Supplementary Files

Old role with new feature: T2SS ATPase as a cyclic-di-GMP receptor to regulate antibiotic production

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Table S1 Strains and plasmids used in this study

Strains and plasmids	Characteristics[a]	Source
Lysobacter enzymogenes		
OH11	Wild-type , Km^R	Qian et al., 2009
Δ*0328*	*0328* in-frame deletion mutant	This study
Δ*0463*	*0463* in-frame deletion mutant	This study
Δ*0770* (Δ*lspE*)	*0770* in-frame deletion mutant	This study
Δ*1020*	*1020* in-frame deletion mutant	This study
Δ*1499*	*1499* in-frame deletion mutant	This study
Δ*1886*	*1886* in-frame deletion mutant	This study
Δ*1890*	*1890* in-frame deletion mutant	This study
Δ*2394*	*2394* in-frame deletion mutant	This study
Δ*2512*	*2512* in-frame deletion mutant	This study
Δ*3049*	*3049* in-frame deletion mutant	This study
Δ*3194*	*3194* in-frame deletion mutant	This study
Δ*3810*	*3810* in-frame deletion mutant	This study
Δ*4096*	*4096* in-frame deletion mutant	This study
Δ*5095*	*5095* in-frame deletion mutant	This study
$OH11_{LspE\text{-}R298A}$	*lspE* site-directed mutation mutant	This study
$OH11_{LspE\text{-}R313A}$	*lspE* site-directed mutation mutant	This study
$OH11_{LspE\text{-}R424A}$	*lspE* site-directed mutation mutant	This study
$OH11_{LspE\text{-}R457A}$	*lspE* site-directed mutation mutant	This study
$OH11_{LspE\text{-}R358A}$	*lspE* site-directed mutation mutant	This study
$OH11_{LspE\text{-}R359A}$	*lspE* site-directed mutation mutant	This study
Escherichia coli		
Trans1-T1	Host strain for plasmid amplification	TransGen Biotech Company, Beijing, China
BL21(DE3)	Host strain for protein expression, Km^R	TaKaRa Company, Shanghai, China
Plasmids		
pEX18GM	Suicide vector for gene in-frame deletion, Gm^R	Hoang et al., 1998

0328-pEX18	Plasmid for in-frame deletion of *0328*	This study
0463-pEX18	Plasmid for in-frame deletion of *0463*	This study
0770-pEX18	Plasmid for in-frame deletion of *0770*	This study
1020-pEX18	Plasmid for in-frame deletion of *1020*	This study
1499-pEX18	Plasmid for in-frame deletion of *1499*	This study
1886-pEX18	Plasmid for in-frame deletion of *1886*	This study
1890-pEX18	Plasmid for in-frame deletion of *1890*	This study
2394- pEX18	Plasmid for in-frame deletion of *2394*	This study
2512-pEX18	Plasmid for in-frame deletion of *2512*	This study
3049-pEX18	Plasmid for in-frame deletion of *3049*	This study
3194-pEX18	Plasmid for in-frame deletion of *3194*	This study
3810-pEX18	Plasmid for in-frame deletion of *3810*	This study
4096-pEX18	Plasmid for in-frame deletion of *4096*	This study
5095-pEX18	Plasmid for in-frame deletion of *5095*	This study
$lspE_{R298A}$-pEX18	Plasmid for site-directed mutation of *lspE*	This study
$lspE_{R313A}$-pEX18	Plasmid for site-directed mutation of *lspE*	This study
$lspE_{R424A}$-pEX18	Plasmid for site-directed mutation of *lspE*	This study
$lspE_{R457A}$-pEX18	Plasmid for site-directed mutation of *lspE*	This study
$lspE_{K358A}$-pEX18	Plasmid for site-directed mutation of *lspE*	This study
$lspE_{T359A}$-pEX18	Plasmid for site-directed mutation of *lspE*	This study
pET30a(+)	Vector for expression His-tag fusion protein	TaKaRa company, Shanghai, China
LspE-pET30a	Plasmid for fusion protein expression of LspE and His-tag	This study
$LspE_{R298A}$-pET30a	Plasmid for fusion protein expression of $LspE_{R298A}$ and His-tag	This study
$LspE_{R313A}$-pET30a	Plasmid for fusion protein expression of $LspE_{R313A}$ and His-tag	This study
$LspE_{R424A}$-pET30a	Plasmid for fusion protein expression of $LspE_{R424A}$ and His-tag	This study
$LspE_{R457A}$-pET30a	Plasmid for fusion protein expression of $LspE_{R457A}$ and His-tag	This study
$LspE_{K358A}$-pET30a	Plasmid for fusion protein expression of $LspE_{K358A}$ and His-tag	This study
$LspE_{T359A}$-pET30a	Plasmid for fusion protein expression of	This study

