Supporting Information

Contribution of the EssC ATPase to the assembly of the type 7b secretion system in *Staphylococcus aureus*

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There are 2 supporting tables, 2 figures with legends, and references list

Supporting tables

Table S1. Strains and plasmids used in this study.

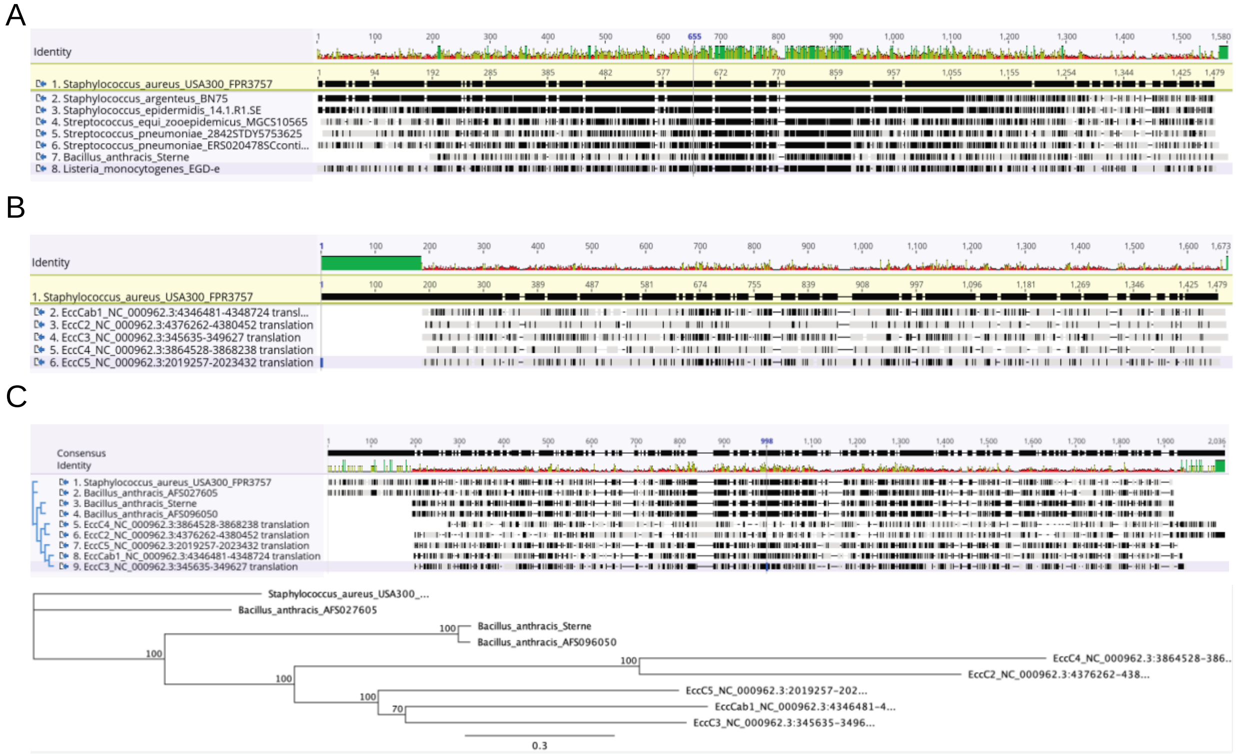
Strain or plasmid	Description	References
Strains		
DH5α	*E. coli fhuA2 lac*Δ*U169 phoA glnV44 Φ80′ lacZ*Δ*M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	Lab collection
USA300 LAC*	*S. aureus* USA300 LAC lacking pUSA03	(1,2)
RN4220	restriction deficient, cloning intermediate	(3)
Δ*essB*	*S. aureus* USA300 LAC* Δ*essB*	(4)
Δ*essC*	*S. aureus* USA300 LAC* Δ*essC*	This study
Δ*essB* Δ*essC*	*S. aureus* USA300 LAC* Δ*essBC*	This study
Δ*esaA* Δ*essC*	*S. aureus* USA300 LAC* Δ*esaA*, Δ*essC*	This study
Δ*esxA-0304*	*S. aureus* USA300 LAC Δ*esxA-SAUSA300_0304*	This study
Plasmids		
pOS1	*E. coli/S. aureus* shuttle vector	(5)
pKOR1	*E. coli/S. aureus* shuttle vector for allelic replacement in *S. aureus*	(6)
