Supplementary material

The homologous components of flagellar type III protein apparatus have acquired a novel function to control twitching motility in a non-flagellated biocontrol bacterium

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Table S1. Strains and plasmids used in this study

Strains and plasmids	Characteristics[a]	source
Lysobacter enzymogenes		
OH11	Wild type (CGMCC No. 1978), Km^R	[1]
Δ*flhA*	*flhA* in-frame deletion mutant, Km^R	This study
Δ*flhB*	*flhB* in-frame deletion mutant, Km^R	This study
Δ*fliI*	*fliI* in-frame deletion mutant, Km^R	This study
Δ*fliP*	*fliP* in-frame deletion mutant, Km^R	This study
Δ*fliQ*	*fliQ* in-frame deletion mutant, Km^R	This study
Δ*fliR*	*fliR* in-frame deletion mutant, Km^R	This study
Δ*pilA*	*pilA* in-frame deletion mutant, Km^R	[2]
Δ*pilB*	*pilB* in-frame deletion mutant, Km^R	[2]
OH11(pBBR)	Wild type harboring plasmid pBBR1-MCS5, Gm^R, Km^R	This study
OH11(*pilA*)	Wild type harboring plasmid pBBR-*pilA*, Gm^R, Km^R	This study
Δ*flhA*(*flhA*)	Δ*flhA* harboring pBBR-*flhA*, Gm^R, Km^R	This study
Δ*flhB*(*flhB*)	Δ*flhB* harboring pBBR-*flhB*, Gm^R, Km^R	This study
Δ*fliI*(*fliI*)	Δ*fliI* harboring pBBR-*fliI*, Gm^R, Km^R	This study
Δ*fliR*(*fliR*)	Δ*fliR* harboring pBBR-*fliR*, Gm^R, Km^R	This study
Δ*flhA*(*pilA*)	Δ*flhA* harboring pBBR- *pilA*, Gm^R, Km^R	This study
Δ*flhB*(*pilA*)	Δ*flhB* harboring pBBR- *pilA*, Gm^R, Km^R	This study
Δ*fliI*(*pilA*)	Δ*fliI* harboring pBBR- *pilA*, Gm^R, Km^R	This study
Δ*fliR*(*pilA*)	Δ*fliR* harboring pBBR- *pilA*, Gm^R, Km^R	This study
Δ*pilB*(*pilA*)	Δ*pilB* harboring pBBR- *pilA*, Gm^R, Km^R	This study
Δ*pilQ*(*pilA*)	Δ*pilQ* harboring pBBR- *pilA*, Gm^R, Km^R	This study
Escherichia coli DH5α	Host strain for molecular cloning	Laboratory collection
Xanthomonas oryzae PX099A	Source of the canonical $FliC_{xoo}$	Laboratory collection
Salmonella		
Δ$flhA_{sal}$	*flhA* in-frame deletion mutant	[3]
Δ$flhB_{sal}$	*flhB* in-frame deletion mutant	[3]
Δ$fliI_{sal}$	*fliI* in-frame deletion mutant	[3]
Δ$fliP_{sal}$	*fliP* in-frame deletion mutant	[3]
Δ$fliQ_{sal}$	*fliQ* in-frame deletion mutant	[3]
Δ$fliR_{sal}$	*fliR* in-frame deletion mutant	[3]

