

SUPPLEMENTAL INFORMATION

A disulfide bond-forming machine is linked to the sortase-mediated pilus assembly pathway in the Gram-positive bacterium *Actinomyces oris*

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*Running title: *Post-translocational folding of Gram-positive pilins*

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Supplemental Data

Figure Legends

Figure S1: Structural analysis of *A. oris* MdbA – (A) Sequence alignment of actinobacterial MdbA and *B. subtilis*, *S. aureus*, and *M. tuberculosis* DsbA-like proteins was performed by ClustalW2. Conserved residues are highlighted. (B) Shown is the secondary structure of the MdbA protein based on the PDBSum server. α -helices and β -strands are labeled as H1 - H7 and β 1 – β 5, respectively. 3_{10} helices are not numbered. Structural motifs, i.e. β -turns, a γ -turn and β -hairpins, are marked as β , γ and \beth , respectively. The active site CSHC motif is outlined in red, and the *cis*-Pro motif is in black. (C) The electrostatic surface potential of the *A.oris* MdbA and *E.coli* DsbA proteins is colored according to charge; red is negative and blue is positive. The active site cysteine residues are shown as (C). The hydrophobic groove, an area that MdbA interacts with its redox partner, i.e. DsbB in *E. coli* or MdbB/VKOR in actinobacteria, is indicated as arrows. The hydrophobic patch of *E.coli* DsbA, an area stabilizing interactions with polypeptide chains, is also highlighted.

Supplemental Tables

Supplemental Table S1: Primers used in this study

Primer	Sequence ^(a)	Used for
FimA_F_C116A	GGTGTGCCGGACAGCGCCGCTGCCAACCCCG GCCCCC	pFimA _{C116A}
FimA_R_C116A	GGGGGCGGCGGGGTTGGCAGCGGCGCTGTC GGCACACC	pFimA _{C116A}
FimA_F_C157A	G TTCAGGCCTACCTCGTGGCTGAGACCACGAC CCTGGT	pFimA _{C157A}
FimA_R_C157A	ACCAGGGGTCGTGGTCTCAGCCACGAGGTAG CCTGAAC	pFimA _{C157A}
FimA_F_C394A	AACGCCTACGCCAACACCGCTTCCAACGAGAA GAGGGC	pFimA _{C394A}
FimA_R_C394A	GCCCTCCTTCTCGTTGGAAGCGGTGTTGGCGT GGCGTT	pFimA _{C394A}
FimA_F_C445A	GTGAATGCCATGGAGCGCGCTTACGTCCTGGT GAGACC	pFimA _{C445A}
FimA_R_C445A	GGTCTCAACCAGGACGTAAGCGCGCTCCATGG ATTCAC	pFimA _{C445A}
VKOR_A_HindIII_F	AAAAGCTTACTGCAACCTCGATGTCATCGCC	pCWU2-VKOR
VKOR_B_R	GAAGAGCCTGGCCAGATCTGTGGGCATGCGC ACAT	pCWU2-VKOR
VKOR_C_F	CGCATGCCACAGATCTGGCCAGGCTCTTCGG TGA	pCWU2-VKOR
VKOR_D_XbaI_R	AATCTAGACGTCGGTGTGCGGCTCAATGG	pCWU2-VKOR
VKOR_F_NdeI	AACATATGACGCCTCGGTAACGGTGG	pVKOR
VKOR_R_XbaI	AATCTAGATTCTTGGGCGCAGTCACC	pVKOR
mdbA _{Ao} _F_XbaI	AATCTAGACCACCCATCGGCCATCCATCATG	pAraC-MdbA _{Ao}
mdbA _{Ao} _F_ATG	ATGGTTCGTCGCTCGTCA	pAraC-MdbA _{Ao}
araC_F_KpnI	AAGGTACCCTACTGTTTCTCCATACCCGTTT	pAraC-MdbA _{Ao}
araC_R	TACCAATTATGACAACCTTGAC	pAraC-MdbA _{Ao}
MdbA _{Ao} _F_XbaI	AATCTAGACCACCCATCGGCCATCCATCATG	pMdbA _{Ao}
MdbA _{Ao} _R_EcoRI	AAGAATTCTCAGCCTTGCTGAGTCGGCTGAGG	pMdbA _{Ao}
MdbA _{Ao} _C216A_F	GCCTCTCACTGCGCCCAGTTTCGAG	pMdbA _{Ao} C216A; pMCSG7-MdbA _{Ao}
MdbA _{Ao} _C216A_R	GGAGTAGTCGAAGTAGATGTCGAGAACGGG	C216A pMdbA _{Ao} C216A; pMCSG7- MdbA _{Ao}
MdbA _{Cd} _BamHI_F	AAAGGATCCCGCCTTCGCACGGTTCTTCAT	C216A pMdbA _{Cd}
MdbA _{Cd} _BamHI_R	AAAGGATCCTTAGTGATGGTG	pMdbA _{Cd}
Lic_MdbA _{Ao} _DAK_F	TACTTCCAATCCAATGCAGACGCCAAGAAGAAC CCA	pMCSG7- MdbA _{Ao}
Lic_MdbA _{Ao} _VQG_R	TTATCCACTTCCAATGTCAGCCTTGCTGAGTCG C	pMCSG7-MdbA _{Ao}