pWWW412	pOS1 derivative herein referred as vector	Laboratory collection
p*essC*	*P*$_{lgt}$-*essC*	This study
p*essC*$_{TS}$	*P*$_{lgt}$-*essC*$_{TS}$	This study
p*essC(1AB)*$_{TS}$	*P*$_{lgt}$-*essC(1AB)*$_{TS}$	This study
p*essC(2AB)*$_{TS}$	*P*$_{lgt}$-*essC(2AB)*$_{TS}$	This study
p*essC(*Δ*D3)*$_{TS}$	*P*$_{lgt}$-*essC(*Δ*D3)*$_{TS}$	This study
p*essC(*Δ*D2-3)*$_{TS}$	*P*$_{lgt}$-*essC(*Δ*D2-3)*$_{TS}$	This study
p*essC(*Δ*D1-3)*$_{TS}$	*P*$_{lgt}$-*essC(*Δ*D1-3)*$_{TS}$	This study
p*essC(*Δ*FHA1)*$_{TS}$	*P*$_{lgt}$-*essC(*Δ*FHA1)*$_{TS}$	This study
p*essC(*Δ*FHA2)*$_{TS}$	*P*$_{lgt}$-*essC(*Δ*FHA2)*$_{TS}$	This study
p*essC(*Δ*DUF)*$_{TS}$	*P*$_{lgt}$-*essC(*Δ*DUF)*$_{TS}$	This study
p*essC(*Δ*HM)*$_{TS}$	*P*$_{lgt}$-*essC(*Δ*HM)*$_{TS}$	This study
p*essBessC*$_{TS}$	*P*$_{lgt}$-*essBessC*$_{TS}$	This study
p*esaAessC*$_{TS}$	*P*$_{lgt}$-*esxA*^{STOP}*esaAessC*$_{TS}$	This study
p*esxA-essC*$_{TS}$	*P*$_{lgt}$-*esxAesaAessAesaBessBessC*$_{TS}$	This study
p*esxA*^{STOP}*-essC*$_{TS}$	*P*$_{lgt}$-*esxA*^{STOP}*esaAessAesaBessBessC*$_{TS}$	This study
p*esaA-essC*$_{TS}$	*P*$_{lgt}$-*esaAessAesaBessBessC*$_{TS}$	This study

