Table S3. Oligonucleotides used in this study

Strain/Gene [1]	Primer name	Sequence (5'->3'; Restriction sites underlined)
For construction of deletion mutants		
Δ*flaABCD*	flaA-upF	ACGCGTCGACGCTAGCGACGTACATCAACGGTC
	flaA-upR	AACTGCAGGCTGTGCGGTCATTGCTGACACG
	flaA-downF	AACTGCAGTCTTAGCTTGTTGGGCTAATACAG
	flaA-downR	GCTCTAGAGACAACTTTGCTGACCATGGAG
	flaB-upF	CCGCTCGAGTGCGAAAGCCGGCGATGATATCGA
	flaB-upR	CGGGATCCTCACTGCCATGATGAATTCTCC
	flaB-downF	CGGGATCCCTCAGCGCTAAGTCTACTAGGCT
	flaB-downR	CGAGCTCGATGTTGGTGGTCTCTGTCATTGC
	flaC-upF	CCCTCGAGCATTCACTGCCATGATGAATTCTCC
	flaC-upR	CGGGATCCCTGATTTTGCGAAGGAAACGACGC
	flaC-downF	CGGGATCCCGTTAGTGCTTACTGTTACAGCC
	flaC-downR	CGAGCTCTCGGTGTCTGATGCCAATGCC
	flaD-upF	ACGCGTCGACCTGTCTCCAGGCTTAAGCGAGATC
	flaD-upR	CGGGATCCGCCTACTAGGCTAATCTCTGAGC
	flaD-downF	CGGGATCCCGCTGTGCTGTCATTGCTGCTACG
	flaD-downR	GCTCTAGAGCTCTCTCATCTCTCACCTGC
Δ*flaEF*	flaE01-upF	ACGCGTCGACCGCAGAGCACTCTAAGTAATCTGC
	flaE01-upR	CGGGATCCGCCCCAGGCAGTGCAATTGGATTGC
	flaE01-downF	CGGGATCCGCCTCTGAGCGACCATCGCAGAC
	flaE01-downR	CGAGCTCGACATGCGCTCTGACAACGTG
	flaF-upF	CCGCTCGAGGCGCCTTCAACGGATGTGGATGTG
	flaF-upR	CGGGATCCCGGTGATCGCCACAACTCTTGGTCC
	flaF-downF	CGGGATCCGGGATAAGCGCCGGAGTGACTGG
	flaF-downR	CGAGCTCGCAATCGTCGTCAAGTCGTTGTCGCC
flaE::*nptI*	flaE-up	ACGCGTCGACGTGAGCAACCTAAGATAGCAATCC
	flaE-down	CGAGCTCGGAGAGGGATATCGATTAAATGG
Δ*flaF*	flaF-upF	CCGCTCGAGGCGCCTTCAACGGATGTGGATGTG
	flaF-upR	CGGGATCCCGGTGATCGCCACAACTCTTGGTCC