	$LspE_{T359A}$ and His-tag	
LspE-Hcp1-pET30a	Plasmid for fusion protein expression of LspE, Hcp1 and His-tag	This study
$LspE_{R298A}$-Hcp1-pET30a	Plasmid for fusion protein expression of $LspE_{R298A}$, Hcp1 and His-tag	This study
$LspE_{R313A}$-Hcp1-pET30a	Plasmid for fusion protein expression of $LspE_{R313A}$, Hcp1 and His-tag	This study

[a]Km^R, Gm^R represent kanamycin and gentamicin resistance, respectively.

Table S2 Primers used in this study

Primer	Sequence[a]	Purpose
Primers used for gene in-frame deletion		
0328F1	GGCCAGTGCCAAGCTTTCGATCCCGGCAAGAACTCG CGCCAATG	To amplify a 524-bp upstream homologue arm of *0328*
0328R1	GGGCTCAGCACTGTTTGGCCTTGATCGCGGAACG	
0328F2	AACAGTGCTGAGCCCCGGGCTCCGATTCC	To amplify a 523-bp downstream homologue arm of *0328*
0328R2	CCGGGGATCCTCTAGATTTCCTGCGATTGCGGGAATT CGGTC	
0463F1	ATTCGAGCTCGGTACCGCGGGAAGGATAGAGGAAT	To amplify a 442-bp upstream homologue arm of *0463*
0463R1	GTCCATCGCCGCCACGAGAAGATGTAGTCGCTGAGCT	
0463F2	CGACTACATCTTCTCGTGGCGGCGATGGACGAA	To amplify a 398-bp downstream homologue arm of *0463*
0463R2	CGACGGCCAGTGCCAGTTCACCACCACCCACTTG	
0770F1	GGCCAGTGCCAAGCTTGCCGTCCGAGGCATGCGTGC GTCCGCGCTCGC	To amplify a 524-bp upstream homologue arm of *0770*
0770R1	ACTGGGTGTGAAAGGGCTGTTGCATGGCCTCGTGCTG CTGTG	
0770F2	CCTTTCACACCCAGTTGCGGGTGACTCC	To amplify a 523-bp downstream homologue arm of *0770*
0770R2	CCGGGGATCCTCTAGAACCGCGCCACGGCCGCGCGG ATG	
1020F1	GGCCAGTGCCAAGCTTCGCCAGGTCGTCGATGCGGA CCA	To amplify a 524-bp upstream homologue arm of *1020*
1020R1	CGTACTCACATGTCTAAAGTTCCGAATGATGGAAGAA C	
1020F2	AGACATGTGAGTACGGTAGATCGGCGCGGGTC	To amplify a 523-bp downstream homologue arm of *1020*
1020R2	CCGGGGATCCTCTAGACGTCAACGTCTTCGCGCCAGC C	
1499F1	ATTCGAGCTCGGTACCGCTATGGCTGACGCTGTT	To amplify a 352-bp upstream homologue arm of *1499*
1499R1	TGTTGCCGTAGATGTCGAGCCTTCCACCAACGC	
1499F2	TTGGTGGAAGGCTCGACATCTACGGCAACAACAGTTT C	To amplify a 264-bp downstream homologue arm of *1499*
1499R2	CGACGGCCAGTGCCACAGTTCGCCCTCGTGCTC	
1886F1	GGCCAGTGCCAAGCTTCGCGCTTCGACGGCGCCGAC	To amplify a 524-bp upstream homologue arm of *1886*
1886R1	CAGCCATGTGAGGGCCGCTTTGGAGGCATT	
1886F2	GCCCTCACATGGCTGTATCACGCCGCGAGT	To amplify a 523-bp downstream

