SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS

Genetic manipulations. To construct a *S. gordonii* DL1 Challis *ΔsdbA* mutant, the mutated *sdbA* gene from *S. gordonii* SecCR1 *ΔsdbA* (1) was amplified by PCR using the primer pair SL756/SL759 (Table S1). This construct contains the regions flanking *sdbA*, separated by an erythromycin resistance cassette, *ermAM* (2). The construct was used to transform *S. gordonii* DL1 Challis as described previously (3), and transformants were selected on BHI agar with 10 μg/ml erythromycin. The mutation was confirmed by PCR.

RT-PCR. Expression of *sthA* was tested by RT-PCR using RNA isolated from cultures grown in BHI with 5% serum to an optical density of OD_{600} = 0.150. Following cDNA synthesis, *sthA* was amplified with the primer pair SL928/SL928, and the total RNA was assessed by amplification of *16S rRNA* using the primers SL525/SL697.

To test for polar effects on the upstream and downstream genes flanking *ciaRH*, *sgo.1071* and *sgo.1074* were amplified from cDNA prepared from the parent, *ΔciaRH, ΔsdbA ΔciaRH,* and the *ΔsdbA ciaRH*-complemented mutant. *sgo.1071* was amplified using the primer pair SL1178/SL1223, and *sgo.1074* was amplified with SL1220/SL1221. The resulting fragments were electrophoresed on a 2% agarose gel.

Transformation efficiency. Transformation efficiency was tested as described previously, with minor modifications (1). Cultures of DL1 Challis, the DL1 *ΔsdbA* mutant, and the SecCR1 *ΔsdbA* mutant were grown in BHI with 5% serum to a density of OD_{600} = ~0.150 and transformed with 150 ng of pDL276 (4). Following transformation, the cells were serially diluted and plated on BHI agar for the total cell count, or on BHI agar with 250 μg/ml kanamycin to count the number of transformants. The transformation efficiency was calculated as the ratio of transformants to total cells and expressed as a percentage of the parent strain, DL1 Challis.

Autolysis. Autolysis was tested as described previously (1). Briefly, overnight cultures of *S. gordonii* were diluted 1:20 into HTVG and grown at 37 °C, 5% CO2, to OD_{600} = 0.9 – 1.0. The cells were pelleted by centrifugation (3000 x *g*, 10 min) and washed with PBS. Pellets were then suspended in pre-warmed (44 °C) 20 mM potassium phosphate buffer (pH 6.5) containing 1 M KCl, 1 mM CaCl2 1 mM MgCl2, 0.4% sodium azide, 0.2% Triton X-100. The cell suspensions

were incubated in a 44 °C water bath, and autolysis was monitored by measuring the OD_{600} at regular intervals.

Cloning and expression of recombinant proteins. Recombinant plasmids to produce SdbA and SdbA C86P/C89A were constructed by cloning in frame fragments of the genes amplified with the primer pair SL763/764 into the expression vector pQE-30 (Qiagen, Valencia, CA) behind an N-terminal His_6-tag. Expression and purification of the recombinant proteins was carried out as described previously (1).

***In vitro* analysis of oxidase activity.** Oxidation of SdbA cysteine mutants was carried out using 0.5 mg/ml protein in 100 mM Tris (pH 8.8), 200 mM KCl, 1 mM EDTA and incubated with 100 mM oxidized glutathione. Excess glutathione was then removed by dialysis against 100 mM sodium phosphate buffer (pH 7), and aliquots were stored at –80 °C. Production of reduced, denatured RNase A (Sigma-Aldrich, Oakville, ON, Canada) and analysis of oxidase activity by RNase A catalyzed cCMP hydrolysis were carried out as described previously (1,5).

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2. **Claverys JP, Dintilhac A, Pestova E V, Martin B, Morrison DA**. 1995. Construction and evaluation of new drug resistance cassettes for gene disruption mutagenesis in *Streptococcus pneumoniae* using an *ami* test platform. Gene **164**:123–128.

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SUPPLEMENTARY FIGURE LEGENDS

FIG. S1. Mutation of *sdbA* and *ciaRH* in *S. gordonii* SecCR1 and *S. gordonii* DL1 Challis produces similar phenotypes in both strains. (A) Bacteriocin activity of culture supernatants from the SecCR1 and DL1 parent and their *ΔsdbA* and *ΔsdbAΔciaRH* mutants. The supernatants were filter sterilized and inoculated with the indicator strain *S. mitis* I18. Results are means ± SD of three experiments. (B) RT-PCR analysis of *sthA* expression in the DL1 parent, DL1 *ΔsdbA* mutant, SecCR1, and SecCR1 *ΔsdbA* mutant. Amplification of 16S rRNA is shown below as a control. (C) Transformation efficiency of the DL1 parent, DL1 *ΔsdbA* mutant, SecCR1, and SecCR1 *ΔsdbA* mutant. Bars represent the percentage of transformed cells relative to the DL1 parent strain. (D) Autolysis of the SecCR1 and DL1 parent strains and their *ΔsdbA* mutants.

