TABLE S1. Strains used in this study

Organism	Serotype	Strain	ATCC	Source[a]	Notes
Bacillus anthracis		Δ sterne		1	
Bacillus cereus		14579		1	
Bacillus subtilis		SL4		1	
Bacillus thuringiensis		HD-73		1	
Enterococcus faecalis		V583		1	
Enterococcus faecium				1	EFSK-2
Escherichia coli		TOP10		1	
Group E streptococci	2	K131	123191	2	
Lactobacillus acidophilus		*Pak*	*4357*	3	
Lactobacillus acidophilus			*11975*	3	
Lactobacillus acidophilus			*4356*	3	
Lactobacillus gasseri			*19992*	3	
Lactobacillus rhamnosus		*LMS2-1*		3	
Lactobacillus rhamnosus			*21052*	3	
Listeria monocytogenes		HER 1184		1	
Listeria monocytogenes	4b	N3013		1	
Listeria monocytogenes	3b	FSLJ 1		1	
Listeria monocytogenes				1	RS823
Listeria monocytogenes				1	RS820
Listeria monocytogenes		HER1083		1	
Listeria monocytogenes			BAA-680	2	
Pseudomonas aeruginosa		RS1		1	
Staphylococcus aureus				4	vancomycin intermediate resistance IV
Staphylococcus aureus				4	vancomycin intermediate resistance III
Staphylococcus aureus		RN4220		1	
Staphylococcus aureus		Newman		5	methicillin sensitive - mutant LyrA
Staphylococcus aureus		Newman		5	methicillin sensitive
Staphylococcus aureus		MW2	BAA-1707	2	methicillin resistant - community acquired
Staphylococcus aureus		192		1	methicillin resistant
Staphylococcus aureus				1	methicillin resistant from patient DS
Staphylococcus aureus				1	highly mupirocin resistant
Staphylococcus aureus				1	D712 - daptomycin resistant
Staphylococcus aureus				1	0325 - daptomycin resisitant
Staphylococcus epidermidis		HER 1292		6	
Staphylococcus simulans				5	TNK3
Streptococcus agalactiae	Type II			1	Group B streptococcus
Streptococcus agalactiae		090R		1	Group B streptococcus
Streptococcus dysgalactiae				1	Group G streptococcus
Streptococcus dysgalactiae equisimilis		26RP66		1	Group C streptococcus
Streptococcus equi			9528	2	
Streptococcus equi zooepidemicus			700400	2	
Streptococcus gordonii			10558	2	
Streptococcus mutans		U159		1	
Streptococcus oralis		35037		1	
Streptococcus pneumoniae	9V	DCC1335		1	
Streptococcus pneumoniae	6	DCC1850		1	
Streptococcus pneumoniae	15	DCC1476		1	
Streptococcus pneumoniae	11			1	
Streptococcus pneumoniae				1	mutant Lyt 4-4
Streptococcus pyogenes	M6	D471		1	
Streptococcus pyogenes	M-neg	D471		1	mutant JRS75
Streptococcus pyogenes	M6	MGAS 10394	BAA-946	2	
Streptococcus pyogenes	M49	NZ131		7	
Streptococcus pyogenes	M4	SmR		1	streptomycin resistant - mucoid
Streptococcus pyogenes	M3	MGAS 315	BAA-595	2	
Streptococcus pyogenes	M18	MGAS 8232	BAA-572	2	
Streptococcus pyogenes	M1	CEM1ΔΦ		1	mucoid – mouse passaged
Streptococcus pyogenes	M1	CEM1ΔΦ		1	
Streptococcus pyogenes	M1	SF370		1	mucoid – mouse passaged
Streptococcus pyogenes	M1	SF370		7	
Streptococcus pyogenes	M1	MGAS 5005	BAA-947	2	
Streptococcus rattus		BHT		1	
Streptococcus sanguinis			10556	2	
Streptococcus sobrinus		6715		1	
Streptococcus suis	9	7997		8	

[a] 1, The Rockefeller University Collection; 2, ATCC; 3, ContraFect Corporation, Yonkers, NY; 4, Alexander Tomasz, The Rockefeller University; 5, Olaf Schneewind, University of Chicago, Chicago, IL; 6, Barry Kreiswirth, Public Health Research Institute, New Jersey, NJ; 7, Joseph Ferretti, University of Oklahoma Health Science Center, Oklahoma City, OK; 8, Jaap A. Wagenaar, Utrecht University, Utrecht, Netherlands.

