Table S1. List of bacterial strains used in this study.

Name	Genotype	Reference
E. coli One Shot™ TOP10	*F- mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 Δ lacX74 recA1 araD139 Δ(araleu)7697 galU galK rpsL (StrR) endA1 nupG.* For clonning	Invitrogen
1448A	*P. syringae* pv. *phaseolicola* wild type strain race 6	Teverson[102]
NLP1	1448A *fliC::GFP3* Km^R	This work
NLP2	1448A *fliC::tdT* Km^R	This work
NLP3	1448A *hrpL::GFP3 fliC::tdT* Km^R	This work
NLP4	1448A *hopAB1::GFP3 fliC::tdT* Km^R	This work
NLP5	1448A *hrcU::GFP3 fliC::tdT* Km^R	This work
IOM7	1448A Δ*hrpL* Km^R	Ortiz-Martín et al.[81]
NLP6	1448A Δ*fleQ* Km^R	This work
PphΔ*fliC*	1448A Δ*fliC* Km^R	Leba et al.[34]
JRP8	1448A eGFP Gm^R	Rufián et al.[37]
NLP7	1448A *fliC::GFP3 eCFP* Km^R Gm^R	This work
NLP8	1448A *hopAB1::GFP3 fliC*::*tdT eCFP* Km^R Gm^R	This work
DLM1	Wild type strain with transcriptional fusion of *hrpL* to *GFP3* Km^R	Rufián et al.[27]
DLM3	Wild type strain with transcriptional fusion of *hopAB1* to *GFP3* Km^R	Rufián et al.[27]
DLM2	Wild type strain with transcriptional fusion of *hrcU* to *GFP3* Km^R	Rufián et al.[27]

Table S2. List of plasmids used and generated in this work.

Name	Description	Reference
pGEM-T vector	*E. coli* expresión vector for cloning. Amp^R	Promega, USA
pGT-GFP^+	*E. coli* expression vector carrying the *GFP3* gene. Amp^R Km^R	Rufián et al.[93]
tdTomato-pBAD	Addgene plasmid # 54856 carrying the *tdTomato* gene sequence. Amp^R	Shaner et al.[91]
pKD4	pANTS derivative containing a FRT-flanked kanamycin resistance gene. Km^R	Datsenko and Wanner[92]
pGT-AB-*fliC*	pGEM-T vector carrying the last 500 pb of the *fliC* ORF (A) followed by an EcoRV site and the 500 bp immediately downstream the *fliC* STOP codon (B). Amp^R	This work
pGT-*fliC*::*tdT*	Allelic exchange vector for the generation of a transcriptional fusion of *fliC* to *tdTomato* in 1448A. Amp^R Km^R	This work
pGT-*fliC*::*GFP3*	Allelic exchange vector for the generation of a transcriptional fusion of *fliC* to *GFP3* in 1448A. Amp^R Km^R	This work
pGT-AB-Δ*fleQ*	pGEM-T vector Carrying the last 500 pb of the *fleQ* ORF (A) followed by an EcoRI site and the 500 bp immediately downstream the *fleQ* STOP codon (B)	This work
pGT-Δ*fleQ*	Allelic exchange vector for the generation of a knockout mutation of the *fleQ* gene. Amp^R Km^R	This work
pHrpL (pBBR-*hrpL*)	pBBR-MCS-4 derivative, contains a promotorless *fleQ* gene expressed from the *lacZ* promoter	Ortíz-Martín et al.[26]

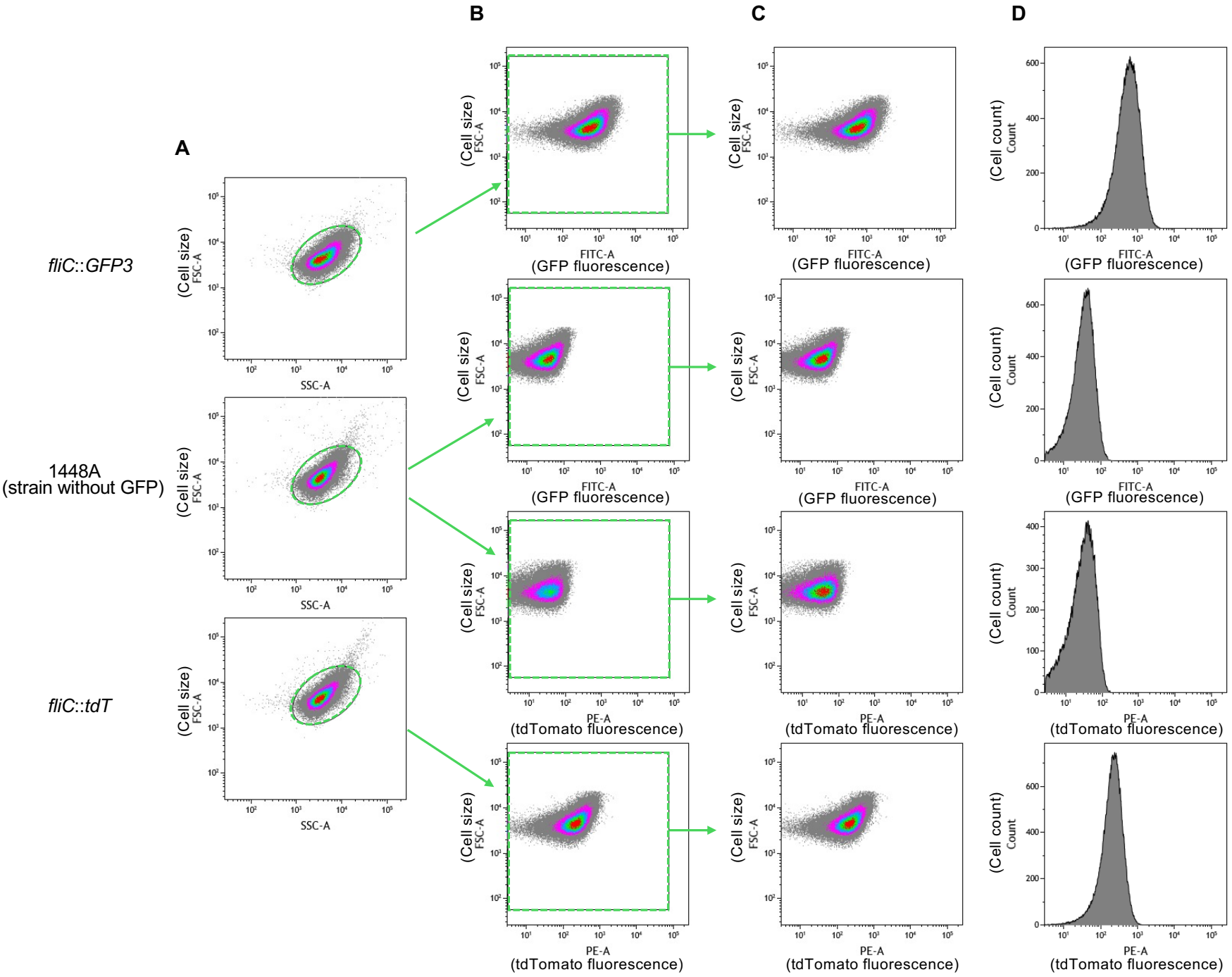
pFleQ (pBBR-*fleQ*)	pBBR-MCS-4 derivative, contains a promotorless *fleQ* gene expressed from the *lacZ* promoter	This work