Δ*flhA*sal (pTrc99A-FlhA)	Δ*flhA*sal harboring pBBR-*flhA*, Amp^R	This study
Δ*flhB*sal (pTrc99A-FlhB)	Δ*flhB*salharboring pBBR-*flhB*, Amp^R	This study
Δ*fliI*sal (pTrc99A-FliI)	Δ*fliI*sal harboring pBBR-*fliI*, Amp^R	This study
Δ*fliP*sal (pTrc99A-FliP)	Δ*fliPA*sal harboring pBBR-*fliR*, Amp^R	This study
Δ*fliQ*sal (pTrc99A-FliQ)	Δ*fliQ*sal harboring pBBR-*fliQ*, Amp^R	This study
Δ*fliR*sal (pTrc99A-FliR)	Δ*fliR*sal harboring pBBR-*fliR*, Amp^R	This study
Plasmids		
pEX18GM	Suicide vector with a *sacB* gene, Gm^R	[4]
pBBR1-MCS5	Broad-host-range vector with a Plac promoter, Gm^R	[5]
pEX18-*flhA*	pEX18GM with two flanking fragments of *flhA*, Gm^R	This study
pTrc99A-FlhA	An expression vector in *Salmonella*, Amp^R	[3]
pEX18-*flhB*	pEX18GM with two flanking fragments of *flhB*, Gm^R	This study
pEX18-*fliI*	pEX18GM with two flanking fragments of *fliI*, Gm^R	This study
pEX18-*fliR*	pEX18GM with two flanking fragments of *fliR*, Gm^R	This study
pEX18-*fliP*	pEX18GM with two flanking fragments of *fliP*, Gm^R	This study
pEX18-*fliQ*	pEX18GM with two flanking fragments of *fliQ*, Gm^R	This study
pBBR-*flhA*	pBBR1-MCS5 cloned with a 2385-bp fragment containing intact *flhA* and its predicted promoter, Gm^R	This study
pBBR-*flhB*	pBBR1-MCS5 cloned with a 1557-bp fragment containing intact *flhB* and its predicted promoter, Gm^R	This study
pBBR-*fliI*	pBBR1-MCS5 cloned with a 1562-bp fragment containing intact *fliI*, FLAG tag and its predicted promoter, Gm^R	This study
pBBR-*fliR*	pBBR1-MCS5 cloned with a 1277-bp fragment containing intact *fliR* and its predicted promoter, Gm^R	This study
pBBR- *pilA*	pBBR1-MCS5 cloned with a 737-bp fragment containing a PilA-Flag tag, Gm^R	This study
pTrc99A-FlhA	pTrc99A cloned with the coding region of *flhA* of strain OH11, Amp^R	This study
pTrc99A-FlhB	pTrc99A cloned with the coding region of *flhB* of strain OH11, Amp^R	This study
pTrc99A-FliI	pTrc99A cloned with the coding region of *fliI* of strain OH11, Amp^R	This study
pTrc99A-FliP	pTrc99A cloned with the coding region of *fliP* of strain OH11, Amp^R	This study
pTrc99A-FliQ	pTrc99A cloned with the coding region of *fliQ* of strain OH11, Amp^R	This study

[a]Km^R, Gm^R and Amp^R: kanamycin-, gentamicin, and Ampicillin-resistance, respectively

Table S2. Primers used in this study

Primer	Sequence[a] (5′-3′)	Purpose
In-frame deletion		
flhA-F1	CGGAATTCGATCGCCAAGAGCCAGGAAC (*Eco*RI)	To amplify a 794-bp upstream homologue arm of *flhA*
flhA-R1	GGGTTTGAAGGAGACGGGCGCGACGATGGTGGGGGCAAGC	
flhA-F2	GCTTGCCCCCACCATCGTCGCGCCCGTCTCCTTCAAACCC	To amplify a 799-bp downstream homologue arm of *flhA*
flhA-R2	GCTCTAGACTGTGCGGATTCGATGCGGCCGACGATGGTGGG GGCAAGC (*Xba*I)	
flhB-F1	CCG*GAATTC*CGTTCCTGGTGTTCCTCGAC (*Eco*RI)	To amplify a 538-bp upstream homologue arm of *flhB*
flhB-R1	CGCCGACCCAGAAAAAGATCGGTCCTGCATTGGGCCGAAG	
flhB-F2	CTTCGGCCCAATGCAGGACCGATCTTTTTCTGGGTCGGCG	To amplify a 464-bp downstream homologue arm of *flhB*
flhB-R2	GCTCTAGACCAGGCATCGGCGTTGAGGG (*Xba*I)	
flip-F1	CGGAATTCGGCTCACGGTCAAGGAACTG (*Eco*RI)	To amplify a 526-bp upstream homologue arm of *fliP*
flip-R1	GCGGCGAGGTCATTCATTTGCGATGGCGGACGAAGAAGGC	
flip-F2	GCCTTCTTCGTCCGCCATCGCAAATGAATGACCTCGCCGC	To amplify a 645-bp downstream homologue arm of *fliP*
flip-R2	GCTCTAGAGGCAGCGAATGGAAATAGTG (*Xba*I)	
fliQ-F1	CGGAATTCGAACATCCTGCTCTCGCTGG (*Eco*RI)	To amplify a 336-bp upstream homologue arm of *fliQ*
fliQ-R1	CCCAAGCTTGCCTGTTTGGTCATTTCGAG (*Hin*dIII)	
fliQ-F2	CCCAAGCTTCGCCGACCGCATCTTCCTCG (*Hin*dIII)	To amplify a 363-bp downstream homologue arm of *fliQ*
fliQ-R2	GCTCTAGACCCAGAACACCATGCCGAAC (*Xba*I)	
fliR-F1	CGGAATTCCACGAGGACGACACCGCGAC(*Eco*RI)	To amplify a 631-bp upstream homologue arm of *fliR*
fliR-R1	CCCAAGCTTCGGCGAGGCGATCAGGAAAC(*Hin*dIII)	
fliR-F2	CCCAAGCTTCCTTGGTGATGCTGACGCTC(*Hin*dIII)	To amplify a 923-bp downstream homologue arm of *fliR*
fliR-R2	GCTCTAGACGTAATGGGTGGGGTTGGTG(*Xba*I)	
fliI-F1	CCGGAATTCTTCTCCAGCGCCTCCAGCCT (*Eco*RI)	To amplify a 389-bp upstream homologue arm of *fliI*
fliI-R1	CCCAAGCTTCGGATGAACAAGCAACGCT (*Hin*dIII)	
fliI-F2	CCCAAGCTTCGACCACTTCGGCGGCGAG (*Hin*dIII)	To amplify a 457-bp downstream homologue arm of *fliI*
fliI-R2	CGGGGTACCCTGCGTGGTGGTGTCGGAAG (*Kpn*I)	
Complementation and heterologous expression		
flhA-F	CCCAAGCTTCACCAACCCCACCCATTACG (*Hin*dIII)	To amplify 2409-bp fragment containing the intact *flhA*
flhA-R	CGGAATTCTCACTTATCGTCGTCATCCTTGTAATCGGCGGCG ACGGGTTTGA (*Eco*RI)	
flhB-F	CCCAAGCTTGATGGACGCAATGTAGCCCC (*Hin*dIII)	To amplify 1331-bp fragment containing the intact *flhB*
flhB-R	CGGAATTCTCACTTATCGTCGTCATCCTTGTAATCGCGCGGC TCGTCGTCGA (*Eco*RI)	
fliI-F	CCCAAGCTT CTGCGTGGTGGTGTCGGAAG (*Hin*dIII)	To amplify 1562-bp fragment containing the intact *fliI*
fliI-R	CGGAATTCTCACTTATCGTCGTCATCCTTGTAATCTCCGAAC AACCTGCGCA (*Eco*RI)	
flip-F	CCCAAGCTTGGCTCACGGTCAAGGAACTG (*Hin*dIII)	To amplify 1101-bp fragment containing the intact *fliP*
flip-R	CGGAATTCTCACTTATCGTCGTCATCCTTGTAATCTTTGAAGC TGAGCACCA (*Eco*RI)	
fliQ-F	CCCAAGCTTCACCGTCAGCGAACTCACCG (*Hin*dIII)	To amplify 676-bp fragment containing the intact *fliQ*
fliQ-R	CGGAATTCTCACTTATCGTCGTCATCCTTGTAATCGTGCCGG ATCATGCCGG (*Eco*RI)	
fliR-F	CCCAAGCTTGAAGGACACGACGAGCAGGC (*Hin*dIII)	To amplify 1300-bp fragment containing the intact *fliR*
fliR-R	CGGAATTCTCACTTATCGTCGTCATCCTTGTAATCTGGCGCG CCCGCCGGGC (*Eco*RI)	
pilA-F	CCCAAGCTTCATCGCAAATCAACCCCCAC(*Hin*dIII)	To amplify 737-bp fragment containing the *pilA*-Flag fusion