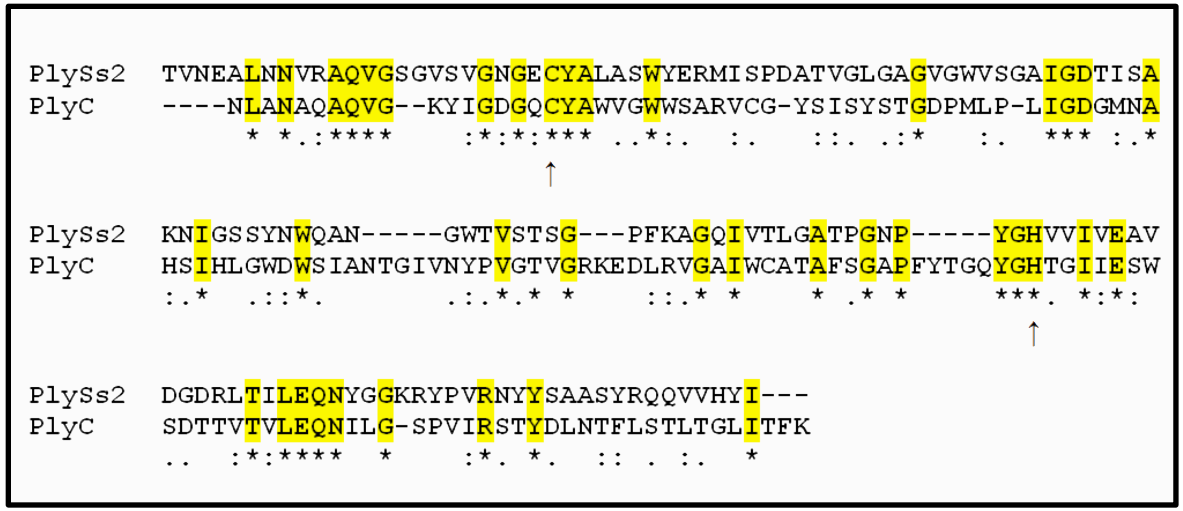
A

MTTVNEALNN VRAQVGSGVS VGNGECYALA SWYERMISPD ATVGLGAGVG WVSGAIGDTI
SAKNIGSSYN WQANGWTVST SGPFKAGQIV TLGATPGNPY GHVVIVEAVD GDRLTILEQN
YGGKRYPVRN YYSAASYRQQ VVHYITPPGT VAQSAPNLAG SRSYRETGTM TVTVDALNVR
RAPNTSGEIV AVYKRGESFD YDTVIIDVNG YVWVSYIGGS GKRNYVATGA TKDGKRFGNA
WGTFK

B

FIG. S1. PlySs2 contains an amine-terminal catalytic domain and a carboxy-terminal binding domain. (A) PlySs2 amino acid sequence. (B) The catalytic domain corresponds to residues 8-146. The binding domain spans residues 162-228.

A



B

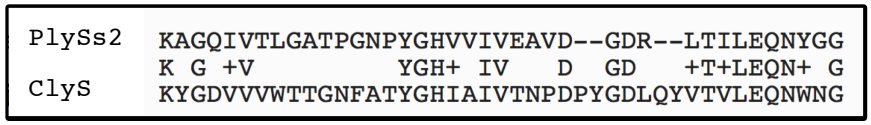


FIG. S2. PlySs2 enzymatic domain alignment. (A) The CHAP domains of the streptococcal lysins PlySs2 and PlyC (subunit A, GenBank no. AAP42310) are aligned here. Amino-acid identities are indicated with underlying asterisks and highlighting. The positions of the presumptive catalytic residues (cysteine and histidine, for which the domain is named is named) are indicated with arrows. (B) The aligned catalytic domains of the staphylococcal lysins PlySs2 and ClyS.