1886R2	CCGGGGATCCTCTAGAGTCGCGCGCCTGGAAACGGTCC	homologue arm of *1886*
1890F1	GGCCAGTGCCAAGCTTGCCCGGCGCGATCGCGTCGATCAC	To amplify a 524-bp upstream homologue arm of *1890*
1890R1	GCACGGCGCACCGCGGAATCGCAGCCGGCGCTGGG	
1890F2	CGCGGTGCGCCGTGCGCGCCGCCCGCGTTGGCCGGACCTG	To amplify a 530-bp downstream homologue arm of *1890*
1890R2	CCGGGGATCCTCTAGAGCTGGTGCACGGTGCGGTAGCTCTCGATC	
2394F1	ATGACCATGATTACGCGCCTTCGTCTGGGTGGT	To amplify a 319-bp upstream homologue arm of *2394*
2394R1	GTAGAACAGCGGGATGCCGCCGAGCATCACCA	
2394F2	GTGATGCTCGGCGGCATCCCGCTGTTCTACCTGCT	To amplify a 490-bp downstream homologue arm of *2394*
2394R2	AGGATCCCCGGGTACCGATGGAGATGTTGCCGTC	
2512F1	GGCCAGTGCCAAGCTTCAGACCCAATGGCGCGGGCCGGTG	To amplify a 524-bp upstream homologue arm of *2512*
2512R1	CCGGCTCACATCGCGCGCTGACCGCTGCGGCGAAC	
2512F2	AGCGCGCGATGTGAGCCGGCCGCGCCGCGCTCAGTGCAC	To amplify a 523-bp downstream homologue arm of *2512*
2512R2	CCGGGGATCCTCTAGAGATCACCGGCTTCCGCATGCG	
3049F1	GGCCAGTGCCAAGCTTCGTTTATTCCAGCGCGTTGAG	To amplify a 524-bp upstream homologue arm of *3049*
3049R1	TTCCCATGTGACGCCGATGTCGCGCCTGC	
3049F2	ACATCGGCGTCACATGGGAACGGCGCGCCCGGGGCGCGTCG	To amplify a 523-bp downstream homologue arm of *3049*
3049R2	CCGGGGATCCTCTAGACGAGTCCGACTTCGACAAGGC	
3194F1	ATGACCATGATTACGGGCGACGAAGGCTGGTG	To amplify a 520-bp upstream homologue arm of *3194*
3194R1	CGCCGCACCCCCTCACACGAGAGCTATCGGCTCA	
3194F2	CCGATAGCTCTCGTGTGAGGGGGTGCGGCGAT	To amplify a 284-bp downstream homologue arm of *3194*
3194R2	AGGATCCCCGGGTACCGACAGCAAGACGGGCGA	
3810F1	GGCCAGTGCCAAGCTTGTCGGCCAGGCGGCT	To amplify a 524-bp upstream homologue arm of *3810*
3810R1	GCCCCGTGTGAGCAACGGCAACGAACGCACGCCCGGCAG	
3810F2	TTGCTCACACGGGGCTGGTGGTTGGGT	To amplify a 523-bp downstream homologue arm of *3810*
3810R2	CCGGGGATCCTCTAGACCCAAGAGCGAAGCCTGGTTCG	
4096F1	ATGACCATGATTACGATCTGCTCGTTCGTCGGC	To amplify a 501-bp upstream