FIG. S2. RT-PCR for the upstream and downstream genes flanking *ciaRH*. RT-PCR was used to amplify *sgo.1071* and *sgo.1074* from the parent (SecCR1) and its mutants, *ΔciaRH*, *ΔsdbAΔciaRH, and ΔsdbA ciaRH*-complemented mutant (*ΔsdbA* CiaRH Compl).

FIG. S3. *In vitro* oxidase activity of SdbA and SdbA C86P/C89A. SdbA catalyzed refolding of reduced and denatured RNase A in the presence of glutathione buffer. RNase A was incubated with affinity purified wild-type SdbA (circles), the double cysteine mutant (C86P/C89A) (squares), or without SdbA (triangles) as a negative control. Refolding was monitored by measuring hydrolysis of cCMP by active RNase A as the increase in absorbance at 296 nm.

Table S1. Primers used in this study

Primer	Gene		Description		Sequence (5'→3')
SL801	*aphA3*	For	Kanamycin resistance	BamHI	TACGGATCC GCAAGGAACAGTGAATTGGA
SL823	*aphA3*	Rev	Kanamycin resistance	KpnI	TACGGTACCCAGTTGCGGATGTACTTCAG
SL752	*degP*	For	DegP mutant		GTTGCTGGAACATGGGGAT
SL753	*degP*	Rev	DegP mutant	BamHI	TACGGATCCATTCCCTGAGTCACTGTATTAGC
SL754	*degP*	For	DegP mutant	EcoRV	TGAGATATCAGCCGTAATGTTTCTTCACGAT
SL755	*degP*	Rev	DegP mutant		TGTTTGCTCTTTTCCATCACG
SL764	*sdbA*	For	Expression	BamHI	TACGGATCCTCAGCTGTAGAACATGAGCTG
SL763	*sdbA*	Rev	Expression	HindIII	TACAAGCTTAAGCTCTCCCTTCTCTTTCTTT
SL756	*sdbA*	For	SdbA point mutants / *sdbA* mutant		ACCTGAACCAAATCGCAGAAT
SL762	*sdbA*	For	SdbA point mutants	BamHI	TACGGATCCTTAAAGGAAAAGTGGTGGCTAC
SL759	*sdbA*	Rev	SdbA point mutants / *sdbA* mutant		CGAACAACTGAAGTCCCCAG
SL803	*sdbA*	Rev	SdbA point mutants	BamHI	TACGGATCCAAGCTCTCCCTTCTCTTTCTT
SL974	*sdbA*	Rev	SdbA point mutants C86P		AGCTGGCCACCAGATTGTCAAAAACAGTT
SL975	*sdbA*	For	SdbA point mutants C86P		TGGTGGCCAGCTAGCCCACTCTAC
SL1038	*sdbA*	For	SdbA point mutants C89A		CCAGATGCTCAAAAACAGTTA
SL1039	*sdbA*	Rev	SdbA point mutants C89A		TAACTGTTTTTGAGCATCTGG
SL1178	*sgo.1071*	For	CiaRH mutant / CiaRH complemented mutant / RT-PCR		AAAACGCTGCAAAATAATCA
SL1179	*sgo.1071*	Rev	CiaRH complemented mutant	HindIII	TACAAGCTTTCTCCTCCTGCTATAAGATA
SL1180	*ciaR*	For	CiaRH complemented mutant	KpnI	TACGGTACCTCCATTTGTTAAAGTCATGAT
SL1220	*sgo.1074*	For	CiaRH mutant / RT-PCR	KpnI	TACGGTACCATGAAAATATTGATTTATGGTGCT
SL1221	*sgo.1074*	Rev	CiaRH mutant / CiaRH complemented mutant / RT-PCR		TTCAACCAATTCGCTAAATC
SL1222	*sgo.1071*	Rev	CiaRH mutant	BamHI	TACGGATCCTCTCCTCCTGCTATAAGATA
SL1223	*sgo.1071*	Rev	RT-PCR		GAATTGCTACCGTTTTCTTG
SL697	*16S*	For	RT PCR / qPCR		ATTTATTGGGCGTAAAGCGAGCGC
SL525	*16S*	Rev	RT PCR / qPCR		GAATTAAACCACATGCTCCACCGC
SL1214	*degP*	For	qPCR		TGGGAATAAGGTTCCTGGTG
SL1215	*degP*	Rev	qPCR		CGGCAGGAATTCTGACTACAG
SL1216	*ciaR*	For	qPCR		CATGCAGGTTTTTGATGGTG
SL1217	*ciaR*	Rev	qPCR		TCAGGAAGCATCAGATCCAG
SL931	*comC*	For	qPCR		AAACAAACAAAATCTATTGCCAAA
SL932	*comC*	Rev	qPCR		AAAGAATATATTTTCCCACCATAATC
SL1212	*comE*	For	qPCR		GCGCAATTTATACGCCAAC

SL1213	*comE*	Rev	qPCR	TCGCAAATTCTGAATGACTCG
SL927	*sthA*	For	RT PCR / qPCR	CCTCACCCTAACCGAAGATG
SL928	*sthA*	Rev	RT PCR / qPCR	AGCAATTCCTCCTGTGAAGC