SUPPLEMENTAL MATERIAL

The Phosphoenolpyruvate:Sugar Phosphotransferase system is involved in sensitivity to the glucosylated bacteriocin sublancin

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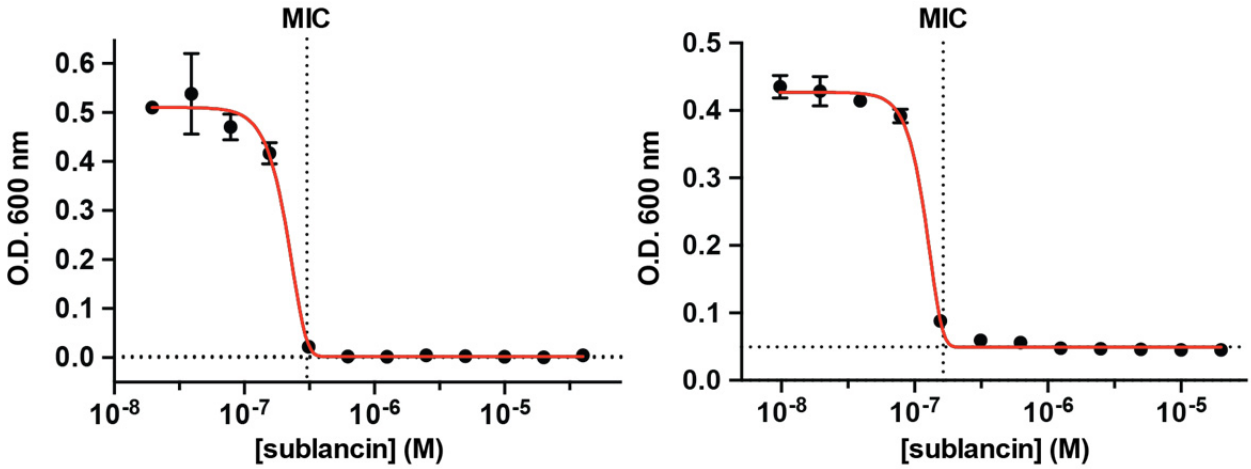
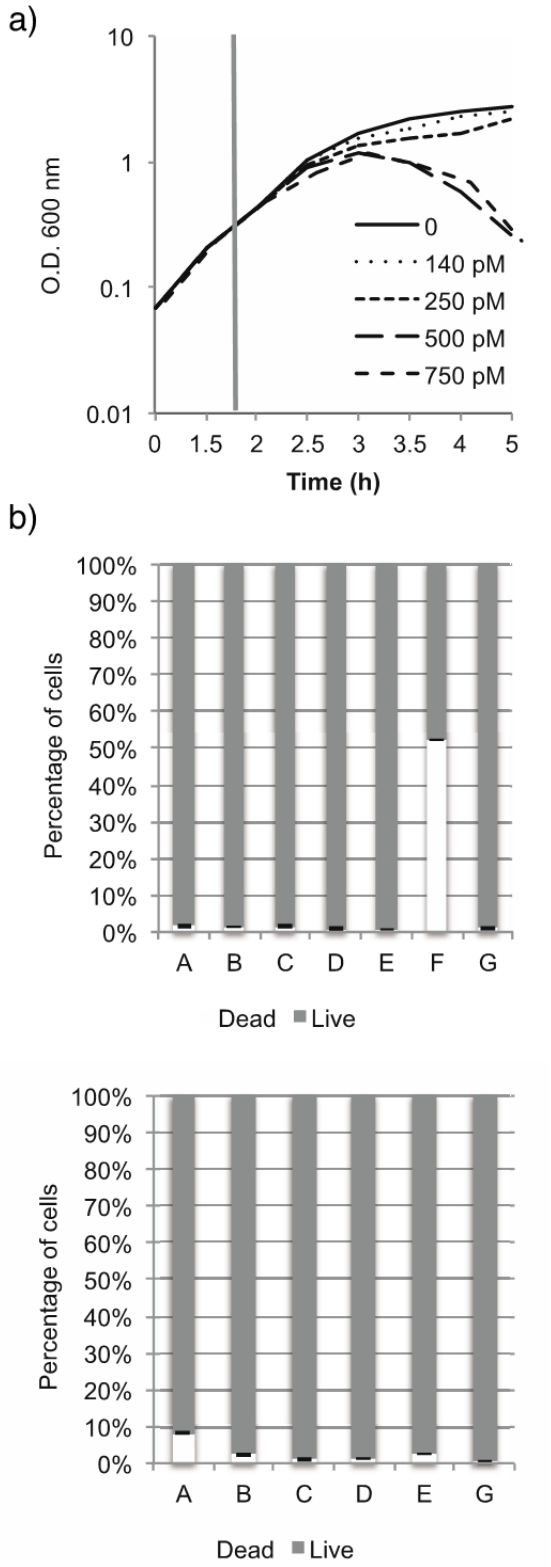
Supplementary Figures and Tables