Table S3. List of primers used in this study.

Name	Sequence	Restriction site	Reference
A1 *fliC*	GATGAGTTCCGAGCTGACCC	-	This work
A2 *fliC*	GATATCCGATATTACTGAAGCAGTTTCAGTA CAGC	*EcoRV*	This work
B1 *fliC*	AGTAATATATCGGATATCGCATGAGTTTTAGC GGGGG	*EcoRV*	This work
B2 *fliC*	CACAGGTCTCGAAAACGG	-	This work
ProtFluorF	GATATCGGAGATATACATATGGTTGAGCAAG GGCG	*EcoRV*	This work
ProtFluorR	GAATTCACAGCCAAGCTTC	*EcoRI*	This work
P1 EcoRV	TCAGATATCGTGTAGGCTGGAGCTGCTTC	*EcoRV*	This work
P2 EcoRI	TCAGAATTCCATATGAATATCCTCCTTAG	*EcoRI*	Zumaquero et al.[90]
A1 Δ*fleQ*	TCAGAATTCCATATGAATATCCTCCTTAG	-	This work
A2 Δ*fleQ*	GAATTCCGATATTACTGCAATAGCAACTTCC CTAGTCAACT	*EcoRI*	This work
B1 Δ*fleQ*	AGTAATATCGGAATTCCGCCTGTCTGTAGCG CCA	*EcoRI*	This work
B2 Δ*fleQ*	CCGACTGATGACGTGACGCCA	-	This work
P1 EcoRI	TCAGAATTCGTGTAGGCTGGA	*EcoRI*	Zumaquero et al.[90]
*fleQ*F	GTAGGTACCTTTATACAGCTGAGATGCCAAG	*KpnI*	This work
*fleQ*R	TATCCGCGGTCAATCATCTGCCTGTTCATCA	*SacII*	This work

Table S4. List of software used in this study.

Software	Reference	Source
Image J/Fiji 2.14.0		https://imagej.net/ij/docs/index.html
GraphPad Prism 9	Prism	https://www.graphpad.com
LAS X	Leica microsystem	https://www.leica-microsystems.com/es/productos/software-de-microscopia/p/leica-las-x-ls/
ZEN 3.4	Carl Zeiss Microscopy	https://www.zeiss.com/microscopy/es/productos/software/zeiss-zen.html
Kaluza Analysis	Beckman Coulter	https://www.beckman.es/flow-cytometry/software/kaluza
DeLTA 2.0	O´Connor et al.[99]	N/A


A
B
C
D
fliC::GFP3
1448A
(strain without GFP)
fliC::tdT
(Cell size)
FSC-A
SSC-A
FITC-A
(GFP fluorescence)
PE-A
(tdTomato fluorescence)
(Cell count)
Count

Supplementary Fig. S1. Gating strategy. (A) Graphs show Forward Scatter Cell (FSC) *versus* Side Scatter Cell (SSC) for *fliC*::*GFP3*, non-fluorescent control 1448A and *fliC*::*tdT* LB cultures. Circle gates are indicated and used to limit the presence in the analysis of cell detritus and cell aggregates. (B) Graphs show GFP or tdT fluorescence (FITC or PE filters) *versus* FSC for the samples mentioned after gating shown in A. Square gates indicated are used to discard zero fluorescence values. (C) Graphs show GFP or tdT fluorescence (FITC or PE filters) *versus* FSC for the samples mentioned after gating indicated in B. (D) Histograms corresponding to graphs shown in C.