33		flaF-downF	CGGGATCCGGGATAAGCGCCGGAGTGACTGG
34		flaF-downR	CGAGCTCGCAATCGTCGTCAAGTCGTTGTCGCC
35	Δ*flaJ*	flaJ-upF	CGTCGACGATCCTACGACCGGTCAAAA
36		flaJ-upR	CGGGATCCCCGCGCATAGTATTCCTCTT
37		flaJ-downF	CGGGATCCGGCTGCGGATATTGGAATGT
38		flaJ-downR	GCTCTAGAAAGAGGGTATTCTTACGTGC
39	Δ*flhA*	flhA-upF	AGTCGGGCCCGGCGGACGTTCCGACCGCGG
40		flhA-upR	AACTGCAGGGCTGGTACTGAACGTTGGGGG
41		flhA-downF	AACTGCAGGGCGACTCAGGAGCAAGAGCTC
42		flhA-downR	GGACTAGTCGTCTTTCCACTTCCTGCCAC
43	**For construction of complementation plasmids**		
44	*flaE*	flaE-comF	CGGGATCCATGGTTTCACTCAATACCAACGTGTCTGC
45		flaE-comR	GGGGTACCGAGCAACCTAAGATAGCAATCCAATTGC
46	*flaF*	flaF-comF	CCCAAGCTTCAGGACCAATATTTGTGGCG
47		flaF-comR	CGGGATCCGTCACTCCGGCGCTTATCCC
48	*flhA*	flhA-comF	CCCAAGCTTATGGCTAAGAAATTTACTCTGCCG
49		flhA-comR	CGGGATCCTCAGTTACCCACAGCCTGTAC
50	**For construction of *luxAB*-based transcription reporters**		
51	*flaA*	flaA_p-F	GGGGTACCCGTCGAAGAGCCGAATCAAAGATAC
52		flaA_p-R	GCTCTAGAGCGGTCATTGCTGACACGTTAGTG
53	*flaB*	flaB_p-F	GGGGTACCCAGATTATGCGAAGGAAACCACGC
54		flaB_p-R	GCTCTAGAGGTAACGCTGTGCTGTCATTGC
55	*flaC*	flaC_p-F	GGGGTACCCGCAGCTCGCATTTGGTAAAAC
56		flaC_p-R	GCTCTAGAGCGGATACGTTAGTGCTTACTG
57	*flaD/E*	flaD_p-F	GGGGTACCGGCAGGTACTTCTATTCTTGCTC
58		flaD_p-R	GCTCTAGACTGTGCTGTCATTGCTGCTACG
59	*flaE*	flaE_p-F	GGGGTACCGGACAACATCAACGAGAACGTGAAC
60		flaE_p-R	GCTCTAGAGCGACCATCGCAGACACGTTGG
61	*flaF*	flaF_p-F	GGGGTACCGGGCTTAAGATTGTTCGTCACAGC
62		flaF_p-R	GCTCTAGAGCGACAAGTGCTGCCACATTGG
63	**For construction of overexpression plasmids for recombinant proteins**		
64	rFlaA	flaAexp-F	CGGGATCCATGCGCGGTTCATTACAGGC

