

Supplemental information

Secretion of flagellar proteins by the

***Pseudomonas aeruginosa* type III secretion-injectisome system**

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Table S1. Bacterial strains used in this study

Strain	Relevant characteristics	Reference
<i>Escherichia coli</i>		
DH5 α	<i>recA</i> cloning strain	(1)
Sm10 λ pir	conjugation to <i>P. aeruginosa</i>	(2)
<i>Pseudomonas aeruginosa</i>		
PAK	wild-type laboratory strain	(3)
PAK Δ <i>fliC</i>	deletion mutant lacking <i>fliC</i>	(4)
PAK Δ <i>exoSTY</i>	deletion mutant lacking <i>exoS</i> , <i>exoT</i> , <i>exoY</i>	(5)
PAK <i>exsA</i> :: Ω	insertion mutant in <i>exsA</i>	(6)
PAK Δ <i>flhB</i>	deletion mutant lacking <i>flhB</i>	This study
PAK <i>exsA</i> :: Ω , Δ <i>flhB</i>	double <i>exsA</i> , <i>flhB</i> mutant	This study
PAK Δ <i>exoSTY</i> , Δ <i>flhB</i>	Δ <i>exoSTY</i> mutant lacking <i>flhB</i>	This study
PAK Δ <i>exoSTY</i> , Δ <i>fliC</i>	Δ <i>exoSTY</i> mutant lacking <i>fliC</i>	This study
PAK Δ <i>pscC</i>	deletion mutant lacking <i>pscC</i>	(7)

Table S2. Plasmids used in this study

Plasmid	Relevant characteristics	Reference
pJN105	Arabinose inducible expression vector	(8)
pEX18Gm	Allelic exchange vector, <i>sacB</i> marker, Gm ^R	(9)
pNMD60	Source of <i>B. pertussis cyaA</i> gene	N. Carbonetti
pUY30	pJN105 with ExsE-CyaA fusion	(10)
pExsE-VSV	pJN105 expressing ExsE-VSV	This study
pExsE-VSV-IAD	pJN105 expressing ExsE-VSV-IAD	This study
pFliC	pJN105 expressing full-length FliC	This study
pFliC-VSV	pJN105 expressing VSV-tagged FliC	This study
pFliC ₁₋₂₀ -CyaA	pJN105 expressing FliC ₁₋₂₀ -CyaA fusion	This study
pFliC ₁₋₃₉₄ -CyaA	pJN105 expressing FliC ₁₋₃₉₄ -CyaA fusion	This study
pFliC ₁₋₁₀₀ -VSV ⁻¹⁰¹⁻³⁹⁴	pJN105 expressing FliC ₁₋₁₀₀ -VSV ⁻¹⁰¹⁻³⁹⁴	This study
pFliC ₁₋₁₀₀ -VSV-IAD	pJN105 expressing FliC ₁₋₁₀₀ -VSV-IAD	This study
pFliC ₁₋₂₀ -VSV-IAD	pJN105 expressing FliC ₁₋₂₀ -VSV-IAD	This study
pFliD	pJN105 expressing FliD	This study
pFliD _{VSV}	pJN105 expressing VSV-tagged FliD	This study
pFliD-IAD	pJN105 expressing FliD-IAD fusion	This study
pFlgK	pJN105 expressing FlgK	This study
pFlgK _{VSV}	pJN105 expressing VSV-tagged FlgK	This study
pFlgK-IAD	pJN105 expressing FlgK-IAD fusion	This study
pFlgL	pJN105 expressing FlgL	This study
pFlgL _{VSV}	pJN105 expressing VSV-tagged FlgL	This study
pFlgL-IAD	pJN105 expressing FlgL-IAD fusion	This study
pFlgE	pJN105 expressing FlgE	This study
pFlgE _{VSV}	pJN105 expressing VSV-tagged FlgE	This study
pFlgE-IAD	pJN105 expressing FlgE-IAD fusion	This study
pFlgB	pJN105 expressing FlgB	This study
pFlgB _{VSV}	pJN105 expressing VSV-tagged FlgB	This study
pFlgB-IAD	pJN105 expressing FlgB-IAD fusion	This study
pEB124	pJN105 derivative expressing <i>exsA</i>	(11)

Table S3. Primers used in this study.