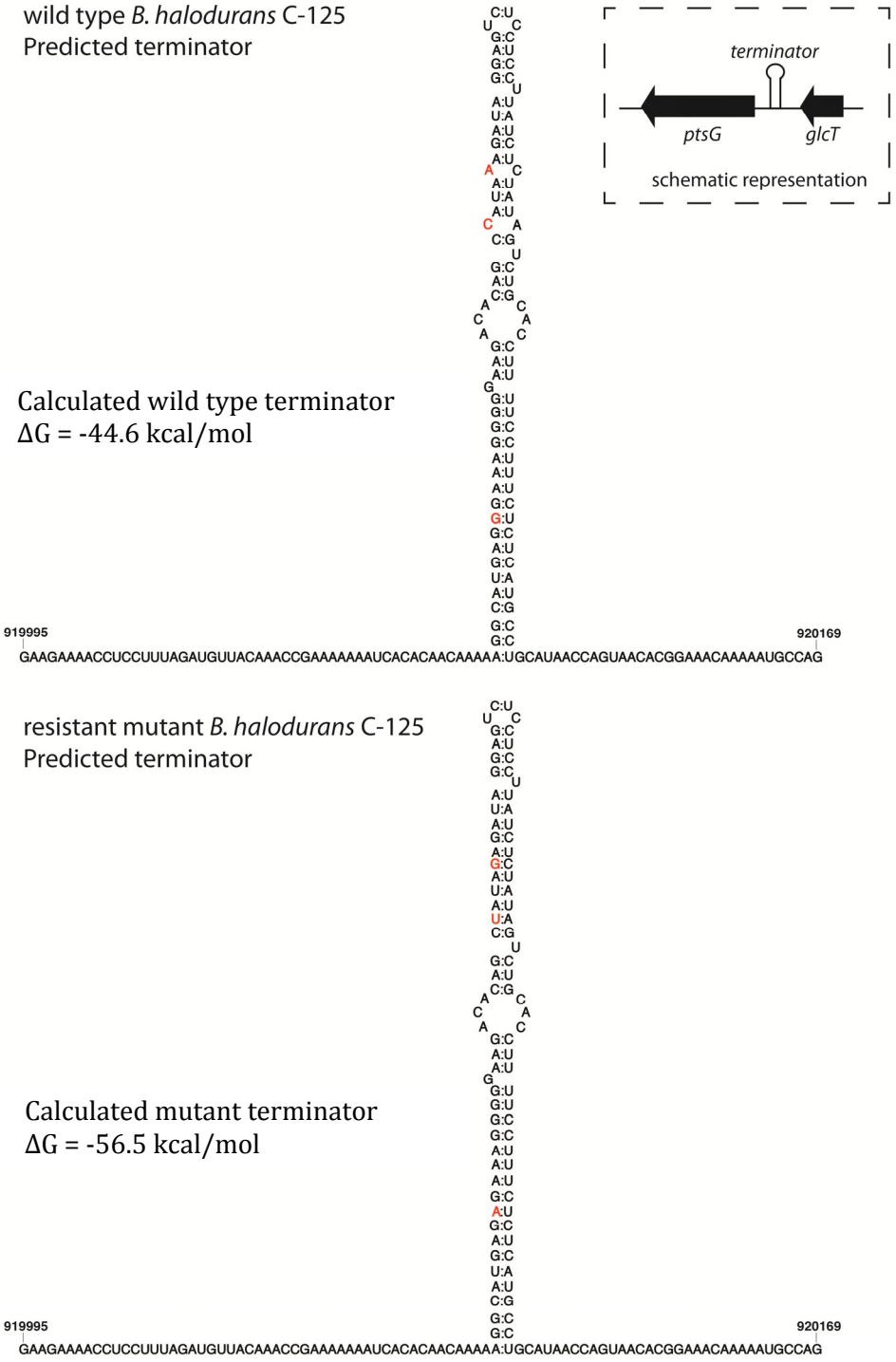


Supplementary figure S1. Determination of the specific activity of sublancin against *Bacillus subtilis* ATCC 6633 (left) and *Bacillus halodurans* C-125 (right) by the broth dilution method. Shown are the means of a single experiment conducted in triplicate as a representative of three independent experiments. For both graphs $R^2 > 0.99$. Error bars indicate standard deviations.