65 flaAexp-R CCCAAGCTTTCACGCCGCTGTATTAGC

66 rFlaB flaBexp-F CGGGATCCATGGCAGTGAATGTAAATACAAACG

67 flaBexp-R AACTGCAGTCTGTCTAGTTAAGGCGATTAGCC

68 rFlaC flaCexp-F CGGGATCCATGACTGCGCAACGTTATCTAAACAAAGCG

69 flaCexp-R AACTGCAGCAGACACAGGATTACTATTAGCCC

70 rFlaE flaEexp-F CGGGATCCATGGTTTCACTCAATACCAACG

71 flaEexp-R GGGGTACCGTGAGCAACCTAAGATAGCAATCC

72 rFlaF flaFexp-F CGGGATCCGTGGCGATCACCGTTAATACCAATGTGG

73 flaFexp-R AACTGCAGAGTCACTCCGGCGCTTATCCCAGC

74 rFlaA$_{vc}$ vcflaA-F CGGGATCCATGACCATTAACGTAAATACC

75 vcflaA-R CCGAGCTCCTACTGCAATAACGAGATTGCAGAGTTTGG

76 rFlaE$_{vc}$ vcflaE-F CGGGATCCATGGCCATGACGGTAAATACC

77 vcflaE-R CCCAAGCTTTTAATTACGCAGCAAAAACAGCAC

78 rFlaC$_{vp}$ vpflaC-F CGGGATCCATGGCTGTAACAGTTAGTACTAACG

79 vpflaC-R CCCAAGCTTCTACAATAGTGACATTGC

80 rFlaE$_{vp}$ vpflaE-F CGGGATCCATGGTCTCTTTAAATACCAATGTTGCCGC

81 vpflaE-R CCCAAGCTTTCAACTGAGCAAGCCTAGTGC

82 rFlaF$_{vp}$ vpflaF-F CGGGATCCTTGGCTATCACCGTTAATACC

83 vpflaF-R CCCAAGCTTCTAGCCAAGCAAGGTAAGAGC

84 **For construction of Bacterial Two-Hybrid system plasmids**

85 *flaB* pKT25-FlaB-F AACTGCAGGGATGGCAGTGAATGTAAATAC

86 pKT25-FlaB-R GATCGGTACCTTAGCCTAGTAGACTTAGCGCTG

87 *flaE* pKT25-FlaE-F GATCTCTAGAGATGGTTTCACTCAATACCATCGTG

88 pKT25-FlaE-R GATCGGTACCTAAGATAGCAATCCAATTGC

89 *flaF* pKT25-FlaF-F GATCTCTAGAGGTGGCGATCACCGTTAATACC

90 pKT25-FlaF-R GATCGGTACCTTATCCCAGCAAGGTCAACGC

91 *flgL* pKT25-FlgL-F GCTCTAGAGATGATTAGCCGTATCGCCAGTTTCCAC

92 pKT25-FlgL-R GGGGTACCCCGTCATCTCATTCAGAGACGGTTCG

93 *fliD* pKT25-FliD-F GCTCTAGAGATGAGTTTAGGCCCTTTGGGG

94 pKT25-FliD-R GGGGTACCGTCATGCGTTACTATCCCAGAGCG

95 *flaB* pUT18c-FlaB-F AACTGCAGTGGAGAATTCATCATGGCAGTG

96 pUT18c-FlaB-R CGGGATCCGTCTAGTTAAGGCGATTAGCC

flaE	pUT18c-FlaE-F	GATCTCTAGAGATGGTTTCACTCAATACCATCGTG
	pUT18c-FlaE-R	GATCGGTACCTAAGATAGCAATCCAATTGC
flaF	pUT18c-FlaF-F	GATCTCTAGAGGTGGCGATCACCGTTAATACC
	pUT18c-FlaF-R	GATCGGTACCTTATCCCAGCAAGGTCAACGC
For RT-PCR of an *flaDE* transcript		
flaD	flaD-F	CCATGCAATCAGCAACTTGGA
	flaD-R	TTAGCCTAGTAGGCTTAGCGCTG
flaE	flaE-F	ATGGTTTCACTCAATACCAACGTG
	flaE-R	TTTTTTTGGGTTTCAGCTAC

[1] Detailed procedures for mutant construction

(i) Construction of *flaABCD* deletion mutant. A *flaB* upstream region of 820-bp was amplified from the genomic DNA of *V. vulnificus* MO6-24/O using two primers, flaB-upF and flaB-upR. The PCR product was then cloned into a plasmid, pBlueScript SKII(+) to produce pMflaB01. A 550-bp DNA fragment containing downstream region of the *flaB* gene was made using primers flaB-downF and flaB-downR, and cloned into the corresponding sites of pMflaB01 to result in pMflaB02. Then, 1.2-kb kanamycin resistance gene was isolated from pUC4K (Pharmacia) and inserted into the BamHI site of pMflaB02 to produce pMflaB03. A 2,570-bp DNA fragment of pMflaB03 digested with ApaI and SacI was ligated to a suicide vector, pDM4 (1), to generate pMflaB04. *E. coli* SM10λ*pir* strain carrying pMflaB04 was conjugated with *V. vulnificus* MO6-24/O, and the exconjugants were selected on the thiosulfate citrate bile sucrose (TCBS) medium supplemented with 4 μg/ml chloramphenicol (2). Colonies with characteristics indicating a double homologous recombination event were isolated (resistance to 5% sucrose, sensitivity to chloramphenicol, and resistance to kanamycin) (2). Deletion of *flaB* gene in candidate colonies was confirmed by PCR with primers, flaB-upF and flaB-downR, and named Δ*flaB*.

A 821-bp PCR product containing the *flaD* upstream region was amplified using primers, flaD-upF and flaD-upR, and then cloned into pBlueScript SKII(+) to produce pMflaD01. A 552-bp PCR product was made to contain downstream region of *flaD* gene using flaD-downF and flaD-downR, and cloned into the corresponding sites of pMflaD01 to produce pMflaD02. The ApaI-XbaI DNA fragment of pMflaD02 was ligated into pDM4 to produce pMflaD03. The resultant plasmid in *E. coli* SM10λ*pir* strain was mobilized to Δ*flaB*, and the exconjugants were selected as described above. Colonies with characteristics indicating a double homologous recombination event were isolated as described above. Deletion of *flaD* gene in candidate colonies was confirmed by PCR with primers,

130 flaD-upF and flaD-downR, and named Δ*flaBD*.