Table S2. Primers used in this study.

Primer	**Sequence (5'⟶3')**
97F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTTAATGGATGTAATTATATGATGAAACTTCTGAAGCAGAGATGG
97R-XbaI	GGGGTCTAGATCTTAATCATTGCCATAACTAGAAACCTCCTG
98F-XbaI	GGGGTCTAGAAGCGACTTATCATAAACATCGTATATTGAAATTTCAAGATTTC
98R	GGGGACCACTTTGTACAAGAAAGCTGGGTGGTAAATAACGTAGTCGAAACGATAAGAACAAAAGATATATTAC
106F	GGGGACAAGTTTGTACAAAAAAGCAGGCTGCTGGCTATAGAGCGAAATTCATCTTAATG
106R	GTTATGATTTTTAACCATCTATTTTTCCTCCTATAGTAACTTC
107F	GAAGTTACTATAGGAGGAAAAATAGATGGTTAAAAATCATAACAGATGGTTTAAATAGCAATGAATTAAATAGGAGGGAG
107R	GGGGACCACTTTGTACAAGAAAGCTGGGTCATAGCTAAATGGTGCAAAATACATATCATTAATTCG
112F	CAGGAGGTTTCTAGTTATGGCAATGATTAAGAGATGGTTTAAATAGCAATGAATTAAATAGGAGGGAGG
112R	GGGGACCACTTTGTACAAGAAAGCTGGGTCAATAAAGGATATGATTGTACAATCGACCACC
121F	GGGGCTCGAGATGGCAATGATTAAGATGAGTCCAGAGG
121R	GGGGAGATCTTTTAAACCATCTAATCTTTTGATAAGCTTGGTTTGC
128F	GGGGACAAGTTTGTACAAAAAAGCAGGCTGAAGAAGTAAATCGTACACGCTACACATTTG
128R-XbaI	GGGGTCTAGACAATTTATGCATTGTCTTTGCCTCAGTCC
135F	GGGGCTCGAGATGAAAAAGAAAAATTGGATTTATGCATTAATTGTCAC
156Fmut	GTTAATCGGAAGTCCAGGATATGcagcAACAACGTTCTTAC
156Rmut	CGAAAATAATGTTGTGTAAGAACGTTGTTgctgCATATCCTGG
157Fmut	CAATTCCGCATGTCTTTATTCTTATTGcgAACTTTGACGC
157Rmut	GAAAGGTGAATCTTTTACTGCGTCAAAGTTcgCAATAAGAATAAAG
173F-P	/5Phos/TCATTTGCGTATCCTTCAATTCAAGATACGATG
173R-P	/5Phos/GACTTCGCCTAACGATTTCAACGTAATATCTGC
174F-P	/5Phos/TTAACACAAGAGATGCCACATGCACAGG
174R-P	/5Phos/TGCATAATCAGCTTCTGTATAAAGCTGTAATGTAATGTCATC
175F-P	/5Phos/GAGATGTATAACGTGAAAGAAGTAGATCAGCTTGATGTG
175R-P	/5Phos/GTAATCTTTCTCTCGTTTTTCAACATCTTTGTTATAC
176F-P	/5Phos/GAATATGTAAACCAAGATTTATCAGAATATGGTATTTCATTAATCTTTG
176R-P	/5Phos/TTGCTCATTACTTCTGCTGCGTTCACGCAC
203F	GGGGCTCGAGATGGCAATGATTAAGATGAGTCCAGAG

230F-P	/5Phos/GCTGAAAGAAAAAAATAGTATAGGACTGAGGCAAAGAC
230R-P	/5Phos/GCTATTCATCAACATTAGATTAATCTCTCTTTCTTAAAGTG
231F-P	/5Phos/ATGGTTAAAAATCATAACCCTAAAAATGAAATGCAAGATATG
231R-P	/5Phos/CTCGAGCATATGTTCACCTCAATTGTATTTATCCC
557	TTTCTCGAGATGCATAAATTGATTATAAAATATAAC
563	TTAAGATCTTATTTTTCAAATTGTGGATGTGACCATGCTGAAGAACCTCCACTACCTCCACCACTTCCACCACCTTTTTCAAATTGTGGATGTGACCATGCGGATCCTTTAAACCATCTAATCTTTTGATAAG
572	TTTGGATCCTATTTAAACCATCTAATCTTTTGATAAG
659	AAAAGGATCCCATCGTTTTGTATGTTTCGTTTTG
660	AAAAGGATCCTGGACGCTTAACTTCTTCGATTTC
661	AAAAGGATCCAGGAATATCATTTGGTGTTTCACC
662	CTTATCAAAAGATTAGATGGTTTAAAGGATCC
663	GCTCGAGATGCATAAATTGATTATAAAATATAACAAACAATTG
664	CAATTTATGCATCTCGAGCATATGTTCACCTC
665	GGGCATCTGAGATTATCCAATCATACATTTTATCTTTAG
666	GGATAATCTCAGATGCCCCTGAACCAGTGGTACCAGC
667	GCAGAGTTTGCCGAATTAAAATCAGAACAACC
668	TTCGGCAAACTCTGCGGAAATAATGAATAAATGTG
669	GGTCACATCCACAATTTGAAAAAGGTGGTGG
670	CAAATTGTGGATGTGACCATGCGGATCCTTTAAAC