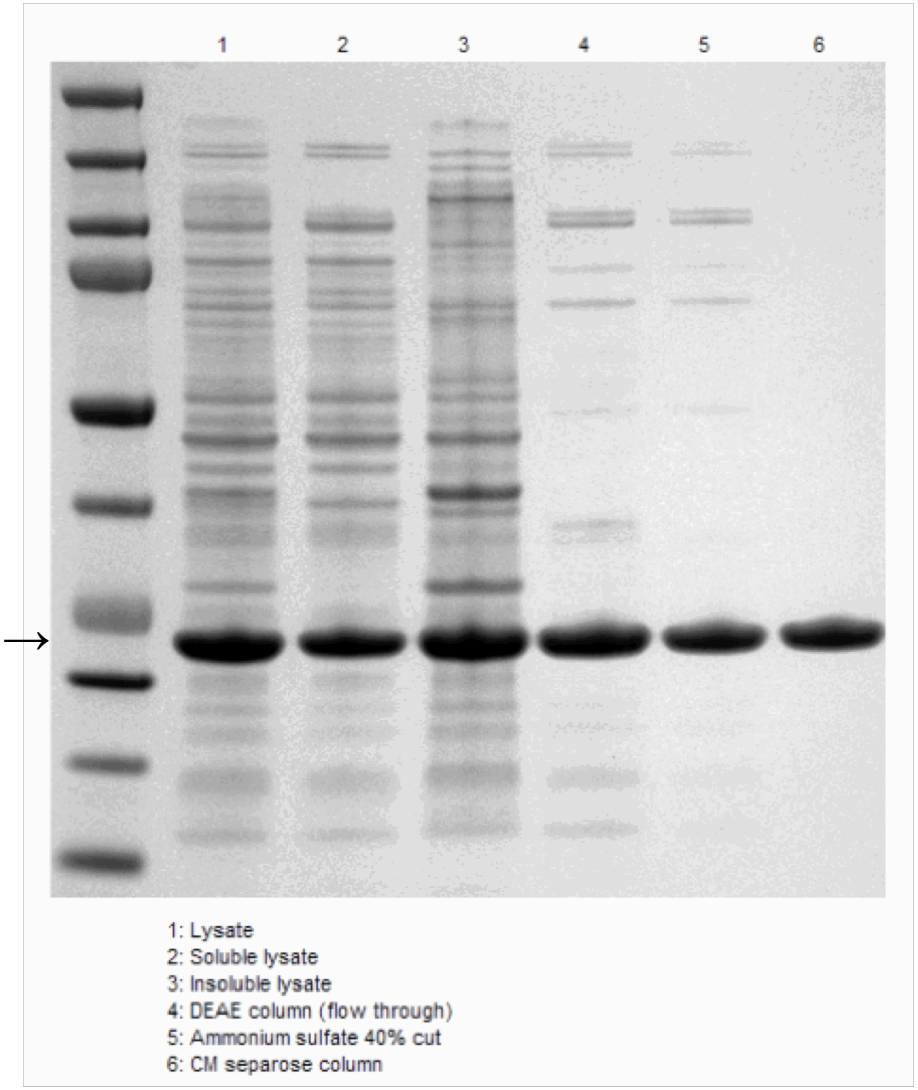


FIG. S3. PlySs2 corresponded to bands at ~26 kDa on SDS-PAGE. All PlySs2 purification samples were run on 4-12% Bis-Tris gels at 200 V for ~40 mins and stained with Coomassie. Lane 1: Whole cell lysate from *E. coli*. Lane 2: Supernatant from lysed *E. coli*. Lane 3: Pellet from lysed *E. coli*. Lane 4: The DEAE column flow through containing PlySs2. Lane 5: Resuspended pellet from 40% ammonium sulphate precipitation. Lane 6: A single band at ~26 kDa indicating the purity of PlySs2 after the CM column.