131 A *flaC* upstream region of 905-bp was amplified using two primers, flaC-upF and flaC-upR. PCR
132 product was cloned into pBlueScript SKII(+) to produce pMflaC01. A 780-bp DNA fragment
133 containing downstream region of *flaC* gene was amplified using primers flaC-downF and flaC-downR,
134 and cloned into the corresponding site of pMflaC01 to produce pMflaC02. A 1,190-bp DNA fragment
135 of pMflaC02 digested with ApaI and SacI was ligated to pDM4, to generate pMflaC03. *E. coli*
136 SM10λ*pir* strain carrying pMflaC03 was conjugated with Δ*flaBD* and the exconjugants were selected
137 as described above. Colonies with characteristics indicating a double homologous recombination
138 event were isolated as described above. Deletion of *flaC* gene in candidate colonies was confirmed by
139 PCR with primers, flaC-upF and flaC-downR, and named Δ*flaBDC*.

140 A 780-bp PCR product containing the *flaA* upstream region was amplified using flaA-upF and flaA-
141 upR, and then cloned into pBlueScript SKII(+) to produce pMflaA01. A 600-bp PCR product was
142 made to contain downstream region of *flaA* gene using flaA-downF and flaA-downR, and cloned into
143 the corresponding sites of pMflaA01 to produce pMflaA02. The SalI-XbaI DNA fragment of
144 pMflaA02 was ligated into pDM4 to produce pMflaA03. The resultant plasmid in *E. coli* SM10λ*pir*
145 strain was mobilized to Δ*flaBDC*, and the exoconjugants were selected as described above. Colonies
146 with characteristics indicating a double homologous recombination event were isolated as described
147 above. Deletion of *flaA* gene in candidate colonies was confirmed by PCR with primers, flaA-upF and
148 flaA-downR, and named Δ*flaABDC*.

149 (ii) Construction of *flaE*::*nptI* mutant. A flanking region of the *flaE* (1,156-bp) was amplified from the
150 genomic DNA of *V. vulnificus* MO6-24/O using the following two primers: flaE-up and flaE-down.
151 The PCR product was the cloned in to pBlueScript SKII(+) to produce pMflaE01. A 1.2-kb
152 kanamycin resistance gene was isolated from pUC4K and inserted into the PstI site of pMflaE01 to
153 produce pMflaE02. About a 2.4-kb DNA insert from pMflaE02, digested with ApaI and SacI, was
154 ligated to a suicide vector pDM4 to generate pMflaE03. An *E. coli* SM10λ*pir* strain carrying
155 pMflaE03 was conjugated with *V. vulnificus* MO6-24/O and the exconjugants were selected on LBS
156 medium supplemented with kanamycin. Colonies with characteristics indicating a double homologous
157 recombination were further confirmed by PCR using flaE-up and flaE-down, and named strain
158 *flaE*::*nptI*. For complementation of the *flaE* mutant, a 1,135-bp DNA fragment containing *V. vulnificus*
159 *flaE* gene, which had been amplified using two primers, flaE-comF and flaE-comR, was cloned into a
160 broad-host-range plasmid pRK415. The resultant plasmid, pRK415-flaE, was transformed into *E. coli*
161 SM10λ*pir* and then transferred to *flaE*::*nptI V. vulnificus* by conjugation.

162 (iii) Construction of *flaF* deletion mutant. A 780-bp PCR product containing the *flaF* upstream region

163 was amplified using primers, flaF-upF and flaF-upR, and then cloned into pBlueScript SKII(+) to
164 produce pMflaF01. A 910-bp PCR product was made to contain downstream region of *flaF* gene
165 using flaF-downF and flaF-downR, and cloned into the corresponding sites of pMflaF01 to produce
166 pMflaF02. *nptI* encoding a kanamycin resistance enzyme was isolated from pUC4K, and inserted into
167 pMflaF02 to generate pMflaF03. The ApaI-SacI DNA fragment of pMflaF03 was ligated into pDM4
168 to produce pMflaF04. The resultant plasmid in *E. coli* SM10λ*pir* strain was mobilized to *V. vulnificus*
169 MO6-24/O, and the exconjugants were selected as described above. Colonies with characteristics
170 indicating a double homologous recombination event were isolated as described above. Deletion of
171 *flaF* gene in candidate colonies was confirmed by PCR with primers, flaF-upF and flaF-downR, and
172 named Δ*flaF*. For complementation of the *flaF* mutant, a 1,161-bp DNA fragment containing *V.*
173 *vulnificus flaF* gene, which had been amplified using flaF-comF and flaF-comR, was cloned into
174 pRK415. The resultant plasmid, pRK415-flaF, was transformed into *E. coli* SM10λ*pir* and then
175 transferred to Δ*flaF V. vulnificus* by conjugation.

