

**A thiol-disulfide oxidoreductase of the Gram-positive pathogen *Corynebacterium diphtheriae* is essential for viability, pilus assembly, toxin production, and virulence**

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Running title: *Gram-positive protein folding required for virulence*

## SUPPLEMENTAL INFORMATION

**Figure S1:** Sequence alignment, based on structure homology, of *C. diphtheriae* MdbA and indicated *M. tuberculosis*, *B. subtilis*, *S. aureus* and *E.coli* DsbA-like proteins performed by DALI server (Holm & Rosenstrom, 2010). Depiction of the secondary structure of the MdbA protein was generated by ESPript/ENDscript server (Robert & Gouet, 2014). PDBs of the DsbA-like proteins are shown on the left.  $\alpha$ -helices,  $\beta$ -strands and  $3_{10}$  helices are labeled as  $\alpha 1 - \alpha 7$ ,  $\beta 0 - \beta 5$ , and  $\eta 1 - \eta 2$ , respectively.

## Supplemental Tables

**Table S1:** Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Reference
<b><i>C. diphtheriae</i> Strains</b>		
NCTC13129	Parental Strain (WT)	(Ton-That & Schneewind, 2003)
HT11	$\Delta spaA$	(Ton-That & Schneewind, 2003)
HT11 pSpaA	HT11 containing pSpaA	(Ton-That & Schneewind, 2003)
HT11 pC383A	HT11 containing pSpaA <sub>C383A</sub>	This Study
HT11 pC483A	HT11 containing pSpaA <sub>C443A</sub>	This Study
NJ2	$\Delta mdbA$	This Study
NJ6	NJ2 containing pMdbA	This Study
MR118	NJ2 containing pDsbA <sub>TM</sub>	This Study
HT3	$\Delta srtA\Delta srtF$	(Swaminathan <i>et al.</i> , 2007)
HT28	$\Delta tox$	This Study
XM5	$\Delta spaA-I$	This Study
<b><i>E. coli</i> Strains</b>		
MR128	BL21 containing pMCSG7-MdbA <sub>Cd</sub>	This Study
<b>Plasmids</b>		
pCGL0243	<i>Corynebacterium/E. coli</i> shuttle vector, Kan <sup>R</sup>	(Ankri <i>et al.</i> , 1996)
pK19MobsacB	<i>Corynebacterium</i> integration plasmid	(Schafer <i>et al.</i> , 1994)
pMCSG7	Ligation-independent cloning for protein expression	(Stols <i>et al.</i> , 2002)

pSpaA	pCGL0243 expressing WT <i>spaA</i>	(Ton-That & Schneewind, 2003)
pSpaA <sub>C383A</sub>	pSpaA harboring a C383A mutation	(Ton-That & Schneewind, 2003)
pSpaA <sub>C443A</sub>	pSpaA harboring a C443A mutation	(Ton-That & Schneewind, 2003)
pMdbA	pCGL0243 expressing WT <i>mdbA<sub>Cd</sub></i>	This Study
pMdbA <sub>C94A</sub>	pMdbA harboring a C94A mutation	This Study
pDsbA <sub>TM</sub>	<i>E. coli</i> DsbA fused to the N-terminal signal peptide and membrane anchor of MdbA	This Study
pK19mobsacB-MdbA <sub>Cd</sub>	pK19mobsacB allelic replacement of <i>mdbA<sub>Cd</sub></i>	This Study
pMCSG7-MdbA	For expression of recombinant MdbA	This Study

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**Table S2** Primers used in this study

