Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or description	Reference or source
E. coli		
DH5α	F^- Φ80d*lacZ* ΔM15 Δ(*lacZYA-argF*)U169 *deoR recA*1 *endA*1 *hsdR*17(r_K^-, m_K^+) *phoA supE*44 λ^- *thi*-1 *gyrA*96 *relA*1	
BL21(DE3)	F^- *ompT hsd*S_B (r_B^- m_B^-) *gal dcm* (DE3)	Novagen
V. alginolyticus		
VIO5	VIK4 (Rif^r Pof^+ $Laf^–$)	(1)
LPN1	VIO5 Δ*flhF* (Rif^r Pof^+ $Laf^–$)	(2)
NMB196	VIO5Δ*fliF* (Rif^r Pof^+ $Laf^–$)	(3)
Plasmids		
pCold I	Amp^r, P_{cspA}	Takara
pMMB206	Cm^r, P_{tac} P_{lac} UV5	(4)
pBAD33	Cm^r, P_{BAD}	(5)
pTY60	Km^r, P_{BAD}	(6)
pTY57	Cm^r, P_{BAD}	(7)
pRO101	pCold I-*his-fliF*	(8)
pRO101-*ΔN30*	pRO101-*his-fliF(31-580)*	This study
pRO101-*ΔN50*	pRO101-*his-fliF(51-580)*	This study
pRO101-*ΔC83*	pRO101-*his-fliF(1-497)*	This study
pRO101-*ΔC110*	pRO101-*his-fliF(1-470)*	This study
pTSK137	pCold I-*his-fliFG*	(9)
pTSK122	pMMB206-*flhF*	(9)
pTY502	pTY57-*fliF*	(8)
pTY502-*ΔN30*	pTY57-*fliF(31-580)*	This study
pTY502-*ΔN50*	pTY57-*fliF(51-580)*	This study
pTY502-*ΔC83*	pTY57-*fliF(1-497)*	This study
pTY502-*ΔC110*	pTY57-*fliF(1-470)*	This study
pHHT103	pBAD33-*his-fliF*	(9)
pHHT103-*ΔN30*	pBAD33-*his-fliF(31-580)*	This study
pHHT103-*ΔN50*	pBAD33-*his-fliF(51-580)*	This study
pYI101	pTY60-*fliF-egfp*	(9)
pYI101-*NΔ30*	pTY60-*fliF*(Δ2-30)-*egfp*	This study
pYI101-*NΔ50*	pTY60-*fliF*(Δ2-50)-*egfp*	This study
Amp^r, ampicillin-resistant; Cm^r, chloramphenicol-resistant; Rif^r, Rifampicin-resistant; Pof^+, possessing a polar flagellum; $Laf^–$, lack of lateral flagella		

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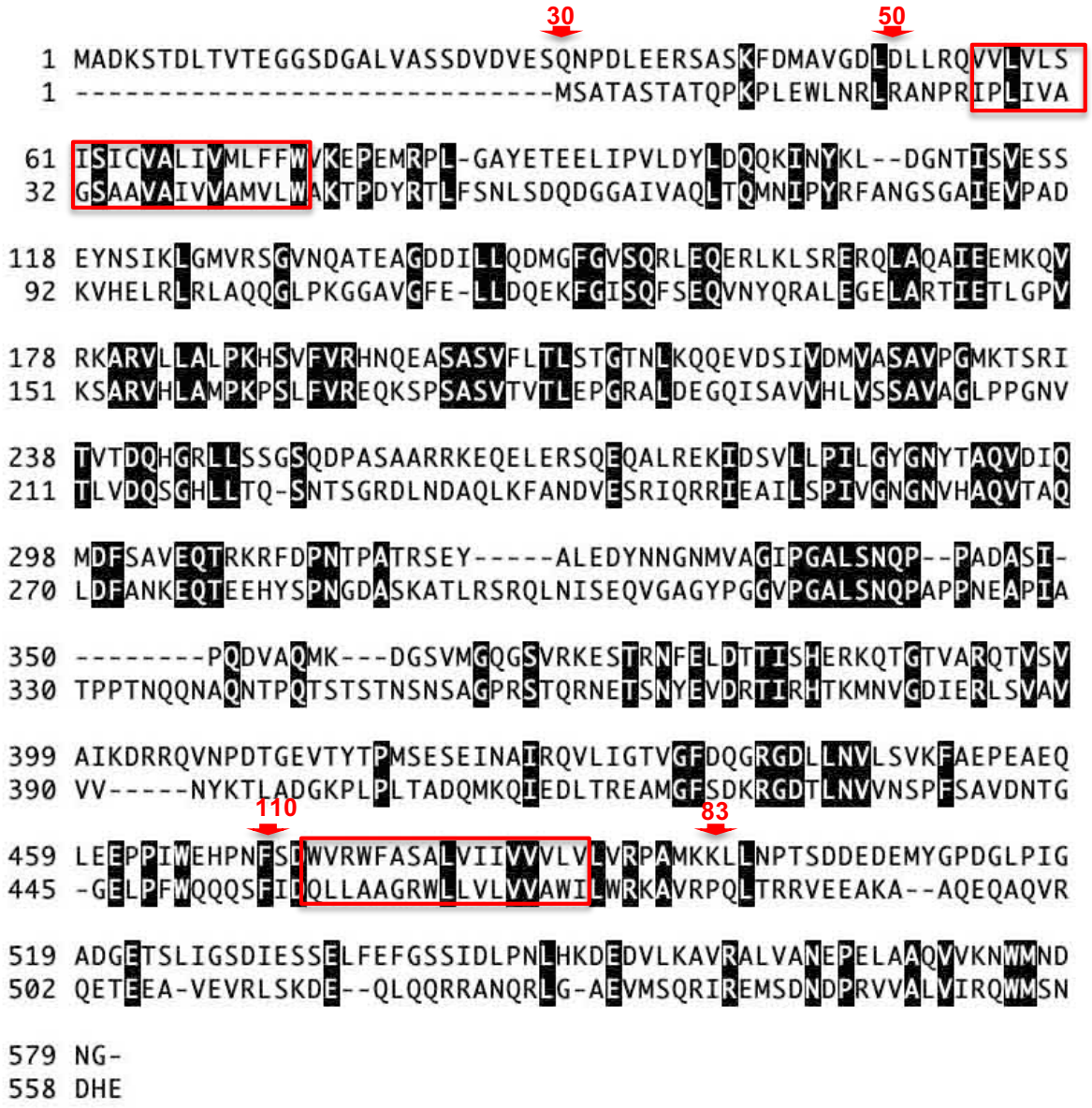