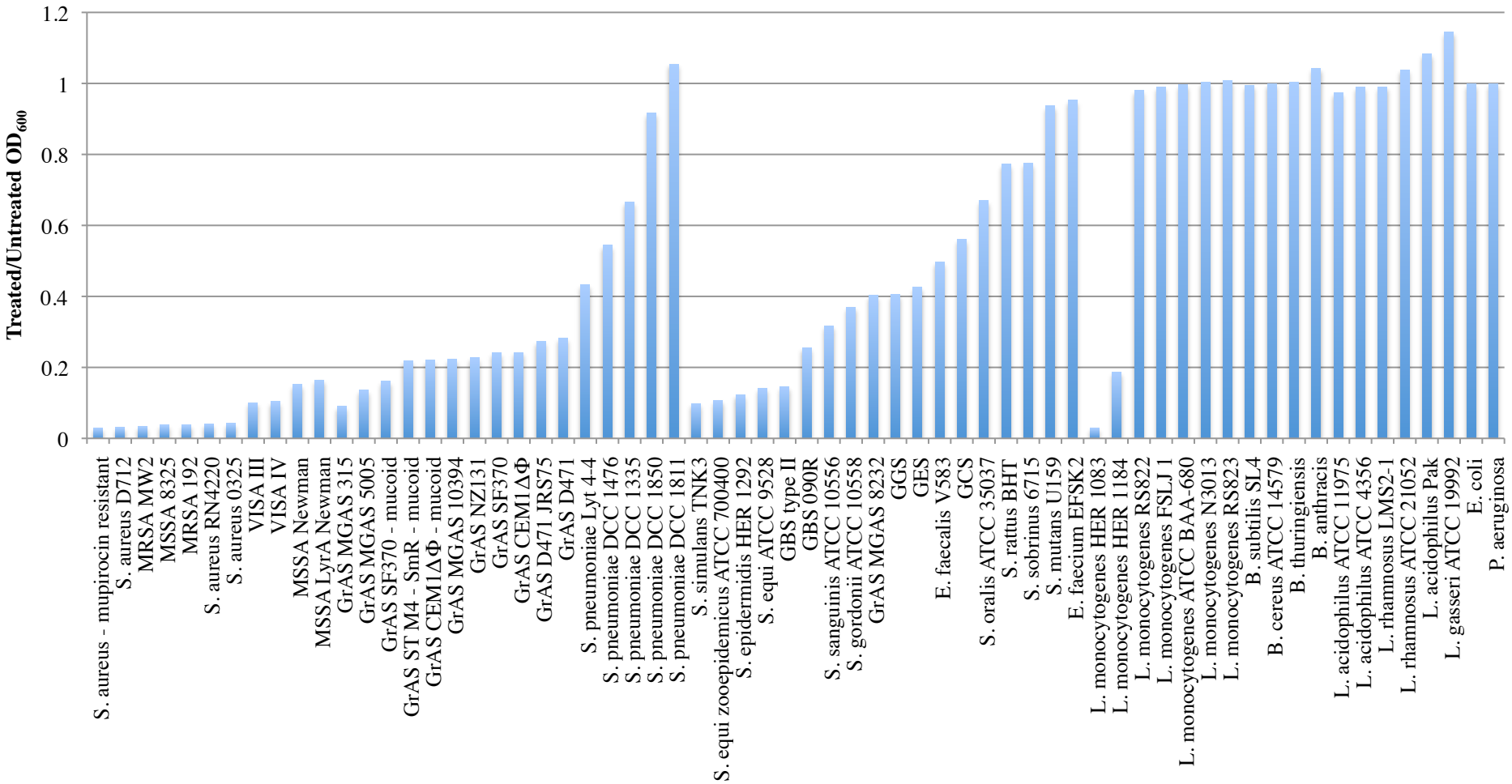


FIG. S4. PlySs2 displayed activity against various species over 60 minutes. Multiple strains of staphylococci (including MRSA, MSSA, and VISA), streptococci, enterococci, *Listeria*, bacilli, and lactobacilli were tested for susceptibility to PlySs2 activity. *Escherichia* and *Pseudomonas* were tested as Gram-negative controls. Log-phase cultures were exposed to 32 µg/ml PlySs2 for 60 minutes in PB. The final OD_{600} of the treated samples was divided by the final OD_{600} of the untreated samples to generate the normalized values. Complete lysis registers a ratio of ~0.02. The following abbreviations apply: ST, serotype; MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*; VISA, vancomycin-intermediate *S. aureus*; GrAS, group A streptococci, *S. pyogenes*; GBS, *S. agalactiae*; GGS, group G streptococci; GES, group E streptococci; GCS, group C streptococci.

.