4096R1	GCGGATCGTTGTCGTAGCCAGTCCTCAGTTTCGGTAT	homologue arm of *4096*
4096F2	AACTGAGGACTGGCTACGACAACGATCCGCTGAG	To amplify a 358-bp downstream homologue arm of *4096*
4096R2	AGGATCCCCGGGTACCGGAGGCGGCGATGGA	
5095F1	GGCCAGTGCCAAGCTTGGGCCCCTAGGCTCGAATCGC	To amplify a 524-bp upstream homologue arm of *5095*
5095R1	CTGGGTCACATCAGGGCATCGGCTTGGG	
5095F2	CCTGATGTGACCCAGGACCACGCCCCGCATGCC	To amplify a 523-bp downstream homologue arm of *5095*
5095R2	CCGGGGATCCTCTAGAGGTAACGGGTTCGCCGTCCAGG	
Primers used for protein expression		
LspE-His-F	AGAAGGAGATATACATATGAATGCGCAGGAGCGTGATGT	To amplify a 1788-bp fragment encoding LspE
LspE-His-R	TCGAGTGCGGCCGCAAGCTTTGCGTCCTCGGTCACCC	
LspE-Hcp1-His-F1	AGAAGGAGATATACATATGAATGCGCAGGAGCGTGATGT	To amplify a 1782-bp upstream homologue arm of LspE--Hcp1
LspE-Hcp1-His-R1	GAACATATCAACAGCCCCACTCGCCAACTTTGCGTCCTCGGTCAC	
LspE-Hcp1-His-F2	GTGACCGAGGACGCAAAGTTGGCGAGTGGGGCTGTTGATATGTTC	To amplify a 483-bp downstream homologue arm of LspE-Hcp1
LspE-Hcp1-His-R2	TCGAGTGCGGCCGCAAGCTTGGCCTGCACGTTCTGGC	
Primers used for qRT-PCR		
Pks-F	ACTATTTGTTGGGCGACGAC	To detect the transcript level of *pks*
Pks-R	GTAACCGAACAGGGTGCAAT	
16s-F	ACGGTCGCAAGACTGAAACT	Reference gene
16s-R	AAGGCACCAATCCATCTCTG	

Table S3 Circular dichroism spectroscopy results of the protein structure

(%)	LspE-Hcp1	$LspE_{K358A}$-Hcp1	$LspE_{T359A}$-Hcp1
Helix	14.7	13.3	15.1
Beta	49.2	49.2	48.0
Turn	13.5	14.7	14.8
Random	22.6	22.8	22.1