Fig. S1. Comparison of amino acid sequences of *Salmonella* FliF and *Vibrio* FliF. Amino acid sequence homology is about 27%. The red frames indicate transmembrane regions. Arrows indicate mutation sites.

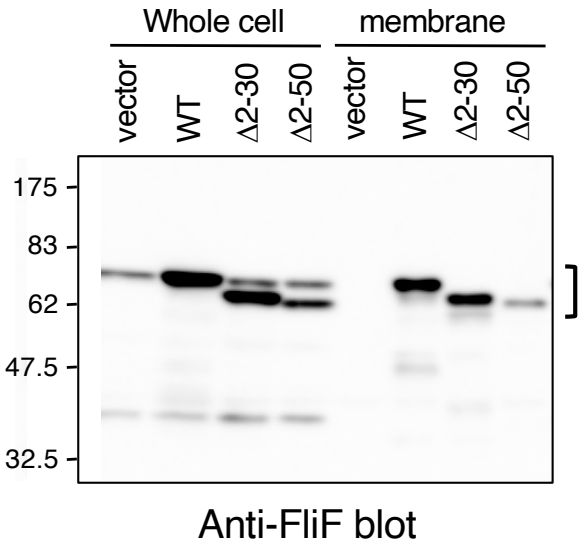


Fig. S2. Detection of N-terminal deletion FliF. The *fliF* deletion mutant (NMB196) producing wild-type FliF, ΔN30FliF, or ΔN50FliF from the pBAD plasmids was grown to mid-log phase in the presence of 0.02% arabinose and cells were disrupted by sonication. The membrane and the soluble fraction were separated by ultracentrifugation. After separating the proteins from the membrane and the soluble fraction by SDS-PAGE, the FliF protein was detected by immunoblotting with an anti-FliF antibody.

