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 β1 – β5, 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 ב, respectively. The active side 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	GTTCAGGCCTACCTCGTGGCTGAGACCACGAC 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_Hindlll_ F	AAAAGCTTACTGCAACCTCGATGTCATCGCC	pCWU2-VKOR
VKOR_B_R	GAAGAGCCTGGCCAGATCTGTGGGCATGCGC ACAT	pCWU2-VKOR
VKOR_C_F	CGCATGCCCACAGATCTGGCCAGGCTCTTCGG TGA	pCWU2-VKOR
VKOR_D_Xbal_R	AATCTAGACGTCGGTGTGCGGCTCAATGG	pCWU2-VKOR
VKOR_F_Ndel	AACATATGACGCCTCGGTAACGGTGG	pVKOR
VKOR_R_Xbal	AATCTAGATTCCTTGGGCGCAGTCACC	pVKOR
mdbA$_{Ao}$_F_Xbal	AATCTAGACCACCCATCGGCCCATCCATCATG	pAraC-MdbA$_{Ao}$
mdbA$_{Ao}$_F_ATG	ATGGTCGTCCGCTCGTCA	pAraC-MdbA$_{Ao}$
araC_F_KpnI	AAGGTACCCTACTGTTTCTCCATACCCGTTT	pAraC-MdbA$_{Ao}$
araC_R	TACCAATTATGACAACTTGAC	pAraC-MdbA$_{Ao}$
MdbA$_{Ao}$_F_Xbal	AATCTAGACCACCCATCGGCCCATCCATCATG	pMdbA$_{Ao}$
MdbA$_{Ao}$ _R_Ecorl	AAGAATTCTCAGCCTTGCTGAGTCGGCTGAGG	pMdbA$_{Ao}$
MdbA$_{Ao}$ _C216A_F	GCCTCTCACTGCGCCCAGTTCGAG	pMdbA$_{Ao\ C216A}$; pMCSG7-MdbA$_{Ao\ C216A}$
MdbA$_{Ao}$ _C216A_R	GGAGTAGTCGAAGTAGATGTCGAGAACGGG	pMdbA$_{Ao\ C216A}$; pMCSG7- MdbA$_{Ao\ C216A}$
MdbA$_{Cd}$ _BamHl_F	AAAGGATCCCGCCTTCGCACGGTTCTTCAT	pMdbA$_{Cd}$
MdbA$_{Cd}$ _BamHl_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- $MdbA_{Cd\text{-}C91A}$
$MdbA_{Cd}$ _C91A_R	GAAGTCCTCGTAGAAGTCGATCTTCTT	pMCSG7-$MdbA_{Cd\text{-}C91A}$

[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
