Characterization of DsbD in *Neisseria meningitidis*

Pradeep Kumar,[1] Soma Sannigrahi,[1] Jessica Scoullar,[2] Charlene M. Kahler[2] and Yih-Ling Tzeng[1*]

1. Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA

2. University of Western Australia, Perth, WA, Australia

*Corresponding author. Mailing address: Emory University School of Medicine, Whitehead Research Building, Rm 245, 615 Michael Street, Atlanta, GA 30322. Phone: (404) 727-8393. Fax: (404) 712-2278. Email: ytzeng@emory.edu

Table S1. Primers used in this study

Name	Sequence (5'-3')	Note
YT143	GGGCAGCCAGTCGGTGTCCAGATG	dsbD promoter
YT144	CCTTTTCCGGCGGCAGCAGAT	dsbD promoter
dsbD-f2	GGGAATTCGGTATTCGGGATGGGCTTCG	dsbD promoter
dsbD-f3	GGGAATTCCGAAAAAGCGGCGGACATCG	dsbD promoter
dsbD-f4	GGGAATTCGGTTCGGATAATGGCGGGTT	dsbD promoter
dsbD-f5	CGGAATTCAATGCAGACAAATTATTTGTCTGA	dsbD promoter
dsbD-f6	CGGAATTCTAAATCCCACCGTCAACCTTAC	dsbD promoter
dsbD-r1	GGGAATTCCCTTTTCCGGCGGCAGCAGAT	dsbD promoter
dsbD-PE1	CGAAAGCTCGTCCGCACAACATCAAAAATAC	Primer extension
dsbD-qF1	GTTGGGACAGCCTTCTTTCA	qRT-PCR
dsbD-qR1	TTGCATAAGGAAAGGCAACC	qRT-PCR
dsbA1-qF1	TTGAGCGAGCACATCAAAAC	qRT-PCR
dsbA1-qR1	TGATTAACCATCGCATCGAA	qRT-PCR
dsbA2-F2a	TATTTTCGATGCGATGGTCA	qRT-PCR
dsbA2-R2	GATTTGGAAGGTTTCGGTCA	qRT-PCR
dsbA3-qF1	TGCGTACATTGCCATCATTT	qRT-PCR
dsbA3-qR1	GAGACAAAGCCCATTTTTCG	qRT-PCR
dsbB-qF1	TGTTGCGGCATATCAGTTGT	qRT-PCR
dsbB-qR1	CACAACTGATATGCCGCAAC	qRT-PCR
dsbC-qF1	CTATTCCGCCCAAGATTTGA	qRT-PCR
dsbC-qR1	TTCTTCGGTCAGGTTTTTGC	qRT-PCR
16sRTF	CCATCGGTATTCCTCCACATCTCT	qRT-PCR
16sRTR	CGTAGGGTGCGAGCGTTAATC	qRT-PCR
5Dkan-F	CGCCAGTGCCTCCATCATTTCGTG	dsbD mutation
5Dkan-R	TTCCTCCTAGTTAGTCACCCCCTTTTCCGGCGGCAGCAGATC	dsbD mutation
3Dkan-F	CCTGGAGGGAATAATGACCCCCGCGCTGCACCATTTCCTGA	dsbD mutation
3Dkan-R	TTGCCTCGCCTTGCCGTACTATCTGT	dsbD mutation