pilA-R	CGGAATTCTTACTTATCGTCGTCATCCTTGTAATCGGTCGCA GCCTTGGTGC(*Eco*RI)	
fliC$_{Xoo}$ F2	CCCAAGCTTATGTCGCTGAACGCTCAGCG (*Hin*dIII)	To amplify *fliC*$_{Xoo}$ terminally fused with FLAG tag
fliC$_{Xoo}$ F2	CGGAATTCTTACTTATCGTCGTCATCCTTGTAATCCTGCAGCA GGCTCAGCA(*Eco*RI)	

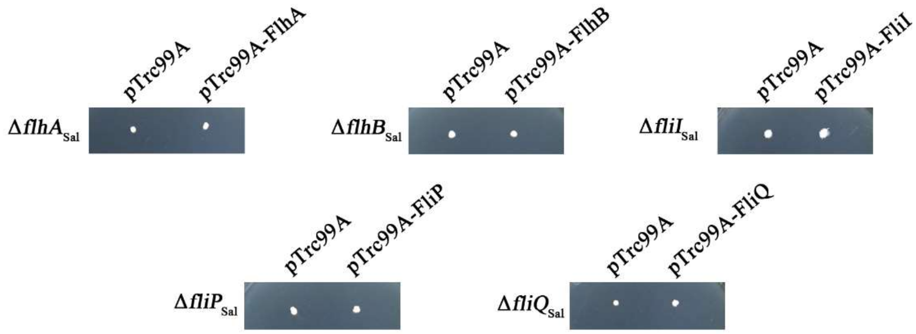

Figure S1. Cross-complementation of the *L. enzymogenes* FT3SS-like genes in the *Salmonella* mutants lacking the respective flagellar counterparts. The FlhA, FlhB, FliP, FliQ and FliI from strain OH11 failed to restore the swimming motility of the respective *Salmonella* mutants, indicated by Δ*flhA*$_{sal,}$ Δ*flhB*$_{sal,}$ Δ*fliP*$_{sal,}$ Δ*fliQ*$_{sal}$ and Δ*fliI*$_{sal,}$ respectively. The photograph was taken after incubation of the transformed strain at 30ºC for 24 hours.

References

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