Primer ID	Primer Sequence
35521313	5'-AAAATCTAGAGCGCAGCAGGCTCAGAACC
35521314	5'-AAACATATGGCCTTGACCGTCAACACC
36637503	5'-AAAATCTAGAGGCCGACTGCAGGGACAGG
36965155	5'-TTTTCTAGAGTTGTTTCAGGTTCCGC
37090298	5'-GGTCATATGAGTTTCAACATCGGCCTGAGCGG
37132649	5'-AAAACATATGGCGAACAGTACGACGATCAA
37794979	5'-CCAGCATATGTCCGACCTACTCTCGATAGGCCT
37975890	5'-AGTACATATGAGCATCAGTTTTCGACAGAGCAC
37975891	5'-GCATGAATTCCCGTAGATGAAGGACACCGCGCTGA
37975892	5'-ACGAGGATCCGACGAGTGACCTGGCTGGAGCTAC
37975893	5'-ACGAGGATCCCTCGGCCATGGCTATTTTCGCCCGC
37975894	5'-GAAGAAGCTTACGGCGATGATCGTCGGCGTGCTG
38040753	5'-CCAGCATATGCGCATTTCACCCATCCAGGCCT
39481304	5'-TCTAGAGCTCTCATTTTCCTAATCTATTTCATTTCAATATCTGTATATCTAGAG CGCAGCAGGCTCAGAACGG
43468728	5'-AAAATCTAGAGCGCAGGTTGATGATGGTCTGG
43468729	5'-AAAATCTAGACTCTCCACGCAGGGCGCTGACC
43468730	5'-TTTTTCTAGACTTCAGGTAGTTGAACAGGCTCAACC
43468731	5'-AAAATCTAGAACGGAAAGGGTATCGAACAAAGTGC
43468732	5'-TTTTTCTAGAGCTTTTCTTCACAAGGCCAGGCAGG
43812189	5'-GATAGAATTCTCGAGGCCATGCGGCGGATGC
43933210	5'-GTGAAAGCTTAGAGCTCGAACATCGGTACCTTGC
47100509	5'-TTTTGAGCTCTTAGCGCAGCAGGCTCAGAACCGAC
47481608	5'-AAAAGAGCTCAGCGCAGGTTGATGATGGTCTGG
47481609	5'-AAAAGAGCTCTTACTCTCCACGCAGGGCGCTGACC
47481610	5'-TTTTGAGCTCACTTCAGGTAGTTGAACAGGCTCAACC
47481611	5'-AAAAGAGCTCAACGGAAAGGGTATCGAACAAAGTGC
47481612	5'-TTTTGAGCTCAGCTTTTCTTCACAAGGCCAGGCAGG
53931313	5'-AAAATCTAGATATACAGATATTGAAATGAATAGATTAGGAAAAGACACCGA CTTCGCAGCCGAAACC
54300848	5'-AAAATCTAGATATACAGATATTGAAATGAATAGATTAGGAAAAACGGCTC CAACAGCGACTCCGAGC

Table S4. Construction of plasmids used in this study.

Figure	Product	Primer Pair(s)	Cloning vector
N/A	pEX18Gm Δ <i>fliB</i>	37975891-37975892 37975893-37975894	pEX18Gm
N/A	pEX18Gm Δ <i>fliC</i>	43812189-43933210	pEX18Gm
N/A	pFliC-VSV	35521314-39481304	pUY30
Fig. 2-3	pFliC	35521314-47100509	pUY30
Fig. 4	pFliC ₁₋₂₀ -CyaA	35521314-36965155	pUY30
Fig. 4	pFliC ₁₋₃₉₄ -CyaA	35521314-35521313	pUY30
Fig. 5	FliC ₁₋₁₀₀ -VSV ₋₁₀₁₋₃₉₄	35521314-36637503 54300848-47100509	pUY30
Fig. 5	FliC ₁₋₁₀₀ -VSV-IAD	53931313-47100509	FliC ₁₋₁₀₀ -VSV ₋₁₀₁₋₃₉₄
Fig. 5	FliC ₁₋₂₀ -VSV-IAD	35521314-36965155	FliC ₁₋₁₀₀ -VSV-IAD
Fig. 6	pFliD _{vsv}	37132649-43468732	pFliC-VSV
Fig. 6	pFlgK _{vsv}	37794979-43468731	pFliC-VSV
Fig. 6	pFlgL _{vsv}	38040753-43468730	pFliC-VSV
Fig. 6	pFlgE _{vsv}	37090298-43468728	pFliC-VSV
Fig. 6	pFlgB _{vsv}	37975890-43468729	pFliC-VSV
Fig. 7	pFliD	37132649-47481612	pUY30
Fig. 7	pFlgK	37794979-47481611	pUY30
Fig. 7	pFlgL	38040753-47481610	pUY30
Fig. 7	pFlgE	37090298-47481608	pUY30
Fig. 7	pFlgB	37975890-47481609	pUY30
Fig. 7	pFliD-IAD	37132649-43468732	FliC ₁₋₁₀₀ -VSV-IAD
Fig. 7	pFlgK-IAD	37794979-43468731	FliC ₁₋₁₀₀ -VSV-IAD
Fig. 7	pFlgL-IAD	38040753-43468730	FliC ₁₋₁₀₀ -VSV-IAD
Fig. 7	pFlgE-IAD	37090298-43468728	FliC ₁₋₁₀₀ -VSV-IAD
Fig. 7	pFlgB-IAD	37975890-43468729	FliC ₁₋₁₀₀ -VSV-IAD

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