Supplementary figure S2. Sublancin does not affect the integrity of the bacterial membrane. (a) Growth curve of the *B. subtilis* Δ*SPβ* strain in the presence of different concentrations of sublancin. Sublancin was added at $O.D._{600nm}$ 0.5 (vertical grey line). Measurements were performed in a Synergy 4 plate reader every 10 min in triplicate and the means of the growth curve was plotted. (b) LIVE/DEAD® *Bac*Light™ bacterial cell viability assay of the Δ*SPβ* strain 30 (top) and 90 (bottom) min after exposure to sublancin.

Grey bars depict bacteria with an intact membrane and white bars depict bacteria with a compromised membrane, error bars depict standard deviation of triplicate experiments. A: 100 nM sublancin, B: 200 nM sublancin, C: 300 nM sublancin, D: 400 nM sublancin, E 500 nM sublancin, F Nisin (10 nM) was used as a positive control that does affect the integrity of the membrane, G negative control, no addition of an antimicrobial agent.



Supplementary figure S3. Predicted secondary structures of the terminator of (top) wild-type *B. halodurans* C-125 (accession no. NC_002570.2) and (bottom) the sublancin resistant mutant *ptsG* leader mRNA. Inset shows a schematic representation of the genomic locus. Terminators are shown in the antisense strand since flanking genes translate in an antisense fashion. The terminator starts at position 920136 and ends at position 920046. The ptsG starts at position 919994. Single nucleotide polymorphisms (SNPs) are labelled in red, and the location in the genome is shown. The RNAstructure Web Server (http://rna.urmc.rochester.edu/RNAstructure Web/index.html) at the University of Rochester Medical Center and the RNAfold Web Server (http://rna.tbi.univie.ac.at/) at

the University of Vienna were used to predict the secondary structures using default parameters. All three mutations result in Watson-Crick base-paired bases that increase the strength of the terminator. The predicted free energy values for the terminator loops were calculated using the RNAstructure Web Server.

M9-Glucose M9-Citrate M9-Malate

Supplementary figure S4. *B. subtilis* ΔSPB was spread over M9 agar plates containing 0.3% glucose, 0.4% citrate or 0.4% malate. Sublancin (2 µL of 100 nM) was spotted on the plates. The presence of glucose in the media resulted in resistance to sublancin.

yvkS-ykvWP1	ttctacacgaggcattacatt
yvkS-ykvWP2	CGACCTGCAGGCATGCAAGCTccctttctttctattgacga
yvkS-ykvWP3	CGAGCTCGAATTCACTGGCCGTCGaggctgctggctttttat
yvkS-ykvWP4	cacatgggtttcttcatttt
ykvY-glcTP1	ctttgataaaaccggaactg
ykvY-glcTP2	CGACCTGCAGGCATGCAAGCTtgttcctccctgccattt
ykvY-glcTP3	CGAGCTCGAATTCACTGGCCGTCGattcagtttatccttataacgtg
ykvY-glcTP4	gtgtttgatgttttcctggt
ptsG-ptsIP1	ctacaaagaggcattggaag
ptsG-ptsIP2	CGACCTGCAGGCATGCAAGCTaagaattgacctcctcttttt
ptsG-ptsIP3	CGAGCTCGAATTCACTGGCCGTCGtaacatggctaggagggata
ptsG-ptsIP4	aggaaaaacgaccctgtg

Supplementary table S1. Oligonucleotides used in this study to generate deletion mutants described in Table 2. Capital letters indicate overlapping regions with phleomycin resistance cassette, lower case letters indicate regions complementary to *B. subtilis* genome.

Nucleotide position	Locus tag	Genetic component	Frequency	SNP mutation
919515	BH0844	PTS system glucose-specific transporter subunit IIC	3/4	G → T
920055 **920075** **920079**	Between BH0844 BH0845	Intergenic region between transcriptional antiterminator and PTS system glucose-specific enzyme II, ABC components	1/4	G → A C → T A → G
3977494	BH3851	Mannitol-1-phosphate 5-dehydrogenase	1/4	T → C