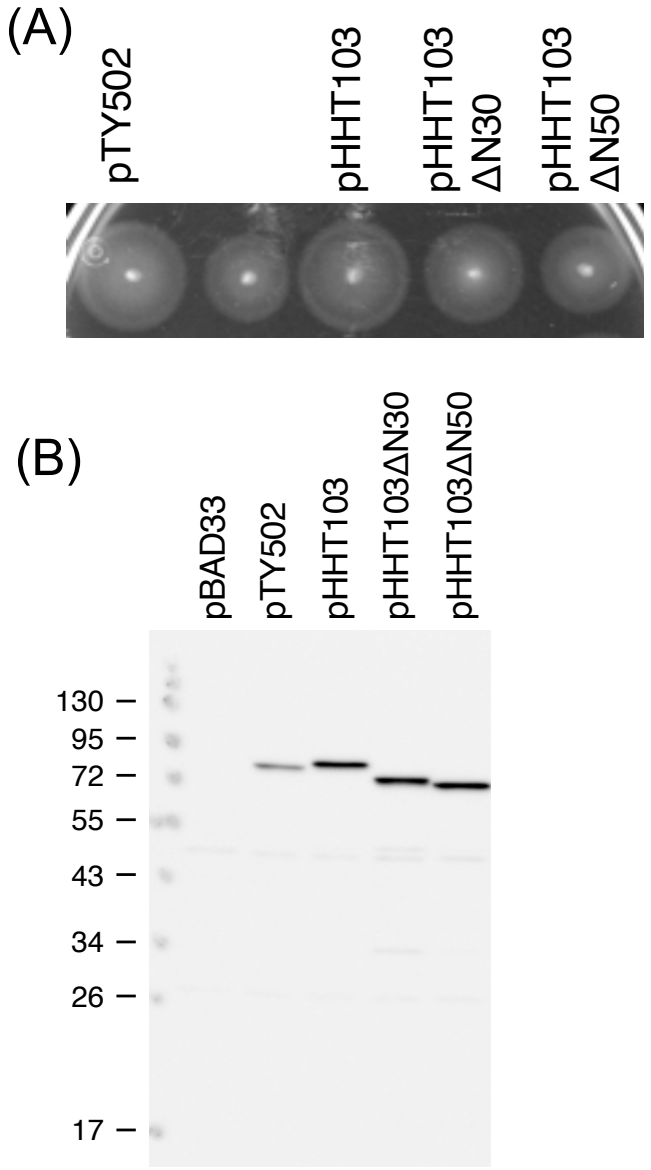
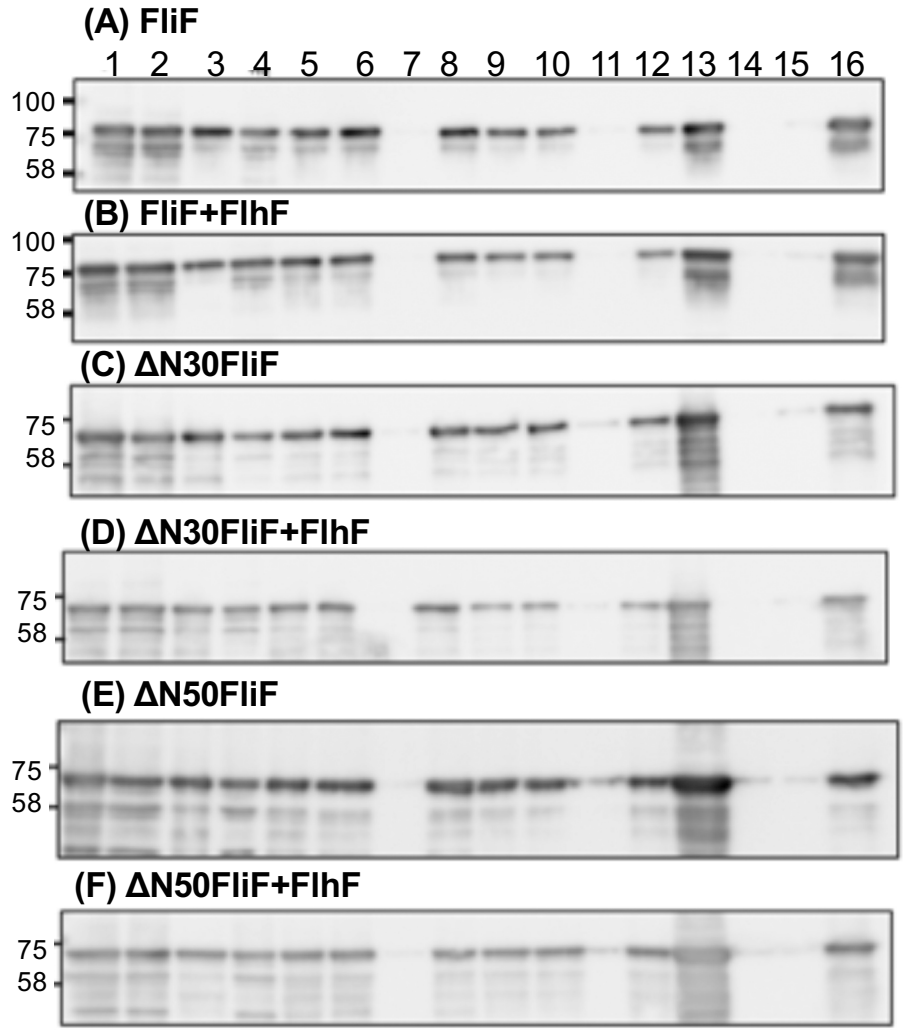


Fig. S3. (A) Motility of N-terminal deletion His-FliF in soft agar plate. Fresh single colonies of the *fliF* deletion mutant (NMB196) containing pTY502 (FliF), pHHT103 (His-FliF), pHHT103-*ΔN30* (His-ΔN30FliF), or pHHT103-*ΔN50* (His-ΔN50FliF) inoculated by toothpicks on the VPG-Cm 0.25% soft agar containing 0.02% arabinose, and incubated at 30 ºC for 4.5 hours. (B) Detection of N-terminal deletion FliF. The *fliF* deletion mutant (NMB196) containing the same plasmids and pBAD33 was grown to mid-log phase and the whole cells were subjected to SDS-PAGE, and the separated FliF protein was detected by immunoblotting with an anti-FliF antibody.