aphA3-cla-f2	AAAGCGATCGATCCCGGGTGACTAACTAGGAGGAA	dsbD mutation
aphA3-cla-r2	AAAGCGATCGATCCCGGGTCATTATTCCCTCCAGG	dsbD mutation
dsbD-5'-PacI	CCTTAATTAATTGTCTGAAACGGATTCGATTATGAA	complementation
dsbD-3-PmeI	AGCTTTGTTTAAACTCAGCGGTTTTGTTCATACCACTCGA	complementation
dsbD-For	GCGCGGGCGTTTGTGCTGTC	Southern blot
dsbD-Rev	GCCGGTGCCCAACGCCAAAGT	Southern blot
ΔdsbD-F	ATTTCATGCGGGTTCAATCTGGCT	dsbD mutation
ΔdsbD-R	CGTACTATCTGTACTGTCTGCGGCTTCG	dsbD mutation
DAP275	CGGAATTCCGTTGGAAGCCGAGTTGCAAAATGC	dsbA1 mutation
DAP263	CCCAAGCTTGCTGTTTGACATGGTCAATGTGCC	dsbA1 mutation
DAP418	TGTTTCATCACGCGGTGACCG	dsbA1 mutation
DAP140	CCTGTCTAGCTTCAAGTATGACG	Ω specific primer
DAP201	GATGAAGATGTTCAGCGGCAACG	dsbA2 mutation
DAP292	GACCGTAGGCATGGATTACAGC	dsbA2 mutation
DAP285	CTACGATATGCGCGGTGAAAGC	dsbA2 mutation
DAP420	CGACGTATTGACCCTGATTGAAGACG	dsbA2 mutation
DAP268	CGGAATTCCCAGCATCGAAAAAGCGTGCAGTGC	DsbA3 mutation
DAP269	CCCAAGCTTGGCAAACACTACCGCCAAAACTGC	DsbA3 mutation
DAP267	CAAACCAACCCCGGAACAAATCC	DsbA3 mutation
DAP265	CCAGCATGACTGCAACACTCAAGG	DsbA3 mutation

Fig. S1. PCR analyses of the *dsbD* locus. Primers annealing to the ends of *dsbD* coding sequence (dsbD-PacI and dsbD-PmeI) were used in PCR amplification. The incorrect *dsbD* mutants display a doublet pattern, while the correct *dsbD* mutants yield a single shorter PCR product resulting from the *dsbD::aphA3* deletion-insertional mutation. 1) wild type control, 2) the incorrect (*dsbD**) mutant obtained from the wild type strain, 3) the correct *dsbD* mutant created in the complemented strain, 4) the *dsbD** mutation in the *dsbA1* strain, 5) *dsbD** mutation in the *dsbA2* strain, 6) *dsbD** mutation in the *dsbA2A3* strain, 7) *dsbD* mutation in the *dsbA2A1* strain, 8) *dsbD* mutation in the *dsbA2A1A3* strain.

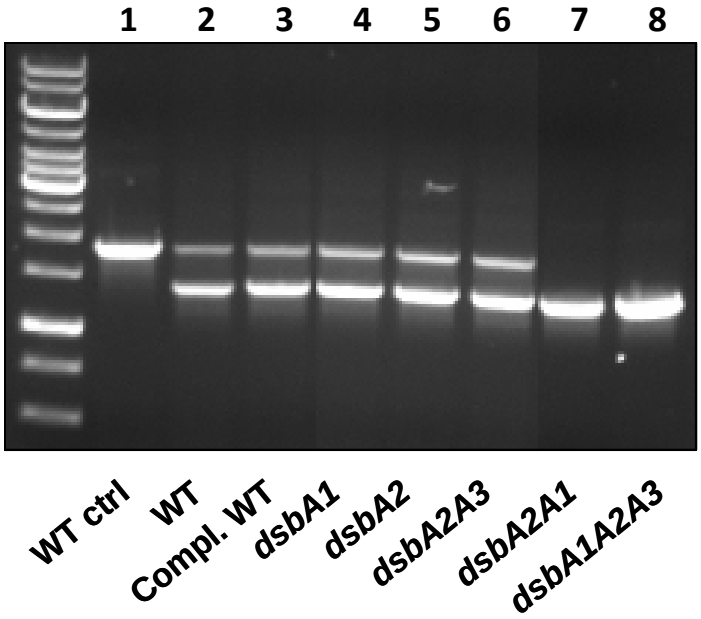

Fig. S2. The correct *dsbD* mutation cannot be obtained from transformation of the wild type strain in the presence of DTT supplementation. The transformants were selected on agar plates supplemented with varied concentrations of DTT, and examined with colony PCR using primers dsbD-PacI and dsbD-PmeI. All Kan^R transformants isolated from various DTT supplemented plates showed the doublet patterns indicative of the incorrect *dsbD* mutation.