Supplementary table S2. SNPs in genes of sublancin-resistant *B. halodurans* C-125 mutants determined by Illumina sequencing. The mutation in BH0844 results in a stop codon instead of a Tyr codon at position 160. The predicted domain organization of PtsG of *B. halodurans* C-125 is: 1-424 domain IIC, 425-524 domain IIB, and 525-675 domain IIA (see schematic drawing of the predicted domain organization in Supplementary table S4). Hence, the stop codon would delete most of PtsG and the respective truncated product would likely not be expressed as a stable protein. The mutation in BH3851 results in a His to Arg mutation. As shown in Supplementary figure 3, the mutations in the intergenic region result in a change in the predicted antiterminator structure that may affect transcription of *ptsG.*

Category and Locus Tag	Entrez Gene ID	Gene	Name	Fold Change in transcript level
Sulfur metabolism				
BH1487	890521	*sat*	Sulfate adenyltransferase	16
BH1486	890994	*cysH*	3'-phosphoadenosine 5'-phosphosulfate reductase	14
BH0088	891637	*cysK*	cysteine synthase A	11
BH1489	890544	*NA*	adenylylsulfate kinase	9
Transmembrane transporter activity				
BH1488	890630	*NA*	sodium-dependent phosphate transporter	12
BH3129	890493	*cysW*	sulfate ABC transporter (permease)	7
BH3128	890497	*cysT*	sulfate ABC transporter (permease)	5
BH3127	894370	*NA*	sulfate ABC transporter (sulfate-binding protein)	5
BH3130	890510	*cysA*	sulfate ABC transporter (ATP-binding protein)	5
Amino sugar and nucleotide sugar metabolism				
BH0421	892066	*nagA*	N-acetylglucosamine-6-phosphate deacetylase	9
BH3130	894561	*nagB*	N-acetylglucosamine-6-phosphate isomerase	5
BH0675	893039	*NA*	beta-hexosamidase A precursor	5
BH1086	894063	*glgD*	required for glycogen biosynthesis	4
BH1087	892001	*glgC*	glucose-1-phosphate adenylyltransferase	4
BH0422	893420	*NA*	PTS system, N-acetylglucosamine-specific enzyme II, ABC component	3
BH1874	892569	*XSA*	alpha-L-arabinosidase	3

Continued on next page

Category and Locus Tag	Entrez Gene ID	Gene	Name	Fold Change in transcript level
BH0673	893046	*NA*	PTS system, n-acetylglucosamine-specific enzyme II, ABC component (EIIABC-Nag)	2
BH3784	894582	*murZ*	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	-2
BH0065	892269	*gcaD*	UDP-N-acetylglucosamine pyrophosphorylase	-2
BH2564	894513	*murB*	UDP-N-acetylenolpyruvoylglucosamine reductase	-2
BH0797	892380	*NA*	Glucose kinase	-2
BH0267	894435	*NA*	phosphoglucosamine mutase	-3
BH0844	892408	*ptsG*	PTS system, glucose-specific enzyme II, ABC component	-4
BH3715	894300	*NA*	UDP-glucose 4-epimerase	-6
BH3343	891275	*pgi*	glucose-6-phosphate isomerase	-8
BH3074	890548	*ptsH*	PTS system, histidine-containing phosphocarrier protein (HPr)	-8
BH1515	890569	*ypqE**	PTS system, glucose-specific enzyme II, A component	-9

** Closest homolog in B. subtilis is a IIA stand-alone domain YpqE with unknown specificity.*
NA: specific gene name not available for B. halodurans C-125.

Supplementary table S3. Changes in transcript levels in *B. halodurans* C-125 in response to the addition of sublancin. Positive numbers indicate up-regulated transcripts, negative numbers indicate down-regulated transcripts.

Begin	End	Length	Localization
1	11	11	Inside
12	32	21	Membrane
33	43	11	Outside
44	60	17	Membrane
61	66	6	Inside
67	83	17	Membrane
84	88	5	Outside
89	109	21	Membrane
110	120	11	Inside
121	149	29	Membrane
150	168	19	Outside
169	189	21	Membrane
190	209	20	Inside
210	232	23	Membrane
233	276	44	Outside
277	295	19	Membrane
296	301	6	Inside
302	319	18	Membrane
320	324	5	Outside
325	349	25	Membrane
350	355	6	Inside
356	373	18	Membrane
374	378	5	Outside
379	401	23	Membrane
402	675	274	Inside

Supplementary table S4. Predicted topology of PtsG from *B. halodurans* C-125 (BH0844). The multidomain PtsG is 675 amino acids in length. PtsG IIC (region 1-424) is a transmembrane domain. PtsG IIB (region 425-524) and PtsG IIA (region 525-675) are cytoplasmic domains. Prediction obtained from the CAMPS (Computational Analysis of the Membrane Protein Space) database (identifiers: Q9KEK8_BACHD, GI:15613407).