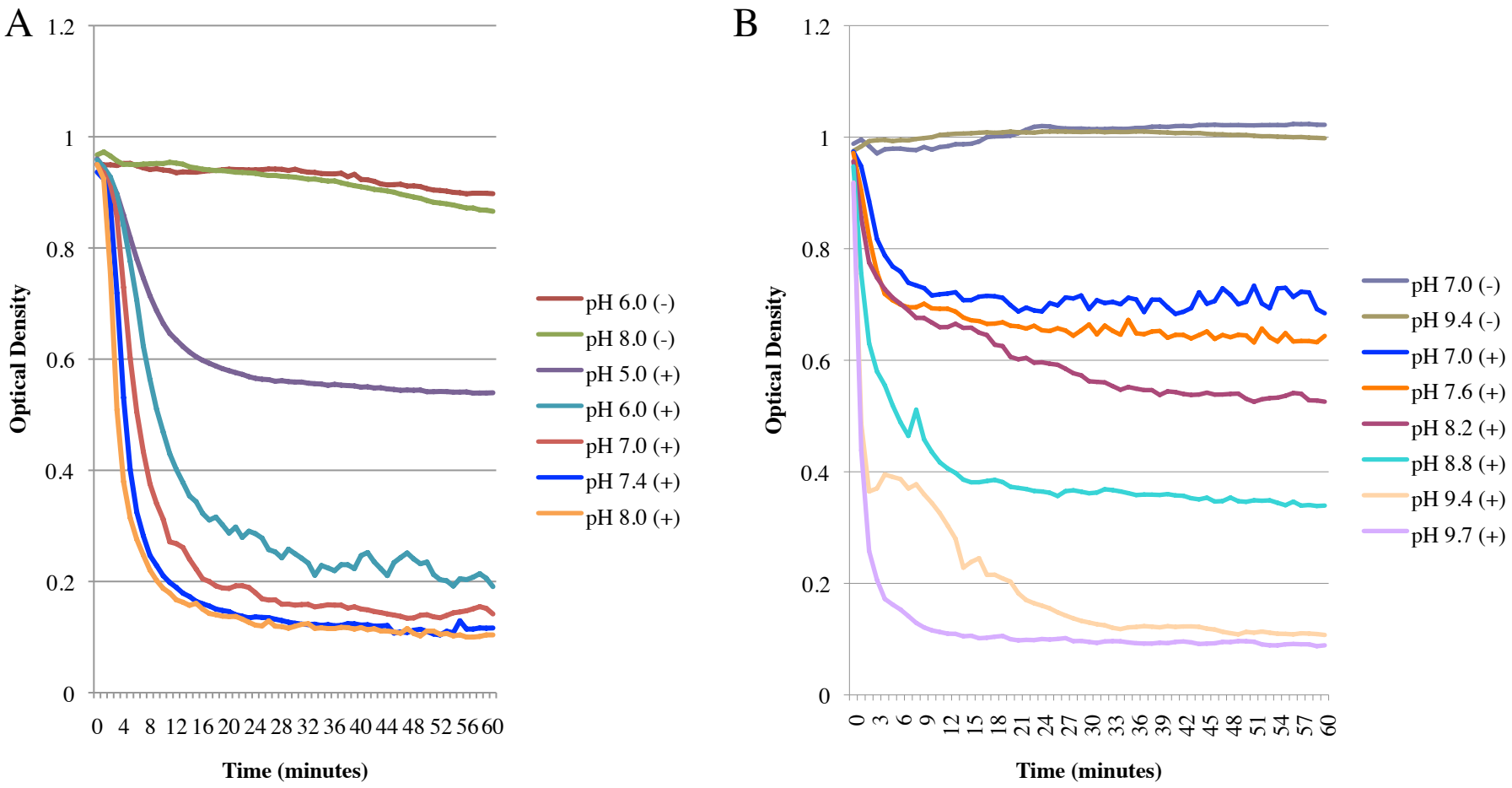


FIG. S5. PlySs2 was found to have the most acute activity in basic pH levels. To test the optimal pH for PlySs2 activity, 32 µg/ml PlySs2 was mixed with log-phase *S. suis* 7997 suspended to a final OD_{600} of 1.0 in phosphate/citrate buffer (A) or bis-tris propane (B) at various pH levels. In controls (-), ddH_2O replaced PlySs2. Spectrophotometric readings were taken at OD_{600} every minute over an hour.

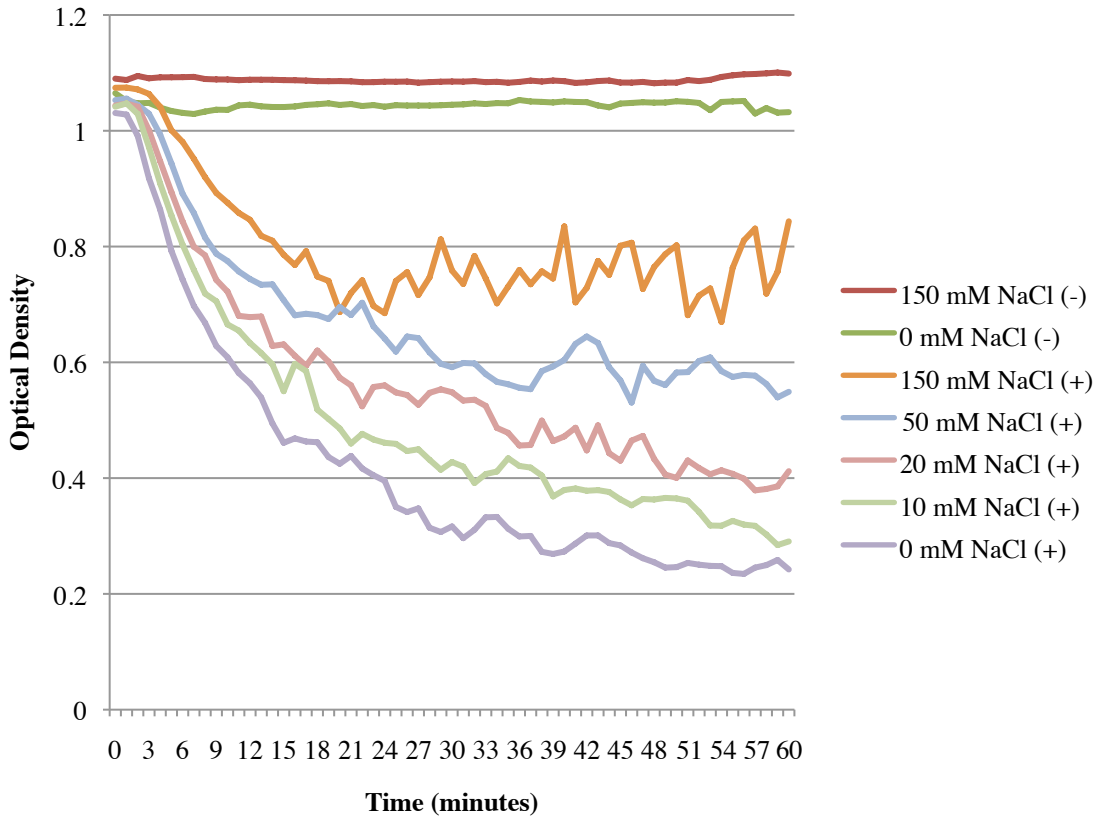


FIG. S6. The greatest PlySs2 activity registered at 0 mM NaCl. Log-phase *S. suis* 7997 were centrifuged and resuspended in PB to OD_{600} ~1.0. To test the optimal salinity for PlySs2 activity, 32 µg/ml PlySs2 was mixed with *S. suis* strain 7997 in phosphate buffer at different NaCl concentrations. In controls (-), PB replaced PlySs2. Spectrophotometric readings were taken at OD_{600} every minute over an hour.