Supporting figures



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FHA1 -----------------------------MHKLIIKYNKQLKMLNLRDGKTYTISEDERADITL-----------KSLGEVIHLEQNNQGTWQANHT---------SINKVLVRKGDLDDIT LQLYTEA    1-80
FHA2 DYASFAYPSIQDTMTIGPNAYDDMVIQSLMNAIIIKDFQSI-----QESQYVRIVHDKNTDVYINYELQEQLTNKAYIGDHI----YVEGIWLEVQADGLNVLSQNTVASSLIR-------- -------    81-185
                                  *: :***  :.:     ::.:   * .*:.:*: :            :*: *      :* *   ::         :: . *:*
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Figure S1. Comparison of EssC homologues. A) Amino acid sequence alignment of EssC orthologues from *Staphylococcus aureus* USA300 (1), *Staphylococcus argenteus* BN75 (2), *Staphylococcus epidermidis* 14.1.R1 (3), *Streptococcus equi zooepidemicus* MGCS10565 (4), *Streptococcus pneumoniae* 2842STDY5753625 (5), *S. pneumoniae* ERS020478SC (6), *Bacillus anthracis* Sterne (7) and *Listeria monocytogenes* EGD-e. **B)** Amino acid sequence alignment of EssC from *S. aureus* USA300 (1) with EccC1-5 from *Mycobacterium tuberculosis* H37Rv (2-6). **C)** Amino acid sequence alignment of EssC orthologs from *S. aureus* USA300 (1), *B. anthracis* AFS027605 (2), *B. anthracis* Sterne (3) and *B. anthracis* AFS096050 (4) with EccC1-5 of *M. tuberculosis* H37Rv (5-9). Below, is the neighbor-joining consensus tree based on the sequence alignment above. Residues with 100% identity are marked black, >80% similar are dark grey, 60-80% similar are light grey and <60% similar is near white. **D)** Alignment of EssC domains FHA$_1$ and FHA$_2$ of *S. aureus* USA300. Identical residues are denoted (*), conserved (:) and semi-conserved (.).

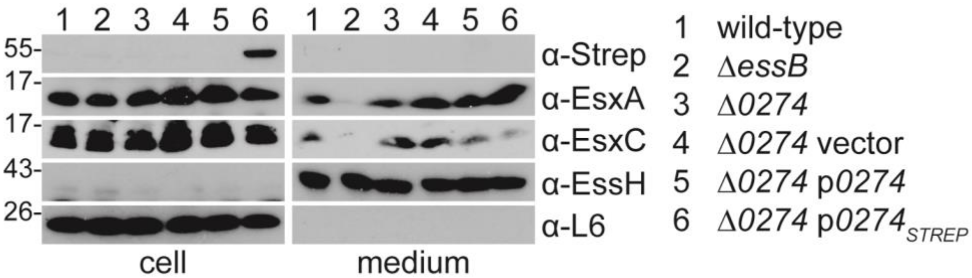

Figure S2. T7b-dependent secretion in the Δ*0274* mutant strain. Cultures of *S. aureus* USA300 (wild-type), Δ*essB*, Δ*0274*, Δ*0274* vector, Δ*0274* p*0274* and Δ*0274* p*0274*$_{STREP}$ strains were grown to A_{600} 3.0 and proteins in the extracellular medium (medium) fractionated from staphylococci in the sediment (cell) by centrifugation. Protein extracts from each fraction were evaluated by western blot using rabbit polyclonal antibodies α-EsxA, α-EsxC, α-EssH, α-L6 (ribosomal protein L6) as control, and mouse monoclonal antibodies α-Strep (Strep tag).

References cited

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