Lic_MdbA _{Cd} _ANK_F	TACTTCCAATCCAATGCAGTGCAGGGCAAAGCAC AC	pMCSG7- MdbA _{Cd}
Lic_MdbA _{Cd} _ATS_R	TTATCCACTTCCAATGTTAAGAGGTTGCTTGCTCA ACCC	pMCSG7- MdbA _{Cd}
MdbA _{Cd} _C91A_F	TCGGCACCACATTGCGCCGAGCTTGGC	pMCSG7- MdbA _{Cd} - C91A
MdbA _{Cd} _C91A_R	GAAGTCCTCGTAGAAGTCGATCTTCTT	pMCSG7-MdbA _{Cd} - C91A

^(a) Underlined are the restriction sites in the primers.

Supplemental Table S2: Bacterial strains and plasmids used in this study

Strain & Plasmid	Genotype and description	Reference
<i>Strain</i>		
<i>A. oris</i> MG-1	Parental Strain	(1)
<i>A. oris</i> CW1	$\Delta galK$; an isogenic derivative of MG1	(1)
<i>A. oris</i> AR4	$\Delta fimA$; an isogenic derivative of CW1	(2)
<i>A. oris</i> MR108	$\Delta vkor$; an isogenic derivative of CW1	This study
<i>A. oris</i> MR111	MR108 containing pMdbA _{Ao}	This study
<i>S. oralis</i> So34	RPS-positive	(3)
<i>S. oralis</i> OC1	RPS-negative	(3)
<i>Plasmid</i>		
pJRD215	<i>Actinomyces/E. coli</i> shuttle vector, Kan ^R	(4)
pFimB	pJRD215 containing the <i>fimB</i> promoter	(2)
pJRD508FimB	pJRD215 containing the <i>fimB</i> promoter	(2)
pFimA	pJRD215 expressing wild-type <i>fimA</i> under control of the <i>fimB</i> promoter	(2)
pFimA-C116A	Derivative of pFimA harboring a C116A mutation	This study
pFimA-C157A	Derivative of pFimA harboring a C157A mutation	This study
pFimA-C394A	Derivative of pFimA harboring a C394A mutation	This study
pFimA-C445A	Derivative of pFimA harboring a C445A mutation	This study
pVKOR	pJRD215 expressing wild-type <i>vkor</i>	This study
pMdbA _{Ao}	pJRD215 expressing <i>A. oris</i> wild-type <i>mdbA</i>	This study
pAraC-MdbA _{Ao}	pJRD215 expressing <i>A. oris mdbA</i> under the control of an arabinose-inducible promoter	This study
pJRD-MdbA _{Cd}	pJRD215 expressing <i>C. diphtheriae</i> wild-type <i>mdbA</i>	This study
pMdbA _{Ao} -C136A	pJRD215 expressing <i>mdbA_{Ao}</i> C136A	This study
pCWU2	Integrative plasmid expressing gGalK under the control of the <i>rpsJ</i> promoter	(2)
pCWU2-VKOR	pCWU2 allelic replacement of <i>vkor</i>	This study
pMCSG7	Ligation-independent cloning for protein expression	(5)
pMCSG7-FimA	For recombinant FimA expression	(2)
pMCSG7-MdbA _{Ao}	For expression of recombinant MdbA _{Ao}	This study

