Supplementary Material

Endolysins from Antarctic *Pseudomonas* display lysozyme activity at low temperature

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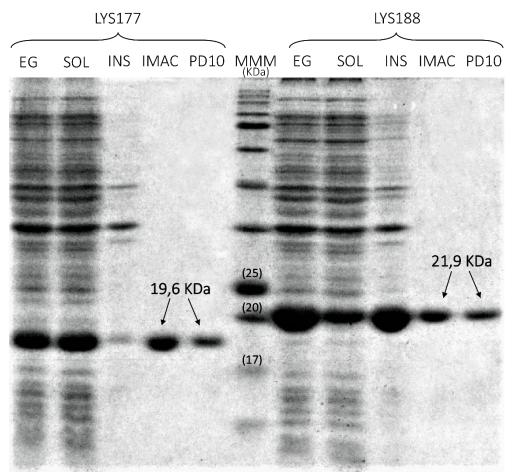


Figure S1. SDS/PAGE of recombinant proteins purified by affinity chromatography (IMAC). For both samples, the first three lanes from the left contain the total (EG), soluble (SOL) and insoluble (INS) fractions obtained from cell lysates after production in Zym-5052 medium (see "Materials and Methods"). \approx 1,5 µg of proteins was loaded after purification (IMAC) and buffer exchanged by double gel filtration (PD10). MMM: molecular mass marker.

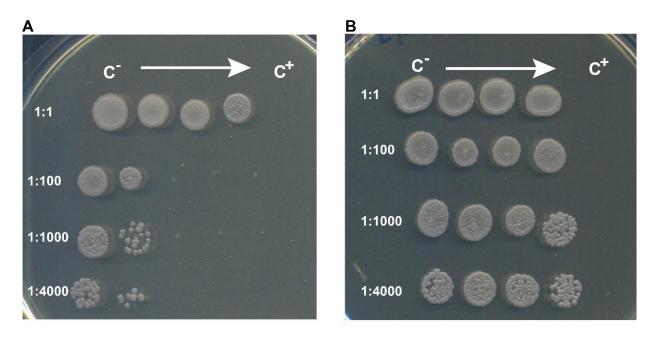


Figure S2. Antimicrobial plate assay. Treatment of different dilutions of exponentially growing *Bacillus subtilis* cultures with LYS177 (**A**) and LYS188 (**B**). The white arrow indicates increasing enzyme amounts: 5, 25 and 50 µg. C: cells treated with buffer only (potassium phosphate 80mM pH 6.5); C+: cells exposed to 5 µg of HEWL. Culture dilution is on the left of each plate row.