(A) FliF
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
100
75
58
(B) FliF+FlhF
100
75
58
(C) ΔN30FliF
75
58
(D) ΔN30FliF+FlhF
75
58
(E) ΔN50FliF
75
58
(F) ΔN50FliF+FlhF
75
58

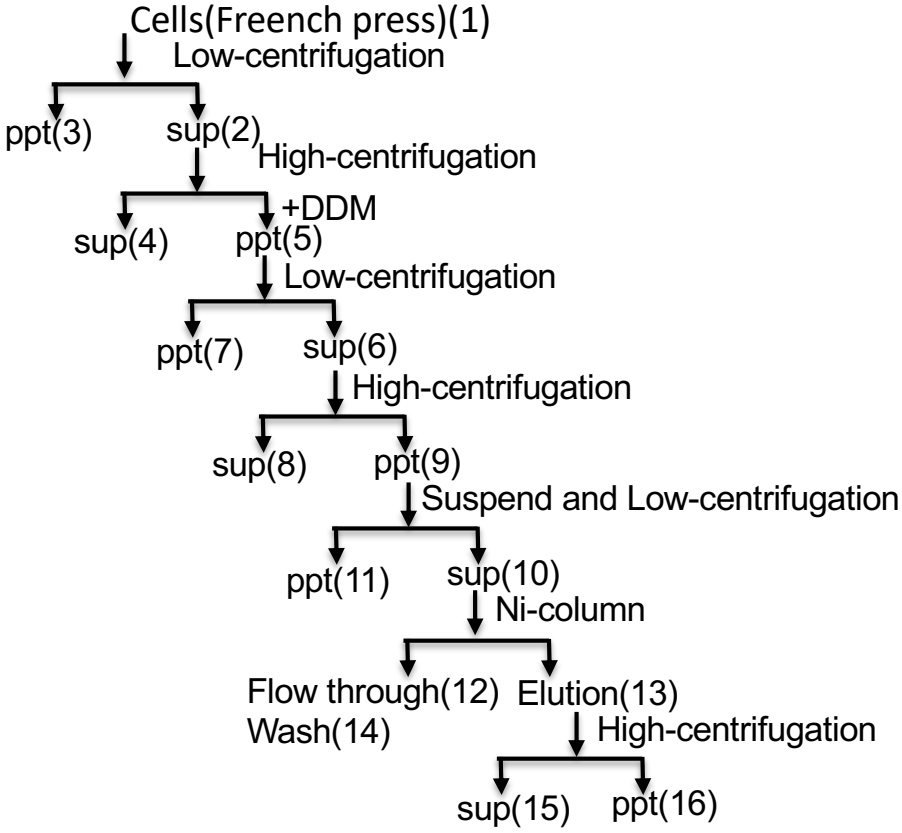

Cells(Freench press)(1)
Low-centrifugation
ppt(3)
sup(2)
High-centrifugation
+DDM
sup(4)
ppt(5)
Low-centrifugation
ppt(7)
sup(6)
High-centrifugation
sup(8)
ppt(9)
Suspend and Low-centrifugation
ppt(11)
sup(10)
Ni-column
Flow through(12)
Wash(14)
Elution(13)
High-centrifugation
sup(15)
ppt(16)

Fig. S4. Purification process of MS ring made by N-terminal deleted FliF. *E. coli* BL21 (DE3) cells harboring pRO101 (A), pRO101 and pTSK22 (B), pRO101-*ΔN30* (C), pRO101-*ΔN30* and pTSK122 (D), pRO101-*ΔN50* (E), or pRO101-*ΔN50* and pTSK122 (F) were cultured and MS ring was isolated. The purification procedure is described in the bottom of the figure. The samples indicated by number in the procedure were subjected to SDS-PAGE, and the separated FliF protein was detected by immunoblotting with an anti-FliF antibody.

Fig. S5. Purification process of MS ring made by C-terminal deleted FliF. *E. coli* BL21 (DE3) cells harboring pRO101-*ΔC83* (A), pRO101-*ΔC83* and pTSK22 (B), pRO101-*ΔC110* (C) were cultured and MS ring was isolated as Fig. S4. The samples indicated by number in the procedure described in Fig. S4 were subjected to SDS-PAGE, and the separated FliF protein was detected by immunoblotting with an anti-FliF antibody.