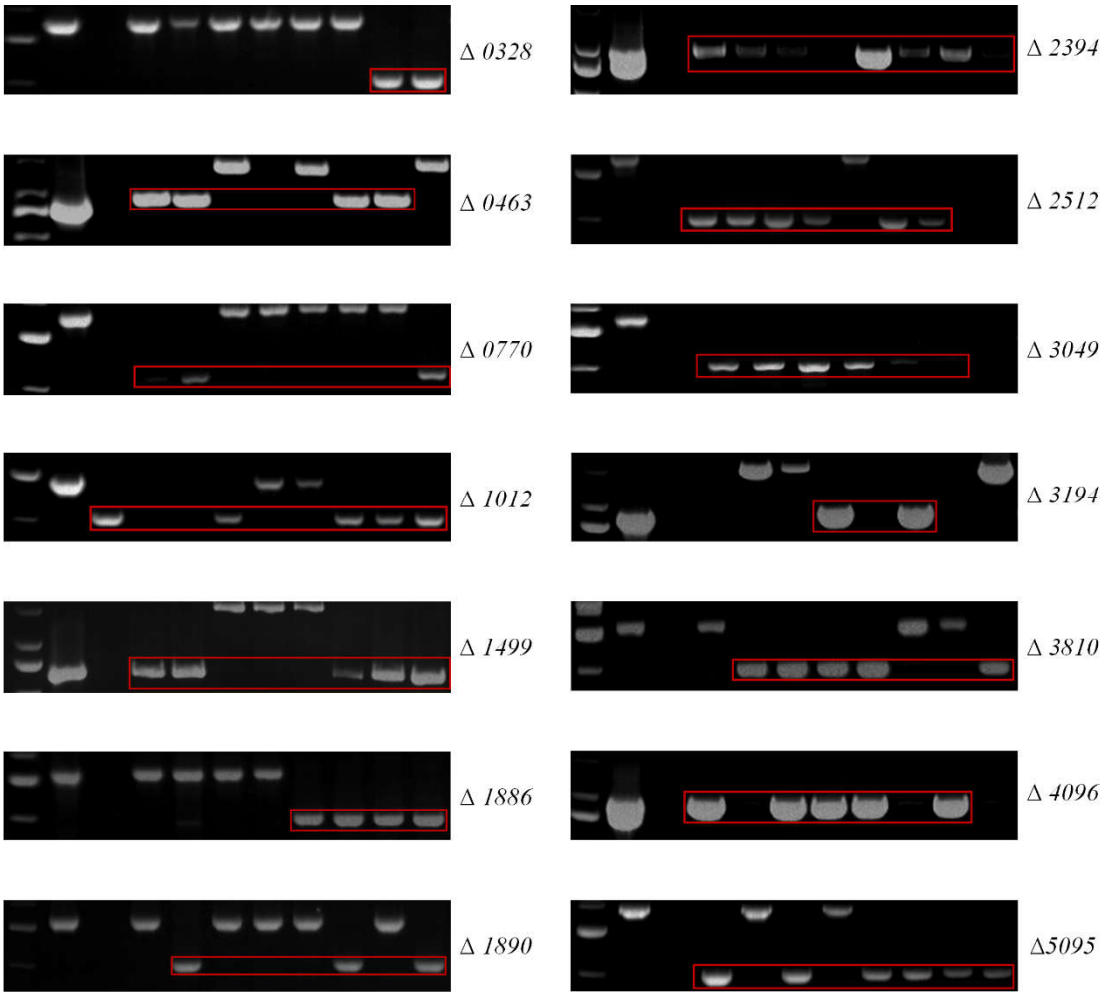

Figure S1. Mutants confirmation by PCR in this study. Red box indicated the expected size from deletion mutant amplified by selected primers, respectively.