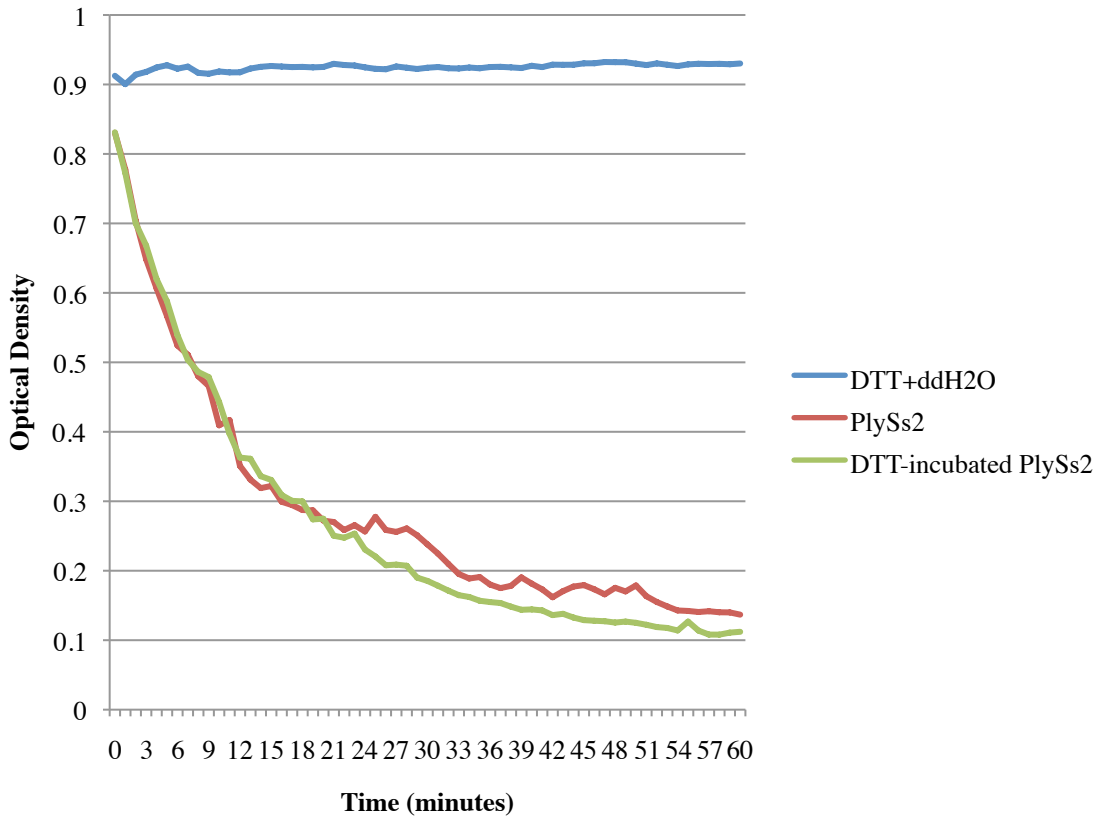


FIG. S7. Dithiothreitol (DTT) does not inhibit PlySs2 activity. Log-phase *S. suis* 7997 were centrifuged and resuspended in PB to OD_{600} ~1.0. PlySs2 was incubated in 5 mM DTT at room temperature for 60 minutes. Incubated or unincubated PlySs2 was added at 32 µg/ml. In controls (-), PB replaced PlySs2. Spectrophotometric readings were taken at OD_{600} every minute over an hour.