Strain		
A. oris MG-1	Parental Strain	(1)
A. oris CW1	Δ*galK*; an isogenic derivative of MG1	(1)
A. oris AR4	Δ*fimA*; an isogenic derivative of CW1	(2)
A. oris MR108	Δ*vkor*; an isogenic derivative of CW1	This study
A. oris MR111	MR108 containing pMdbA$_{Ao}$	This study
S. oralis So34	RPS-positive	(3)
S. oralis OC1	RPS-negative	(3)
Plasmid		
pJRD215	*Actinomyces*/*E. coli* shuttle vector, Kan^{R}	(4)
pFimB	pJRD215 containing the *fimB* promoter	(2)
pJRD508FimB	pJRD215 containing the *fimB* promoter	(2)
pFimA	pJRD215 expressing wild-type *fimA* under control of the *fimB* 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 *vkor*	This study
pMdbA$_{Ao}$	pJRD215 expressing *A. oris* wild-type *mdbA*	This study
pAraC-MdbA$_{Ao}$	pJRD215 expressing *A. oris mdbA* under the control of an arabinose-inducible promoter	This study
pJRD-MdbA$_{Cd}$	pJRD215 expressing *C. diphtheriae* wild-type *mdbA*	This study
pMdbA$_{Ao}$-C136A	pJRD215 expressing *mdbA$_{Ao}$* C136A	This study
pCWU2	Integrative plasmid expressing gGalK under the control of the *rpsJ* promoter	(2)
pCWU2-VKOR	pCWU2 allelic replacement of *vkor*	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-$MdbA_{Ao\ C216A}$	For expression of recombinant $MdbA_{Ao}$ 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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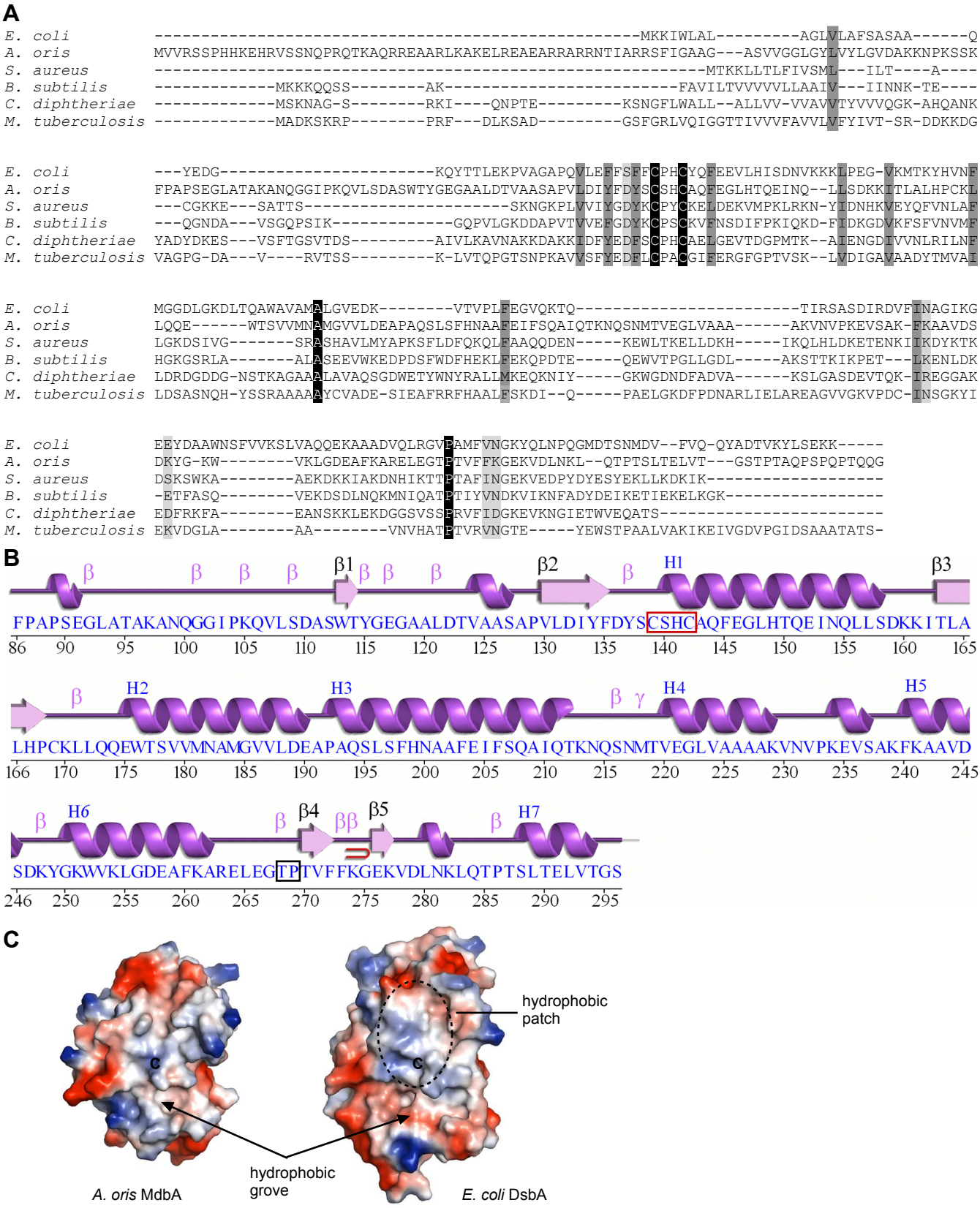

Figure S1: Reardon-Robinson et al.