Primer	Sequence <sup>(a)</sup>	Used for:
<i>spaA</i> _F_C383A	GCCGAGGCCGACGGCAGCCTAGTCAAGTCCGAC	pSpaA <sub>C383A</sub>
<i>spaA</i> _R_C383A	ACGATGCAGCTGGAACGTCGCGGTGCGATCG GC	pSpaA <sub>C383A</sub>
<i>spaA</i> _F_C443A	GGCAAGGGAACCGAATTCGCCCTGGTAGAAAC A	pSpaA <sub>C443A</sub>
<i>spaA</i> _R_C443A	GGCCCACGCGTCCGGTATATTTTCATAACATTGG A	pSpaA <sub>C443A</sub>
<i>dsbA</i> <sub>Ec</sub> _F	GCGCAGTATGAAGATGGTAAACAG	pDsbA <sub>TM</sub>
<i>dsbA</i> <sub>Ec</sub> _R_BamHI	<u>AAGGATCCG</u> CCCGTGAA TATTCA CGGGCTT	pDsbA <sub>TM</sub>
<i>mdbA</i> _A_F_HindIII	<u>AAGCTTA</u> ATGGCACCGTATGGTCTGACT	pK19mobsacB- MdbA
<i>mdbA</i> _B_R	CCCATCCACTAAACTTAAACACCTAGAACCAGC GTTTTTACT	pK19mobsacB- MdbA
<i>mdbA</i> _C_F	TGTTTAAGTTTAGTGGATGGGTGGGTTGAGCA AGCAACC	pK19mobsacB- MdbA
<i>mdbA</i> _D_R_XbaI	<u>TCTAGA</u> AAGAACTCACTGACACAAGCCC	pK19mobsacB- MdbA
<i>mdbA</i> _BamHI_F	AA <u>AGGATCC</u> CGCCTTCGCACGGTTCTTCAT	pMdbA, pDsbA <sub>TM</sub>
<i>mdbA</i> _BamHI_R	AA <u>AGGATCC</u> TTAGTGATGGTG	pMdbA
<i>PmdbA</i> _TM_R	CACAACAACATAGGTCACAAC	pDsbA <sub>TM</sub>
<i>mdbA</i> _C94A_F	GCCGCCGAGCTTGGCGAGGTCACCGATGG	pMdbA <sub>C94A</sub>
<i>mdbA</i> _C94A_F	ATG TGG GCA CGA GAA GTC CTC GTA GAA GTC	pMdbA <sub>C94A</sub>
Lic_ <i>mdbA</i> _ANK_F	TACTTCCAATCCAATGCAGTGCAGGGCAAAGCAC AC	pMCSG7- MdbA
Lic_ <i>mdbA</i> _ATS_R	TTATCCACTTCCAATGTTAAGAGGTTGCTTGCTCA ACCC	pMCSG7- MdbA

\*Restriction sites are underlined

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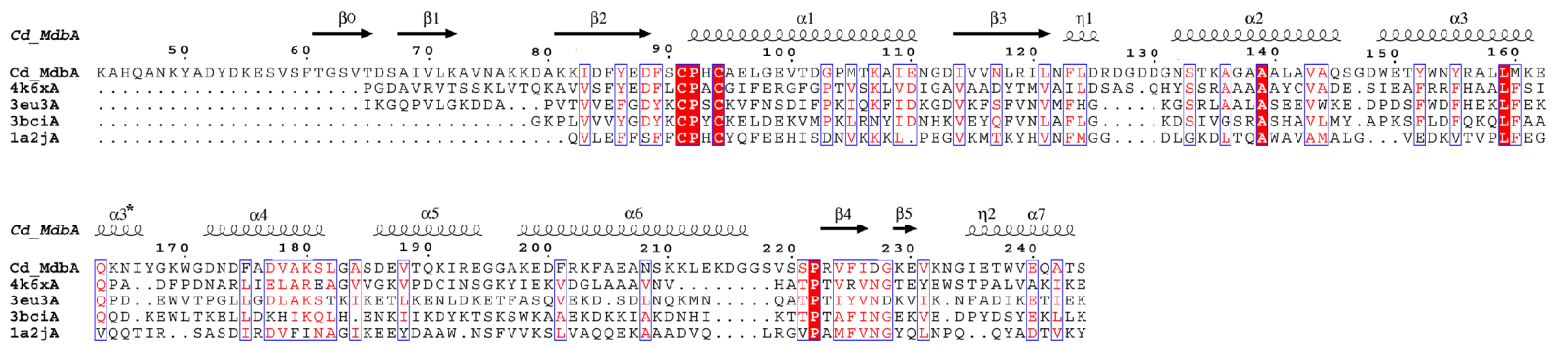


Figure S1: Reardon-Robinson et al.