RTF	CCATTCGCTTGCCTATTCAT
Vp_rpoS-RTB	CCACCAATTGGGGTATCAAC
Vp_leuO-RTF	GCTGTGATGCAAGAGCAAAA
Vp_leuO-RTB	ATAGGGCCAAACAGTTGACG
Vp_gap-RTF	AGTGCAGGTTGAGGGAAATG
Vp_gap-RTB	ACGCCCTGATCGAGATATTG

^a Nucleotides modified for the generation of restriction sites or for site-directed mutagenesis are underlined.

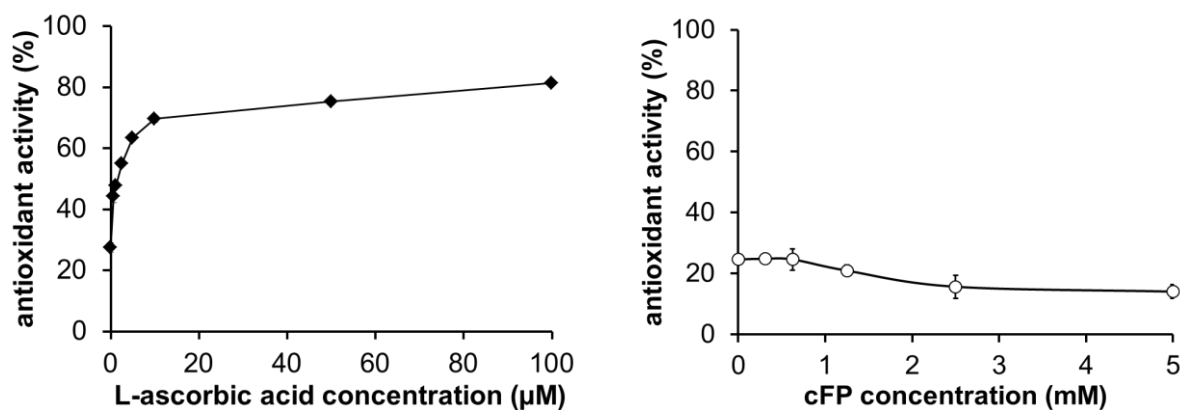


Figure S1. cFP does not have scavenging activity of DPPH radical. Scavenging activity of DPPH radical by various concentrations of L-ascorbic acid (positive control, left side) and cFP (0, 0.3125, 0.625, 1.25, 2.5, 5 mM, right side). Ascorbic acid solution and cFP solution was prepared in distilled water, and DMSO respectively. Anti-oxidant activity of cFP was measured by assessing the ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described previously (Brandwilliams et al., 1995. Food Sci Technol-Leb 28:25-30) with some modifications. Briefly, freshly prepared 100 µl of 30 µM DPPH in DMSO solution was thoroughly mixed with same volume of various concentrations of cFP (0, 0.3125, 0.625, 1.25, 2.5, 5 mM). Optical absorbance of the resultant mixtures were measured at 517 nm using a biophotometer (Eppendorf, Hamburg, Germany) after being incubated for 15 min at room temperature in dark condition. The percentage of scavenging activity was calculated as $[1 - (A_i - A_j) / A_c] \times 100$ (A_i , A_j , and A_c are A_{517} values of sample mixture, each cFP sample without DPPH, and control sample with only DPPH, respectively). Scavenging activity was calculated as described in Materials and Methods. Error bars denote standard deviation of three independent experimental data.