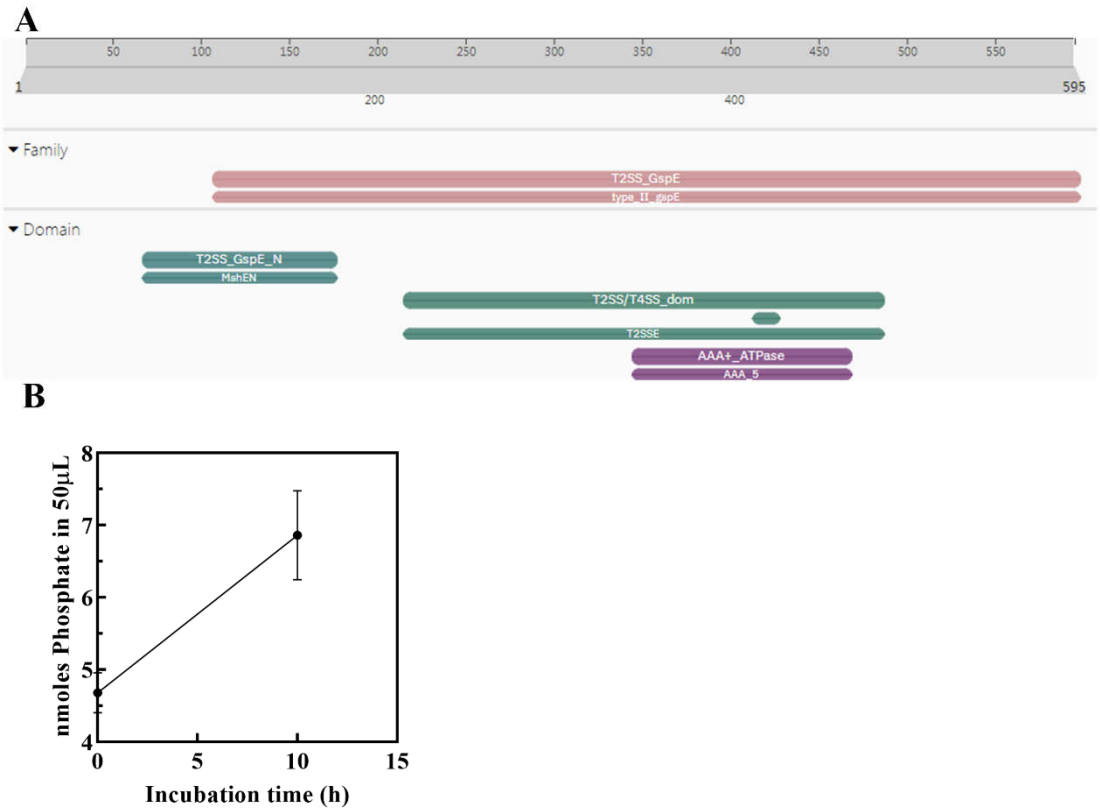

Figure S2. (A) The amino acid sequence of LspE shows homology to the ATPase GspE of T2SS, as indicated by the alignment with the Pfam database. (B) *In vitro* ATPase activity assay of LspE-His showed negative result.

