Pseudopilin residue E5 is essential for recruitment by the type 2 secretion system assembly platform

Supplementary data

Mangayarkarasi Nivaskumar[1,2#]*, Javier Santos-Moreno[2-6,]*, Christian Malosse[7], Nathalie Nadeau[1], Julia Chamot-Rooke[7], Guy Tran Van Nhieu[3-6] and Olivera Francetic[1,8]

[1]Laboratory of Macromolecular Systems and Signalling, Institut Pasteur, Department of Microbiology, CNRS ERL3526, 25 rue du Dr Roux, 75724 Paris, Cedex 15, France
[2]Université Paris Diderot (Paris 7) Sorbonne Paris Cité
[3]Laboratory of Intercellular Communication and Microbial Infections, CIRB, Collège de France, Paris France
[4]Institut National de la Santé et de la Recherche Médicale (Inserm) U1050, France
[5]Centre National de la Recherche Scientifique (CNRS), UMR7241, France
[6]MEMOLIFE Laboratory of Excellence and Paris Science Lettre, France
[7]Structural Mass spectrometry and Proteomics unit, CNRS UMR3528, Institut Pasteur, Paris, France
[#]Present address: Department of Chemistry, National University of Singapore, 3 Science Drive, Singapore 117543
* Co-first authors
[8]Correspondence: ofrancet@pasteur.fr
Tel: 33 1 40 61 36 81
Fax: +33 1 45 68 89 60

Table S1. Oligonucleotides used in this study

Name	Sequence (5'-3')
I-Kpn 5	GCAGGTACCTATGACGCTGATTGAAGTCATGG
I-Eco 3	GCTTTAGCGCGAATTCATGGCGATGTCACGTAGGTGC
H-Kpn 5	CGACAGGTACCCTTTACGTTGCTGGAGATGATGC
H-Eco 3	GGACTACGAATTCATTGCGCCTCCTGCGGTTCG
J-Kpn 5	GCTCGGTACCCTTTACCCTCGTCGAAATGCTG
J-Eco 3	CTGTCAGGATCATCGGCTGTCTCCCGGCGTAAGC
G-Kpn 5	CGACAGGTACCATTCACCTTACTGGAAATCATGGTGG
G-^{E5A} Kpn 5	CGACAGGTACCATTCACCTTACTGGCCATCATGGTGG
G-Eco 3	CAACGAATTCCCGCGCTGTCGCACTATTTC
K-Kpn 5	CCGGGTACCCATCGCCCTGCTCATGGTGCTGCTGATCCTC
K- Eco 3	AGTGAATTCATTCATCGGCTACCCAGTAGATTCC
L-Kpn 5	GCAGGTACCTATGAATAACCACCATACC
L-Eco 3	CACGAATTCCTGTTGCCATAAGGCGAG
F-Kpn 5	CAAGGTACCGCTGTTTCGTTACCAG
F-Eco 3	CCCGAATTCCATTAGAACAATCAC
C-Kpn 5	CCAGGTACCTATGCATAATTCCGTGATGAGATTAAC
C-Eco 3	CCAGAATTCGGAAAATGAGCGGATAACGTTGG
M-Bam 5	CACGGATCCGATGCATAACCTGCTCGCCTTATG
M-Eco 3	CACGAATTCGCAGGCCGATAGTGAGTCTA
pulH^{E5A}-Kpn 5	CGACAGGTACCCTTTACGTTGCTGGCGATGATGC
pulI^{E5A} 5	GGTATGACGCTGATTGCGGTCATGGTCGCCCTG
pulI^{E5A} 3	CAGGGCGACCATGACCGCAATCAGCGTCATACC
pulJ^{E5A} 5	GGCTTTACCCTCGTCGCGATGCTGCTGGCGCTG
pulJ^{E5A} 3	CAGCGCCAGCAGCATCGCGACGAGGGTAAAGCC
pulH^{E5A} 5	GCTTTACGTTGCTGGCGATGATGCTCATTTTG
pulH^{E5A} 3	CAAAATGAGCATCATCGCCAGCAACGTAAAGC
pulK M5 5	ATCGCCCTGCTCGAGGTGCTGCTGATCCTC
pulK M5 3	ATCAGCAGCACCTCGAGCAGGGCGATGCC

Figure S1. (Related to Figure 3)**.** Effect of 5th residue substitutions on pseudopilin function. Pullulanase (PulA) secretion was assayed in *E. coli* strain PAP5207 containing pCHAP8185 derivatives with single deletions of pseudopilin genes was complemented with pSU18 plasmid (-) or its derivatives expressing the wild type (WT) or the mutant (E5A or M5E) allele of the missing pseudopilin gene as indicated above the lanes. **A**. Results of experiment 1 are shown. Equivalents of 0.05 OD_{600nm} of cell (C) or supernatant (S) fractions were analysed on 9% Tris-Tricin SDS-PAGE and detected using anti-PulA antibodies. **B**. Results of experiment 2 are shown. Equivalents of 0.05 OD_{600nm} of cell (C) or supernatant (S) fractions were analysed on 9% Tris-Tricin SDS-PAGE and detected using anti-PulA antibodies.