176 (iv) Construction of *flaEF* deletion mutant. A 768-bp PCR product containing the *flaE* upstream
177 region was amplified using primers, flaE01-upF and flaE01-upR, and then cloned into pBlueScript
178 SKII(+) to produce pMflaE_01. A 854-bp PCR product was made to contain downstream region of
179 *flaE* gene using flaE01-downF and flaE01-downR, and cloned into the corresponding site of pflaE_01
180 to produce pMflaE_02. The SalI-SacI DNA fragment of pMflaE_02 was ligated into pDM4 to
181 produce pMflaE_03. The resultant plasmid in *E. coli* SM10λ*pir* strain was mobilized to *V. vulnificus*
182 Δ*flaF*, and exconjugants were then selected as described above. Colonies with characteristics
183 indicating a double homologous recombination event were isolated as described above. Deletion of
184 *flaE* gene in candidate colonies was confirmed by PCR with primers, flaE01-upF and flaE01-downR,
185 and named Δ*flaEF*. For complementation of *flaE* or *flaF*, *E. coli* SM10λ*pir* containing pRK415-flaE
186 or pRK415-flaF was mobilized to Δ*flaEF V. vulnificus* by conjugation, respectively.

187 (v) Construction of *flaJ* deletion mutant. A 806-bp *flaJ* upstream region (from -798 to +8 relative to its
188 IC) was amplified from the genomic DNA of *V. vulnificus* MO6-24/O using primers, flaJ-upF and
189 flaJ-upR. The PCR product was cloned into pBlueScript SKII(+) to produce pSKflaJ01. A DNA
190 fragment containing 651-bp downstream of the *flaJ* ORF (from +390 to +1,040 relative to its IC) was
191 generated using primers *flaJ*-downF and *flaJ*-downR, and cloned into the corresponding sites of
192 pSKflaJ01 to produce pSKflaJ02. A 1,457-bp DNA fragment from pSKflaJ02, digested with SalI and
193 XbaI, was ligated to a pKAS32 (3). pKAS-flaJ in *E. coli* SM10λ*pir* was mobilized to a *V. vulnificus*,
194 and the conjugates were selected by plating the conjugation mixture of *E. coli* and *V. vulnificus* on
195 LBS supplemented with 4 μg/ml of ampicillin. *V. vulnificus* colonies with characteristics of double
196 homologous recombination event were further confirmed by PCR using primers, flaJ-upF and flaJ-

197 downR. The confirmed cell was named Δ*flaJ*.

198 (vi) Construction of *flhA* deletion mutant. A *flhA* upstream region of 590-bp was amplified from the
199 genomic DNA of *V. vulnificus* MO6-24/O using two primers, flhA-upF and flhA-upR. The PCR
200 product was then cloned into a plasmid, pBluescript SKII(+) to produce pYflhA01. A 890-bp DNA
201 fragment containing downstream region of the *flhA* gene was made using primers flhA-downF and
202 flhA-downR, and cloned into the corresponding sites of pYflhA01 to result in pYflhA02. Then, 1.2-
203 kb kanamycin resistance gene was isolated from pUC4K, and inserted into the BamHI site of
204 pYflhA02 to produce pYflhA03. A 2,680-bp DNA fragment of pYflhA03 digested with ApaI and SpeI
205 was ligated to a suicide vector, pDM4, to generate pYflhA04. *E. coli* SM10λ*pir* strain carrying
206 pYflhA04 was conjugated with *V. vulnificus* MO6-24/O, and the exconjugants were selected as
207 described above. Candidate mutant colonies were confirmed by PCR using primers, flhA-upF and
208 flhA-downR, and named Δ*flhA*. For complementation of the *flhA* mutant, a 2,103-bp DNA fragment
209 containing *V. vulnificus flhA* gene, which had been amplified using two primers, flhA-comF and flhA-
210 comR, was cloned into pRK415. The resultant plasmid, pRK415-flhA, was transformed into *E. coli*
211 SM10λ*pir* and then transferred to Δ*flhA* by conjugation.

212 (vii) References

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