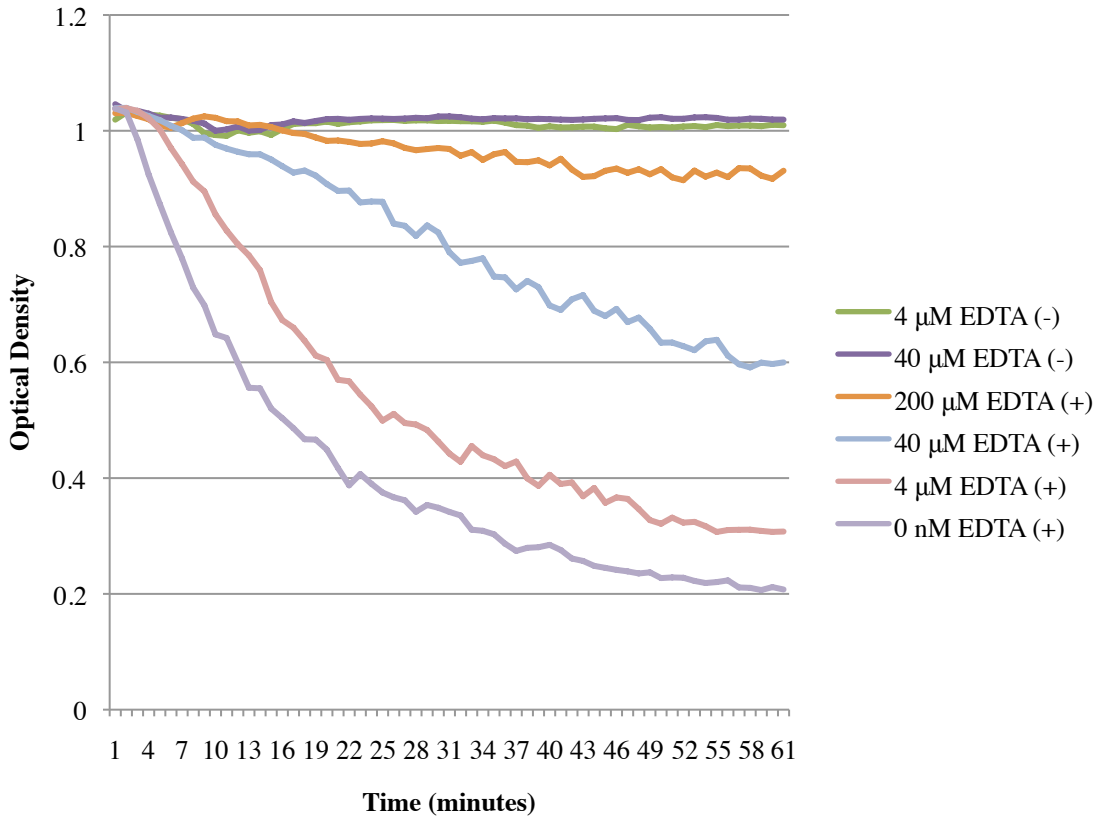


FIG. S8. Minimal ion depletion from ethylenediaminetetraacetate (EDTA) inhibits PlySs2 activity. Log-phase *S. suis* 7997 were centrifuged and resuspended in PB to OD_{600} ~1.0. PlySs2 was added at 32 µg/ml to cells with various concentrations of EDTA. In controls (-), PB replaced PlySs2. Spectrophotometric readings were taken at OD_{600} every minute over an hour.