pMCSG7-MdbA _{A0} C216A	For expression of recombinant MdbA _{A0} harboring a AxxC mutation	This study
pMCSG7-MdbA _{Cd}	For expression of recombinant MdbA _{Cd}	This study
pMCSG7-MdbA _{Cd C91A}	For expression of recombinant MdbA _{Cd} harboring a AxxC mutation	This study

Supplemental References

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A

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E. coli -----MKKIWLAL-----AGLVAFSASAA-----Q
A. oris  MVVRSSPHHKEHRVSSNQPRQTKAQRREAARLKAKELREAEARRARNTIARRSFIGAAG---ASVVGGLGYLVYLGVDAKKNPKSSK
S. aureus -----MTKKLLTLFIVSML---ILT---A---
B. subtilis -----MKKKQSS-----AK-----FAVILTVVVVLLAAIV---IINNKT-TE---
C. diphtheriae -----MSKNAG-S-----RKI---QNPT-----KSNGLFWALL--ALLVV-VVAVVTVVVVQGK-AHQANK
M. tuberculosis -----MADKSKRP-----PRF---DLKSAD-----GSFGRLVQIGGTTIVVVFVAVLVFYIVT-SR-DDKKDG

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E. coli ---YEDG-----KQYTTLEKPVAGAPQVLEFFSFCPHCYQFEVLHISDNVKKKLEPG-VKMTKYHVN
A. oris  FPAPEGLATAKANQGGIPKQVLSASWYEGEALDTVAASAPVLDIYFDYSCSHCQAFEGLHTQEINQ--LLSDKKITLALHPCKL
S. aureus ---CGKKE---SATTS-----SKNGKPLVVIYGDYKCPYCKELDEKVMPLRKN-YIDNHKVEYQFVNLA
B. subtilis ---QGND---VSGQPSIK-----GQPVLGKDDAPVTVEFGDYKCPSCKVFNSDIFPKIQKD-FIDKGDVVKFSFVNMF
C. diphtheriae YADYDKES---VSFTGSVTD-----AIVLKAVNAKDAKKIDFYEDFSCPHCAELGEVTDGPMTK--AIENGDIVVNLRLNF
M. tuberculosis VAGPG-DA---V---RVTSS-----K-LVTQPGTSNPKAVVSFYEDLCPACGIFERGFPGTVSK--LVDIGAVAADYTMVAI

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E. coli  MGGDLGKDLTQAWAVMALLGVEDK-----VTVPLEGVQKTK-----TIRSASDIRDVFINAGIKG
A. oris  LQQE-----WTSVVMNAMGVVLDEAPAQSLSFHNAAFEIFSQAIQTKNQSNMTVEGLVAAA-----AKVNVPKVESAK-FKAAVDS
S. aureus LGKDSIVG-----SRASHAVLMYAPKSFLDFQKQLFAAQDEN-----KEWLTKELLDKH-----IKQLHLDKETENKIKDYKTK
B. subtilis HGKGSRLA-----ALASEEVWKEDPDSFDFHEKLEKQPDTE-----QEWVTPGLLGD-----AKSTTKIKPET---LKENLDK
C. diphtheriae LDRDGDG-NSTKAGAAALAVAQSGDWETYWNYRALLMKEQNIY-----GKWGDNDFADVA-----KSLGASDEVTQK-IREGGAK
M. tuberculosis LDSASNQH-YSSRAAAAAYCVADE-SIEAFRRFHAALSKDI--Q-----PAELGKDFPDNARLIELAREAGVVGKVPDC-INSGYI