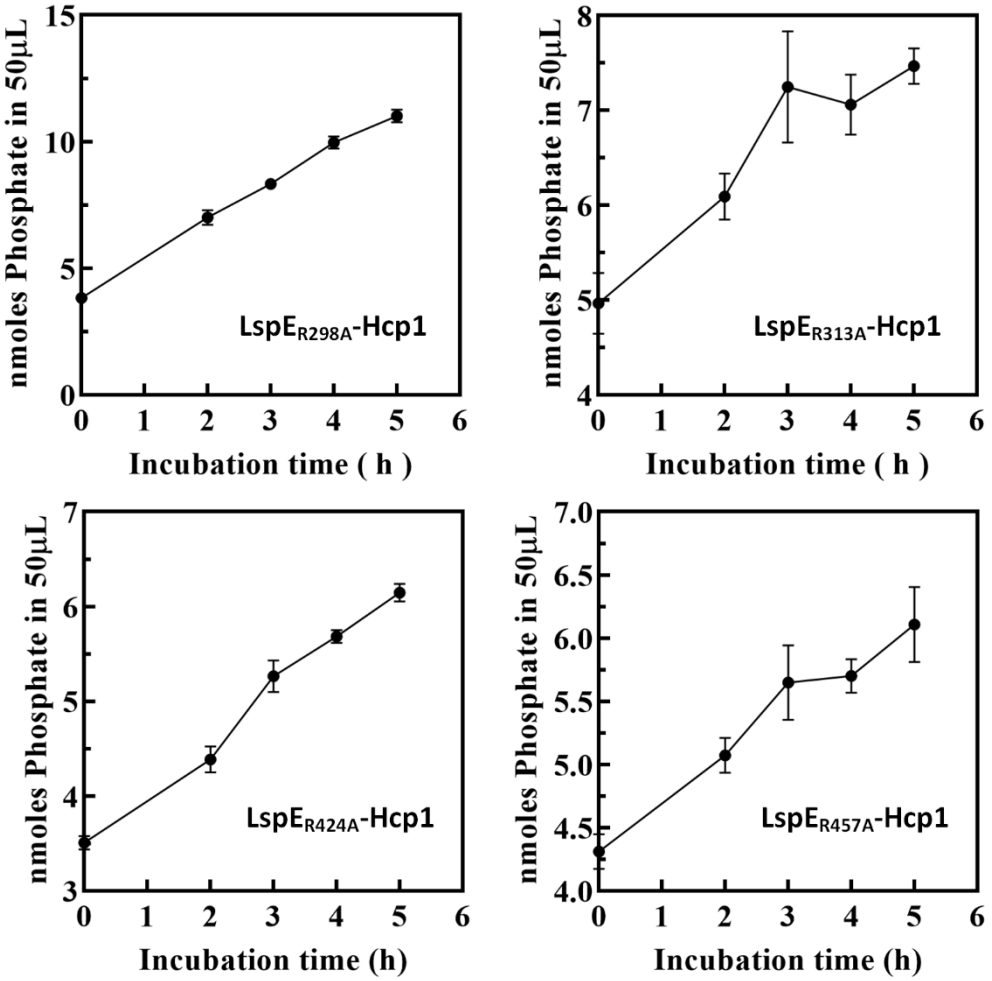

Figure S3. *In vitro* ATPase activity assay of LspE$_{R298A}$-Hcp1, LspE$_{R313A}$-Hcp1, LspE$_{R424A}$-Hcp1 and LspE$_{R313A}$-Hcp1 showed negative results.

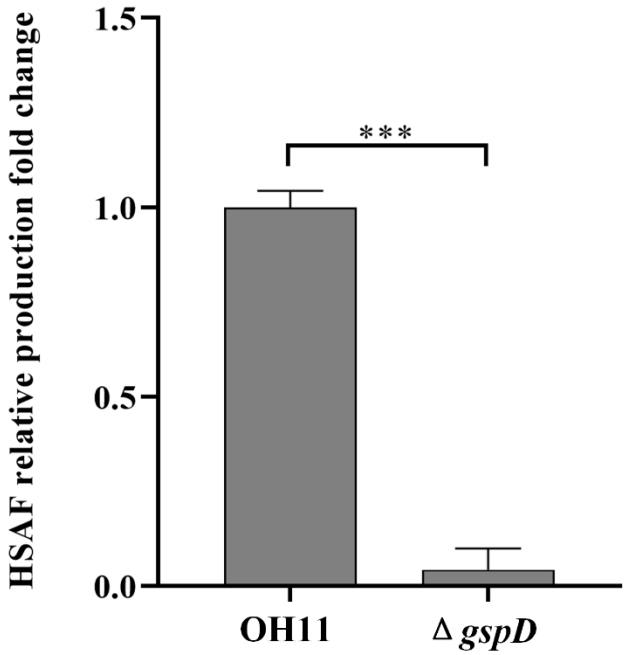

Figure S4. HSAF production detection. Statistical comparisons were performed with GraphPad software (GraphPad, La Jolla, CA) using t-test. The error bars indicate standard errors. ***P<0.001, relative to the wild-type OH11. Biological experiments for each treatment were performed three times and assayed in triplicate.

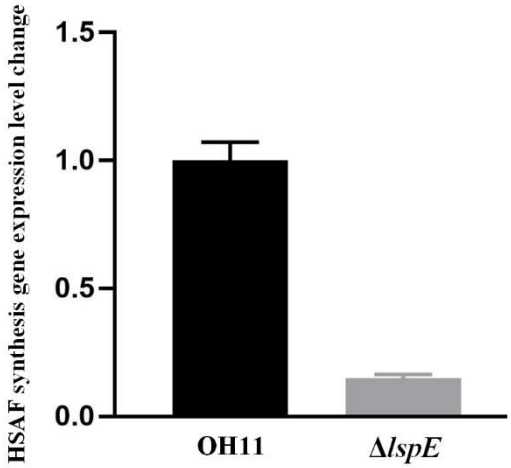

Figure S5. HSAF biosynthesis gene expression level detected by qRT-PCR.

References

Hoang, T.T., Karkhoff-Schweizer, R.R., Kutchma, A.J., and Schweizer, H.P. 1998. A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. *Gene* 212: 77-78.

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