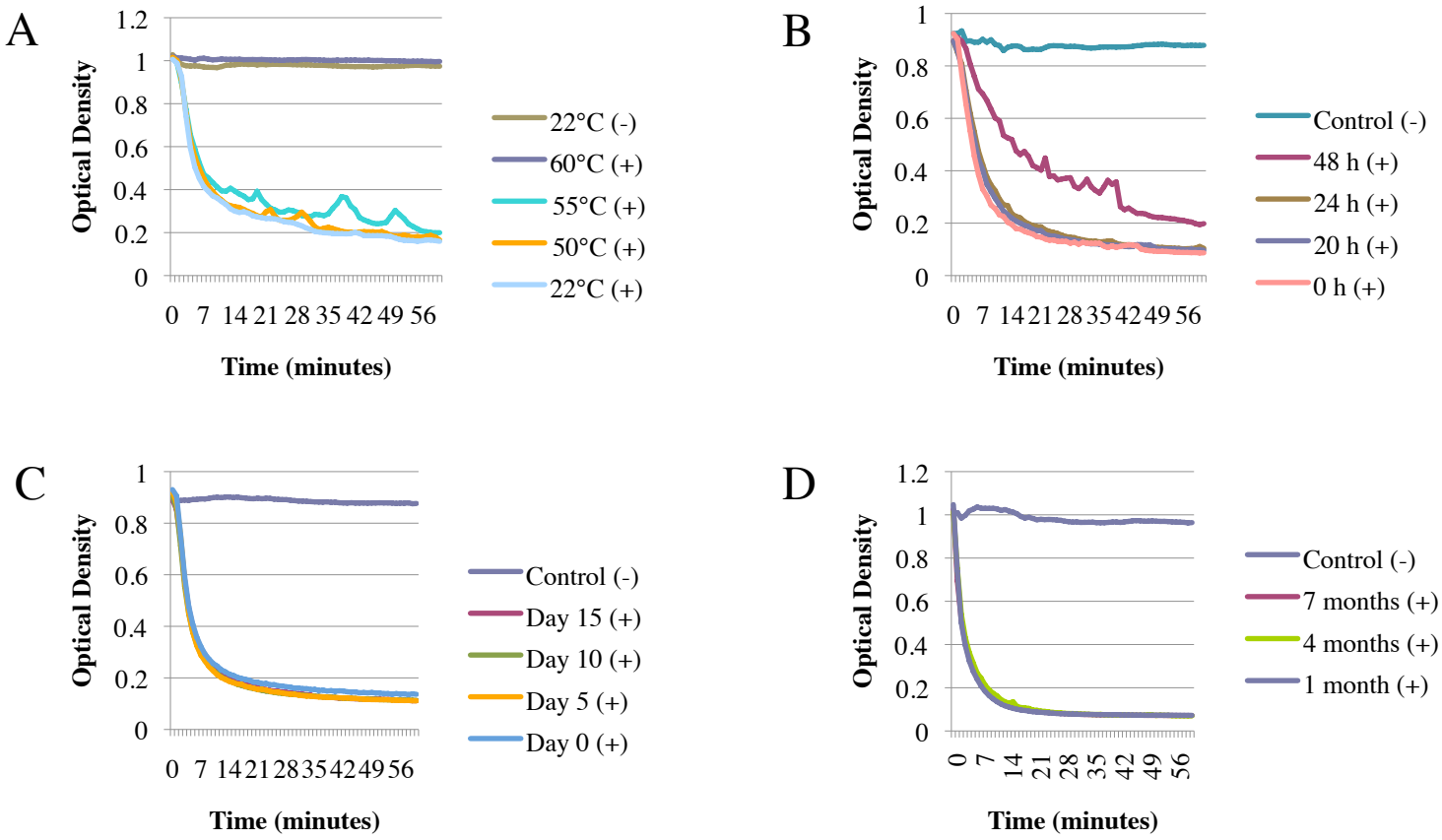


FIG. S9. PlySs2 is stable under a variety of conditions. PlySs2 was incubated: (A) for 30 minutes at various temperatures, then cooled; (B) at 37°C for different increments of time; (C) at 4°C for different numbers of days; (D) at -80°C for different numbers of months. For each test, 32 µg/ml PlySs2 was added to *S. suis* 7997 cells at an OD_{600} of ~1.0. Spectrophotometric readings were taken at OD_{600} every minute over an hour.

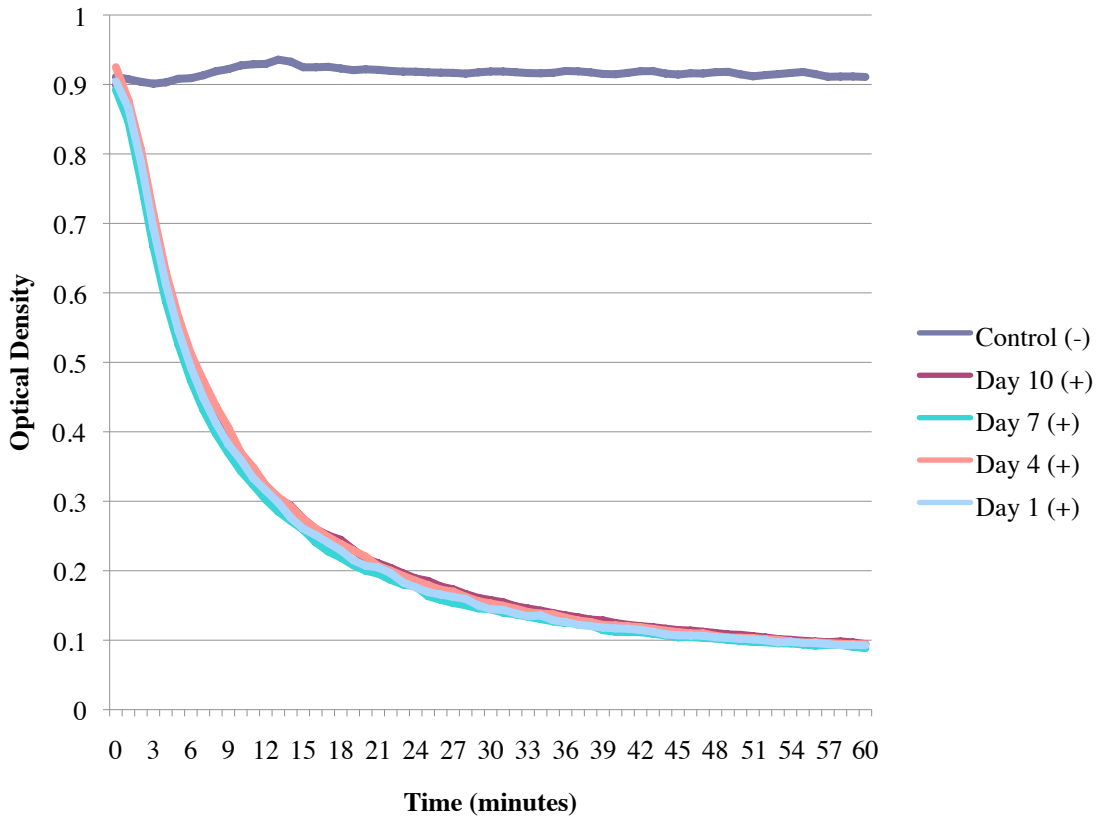


FIG. S10. PlySs2 is stable and active after ten, consecutive freeze-thaws. Log-phase *S. suis* 7997 were centrifuged and resuspended in PB to OD_{600} ~1.0. PlySs2 was taken from -80°C to room temperature and back to -80°C on subsequent days from Day 1 to Day 10. A sample from each day was added to cells at 32 µg/ml. In controls (-), PB replaced PlySs2. Spectrophotometric readings were taken at OD_{600} every minute over an hour.