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E. coli  EEDAAWNSFVVKSLVAQQEKAAADVQLRGVFAMVNGKYQLNPQGMDSNMDV--FVQ-QYADTVKYLSEKK-----
A. oris  DKYG-KW-----VKLGDEAFKARELEGTPTVFFKGEKVDLNLK--QTPTSLTELVT---GSTPTAQSPQPTQQG
S. aureus DSKSWKA-----AEKDKKIAKDNHIKTTPTAFINGEKVEDPYDYESYEKLLKDKIK-----
B. subtilis -ETFASQ-----VEKSDSLNQKMNIQATPTIYVNDKVIKNFADYDEIKETIEKELKGG-----
C. diphtheriae EDFRKFA-----EANSKLEKDGGSVSSPRVFDGKEVKNGIETWVEQATS-----
M. tuberculosis EKVDGLA-----AA-----VNVHATTVRVNGTE-----YEWSTPAALVAKIKEIVGDVPGIDSAATATS-

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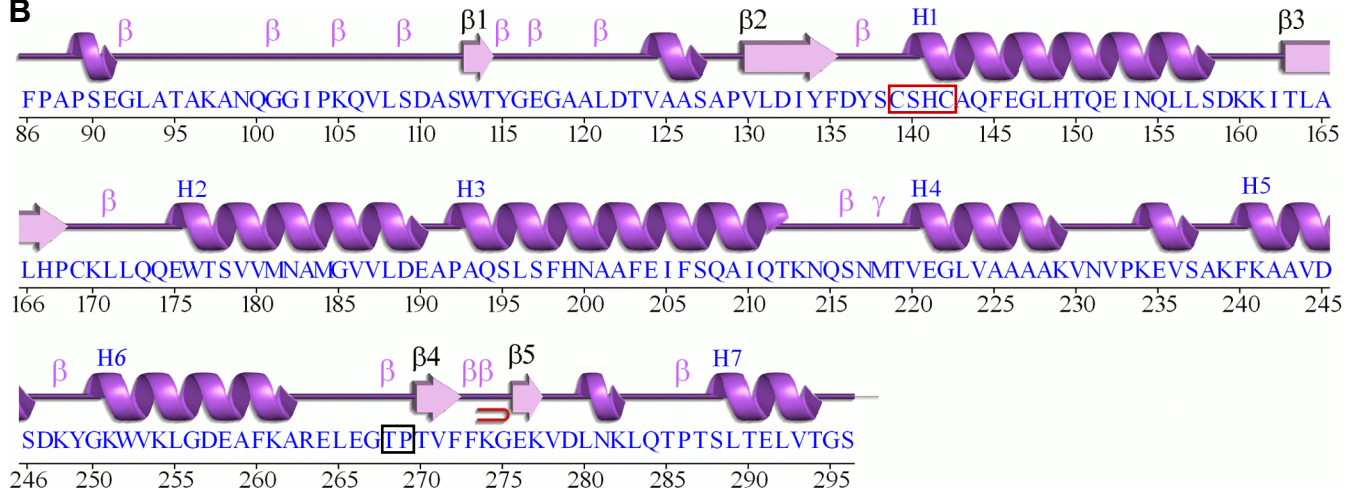
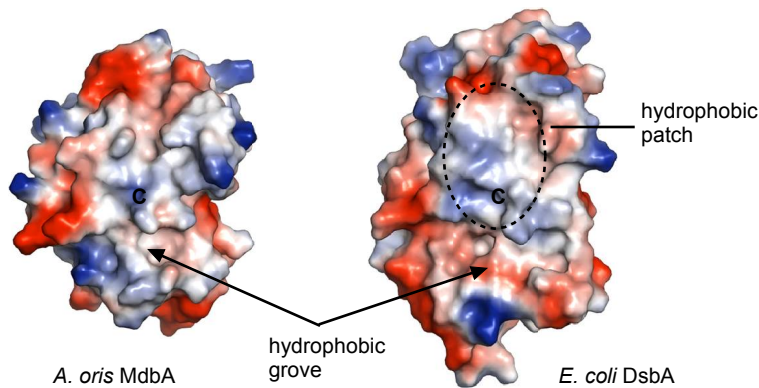
B**C**

Figure S1: Reardon-Robinson et al.