1		+1-1 -1	Unaligned region	Inser
MPITAQQLLLILPNA	TQAGVFVSALNTAMQHYQIVGPKRAA	AFLAQIGHESGQL <mark>R</mark> YV <mark>R</mark> E AFIAQIGHESGQLCYV <mark>R</mark> E	IIWGPTAAQ <mark>R</mark> GYEGRED	T <mark>LGNT</mark> -TV -LGNTV
MPITQQQLLQILPNA MPITQQQLLQILPNA	TQAGVFVSALNTAMQHYQIVGPKRAA TQAGVFVSALNTAMQHYQIVGPQRAA	AFIAQVGHESGQLRYVRE		- LGNT V - LGNT V
1 <mark>PITQQQLLQILPNA</mark> F	T <mark>K</mark> AGVFVSALNTAMQHYQIVGPKRAA	AFIA <mark>QIGHE</mark> SGQL <mark>R</mark> YV <mark>R</mark> E	IWGPTAAORGYEGRED	-LGNTN
			IWGPTDAQRGYEGRKD	- LGNT K
PITQQQLLQILPNA PITQQQLLQILPDA		AFIAQI <mark>GHESGQL</mark> RYV <mark>R</mark> E AFIAQIGHESGQLRYVRE		
PITAQQLLQILPNA	TQAGVFVSALNTAMQHYQIVGPERAA	AFIAQI <mark>GHESGQLC</mark> YV <mark>R</mark> E	IIWGPTAAQRGYEGRVD	
		AFIAQIGHESGQLCYV <mark>R</mark> E AFIAQIGHESGQLHYV <mark>R</mark> E	IWGPTAACRGYEGRED	
	SQAGVFVSPLNTAMARH <mark>RIDTPKR</mark> IA	AFLAQVGHESGQLRYVRE AFLAQVGHESGQLRYVRE		FLGNTPE
4QITENNLIDIMPNA <mark>F</mark>	SQAGVFVSVLNTAMA <mark>R</mark> HRIDTPKRIA	AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E	IIGNNOYLSKYDTGTLAL	HLGNTPE
MOITEKHLIDIMPNAF	SOAGVFVSPLNTAMARORIDTPKRIA	AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E		RLGNTPE. RLGNTPE
MQLTEKHLIDIMPNA <mark>F</mark>	SQAGVFVSPLNTAMARHRIDTPKRIA	AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E AFLAOVGHESGOLRYVRE	ILGNNQYLS <mark>K</mark> YDTGTLAI	HLGNTPE
MQLTEKHLIDIMPNAF	SQAGVFVSPLNTAMARHRIDTPKRIA	AFLAQV <mark>GHESGQL</mark> RYV <mark>R</mark> E	ILGNNQYLSKYDTGTLAI	RLGNTPE.
MQLTEKHLIDIMPNAH MOLTEKHLIDIMPNAH		AAFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E AAFLAOVGHESGOLRYVRE		
QLTEKHLTDIMPNAF	SQAGVFVSPLNTAMARH <mark>RIDTPKR</mark> IA	AFLAQV <mark>GHESGQL</mark> RYV <mark>R</mark> E	ILGNNQYLSKYDTGTLAI	RLGNTPE
4QLTEKHLTDIMPNAF 4QLTEKHLVDIMPTAF		AFLAQV <mark>GHES</mark> GQL <mark>R</mark> YV <mark>R</mark> E AFLAQV <mark>GHESGQLR</mark> YV <mark>R</mark> E	ILGNNQYLSKYDTGTLAL	RLGNTPE
10LTEKHLVDIMPNAF 10LTEKHLIDIMPNAF		AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E		RLGNTPE FLGNTPE
IQLTEKHLIDIMPNAF	SQAGVFVSALNAAMA <mark>RQ</mark> RIDTPKRIA	AFLAQVGH <mark>E</mark> SGQL <mark>R</mark> YV <mark>R</mark> E	ILGNNQYLS <mark>K</mark> YDTGTLAI	RLGNTPE
4QLTEKHLIDIMPNAF 4QLTEKHLIDIMPNAF		AFLAQV <mark>GHES</mark> GQL <mark>R</mark> YV <mark>R</mark> E AFLAQV <mark>GHES</mark> GQL <mark>R</mark> YV <mark>R</mark> E	IIGNNQYLSKYDTGTLAI	HLGNTPE
IQLTEKHLIDIMPNAP IOLTEKHLIDIMPNAP	SLAGVFVSALNAAMA <mark>RQ</mark> RIDTPKRIA	AFLAQVGHESGQLRYVRE AFLAOVGHESGOLRYVRE		R LGNT PE
MQLTERHLIDIMPNAF MQLTERHLIDIMPNAF	SQAGVFVSALNAAMA <mark>RQ</mark> RIDTPKRIA	AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E	IIGNNQYLSKYDTGTLAI	HLGNTPE
4QLTEKHLIDIMPNAF 4OLTEKHLIDIMPNAF		AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E AFLAQVGHESGQLRYVRE		
1QLTEKHLTDIMPNAF	SQAGVFVSPLNTAMA <mark>KQ</mark> RIDTPKRIA	AFLAQV <mark>GHESGQL</mark> RYV <mark>R</mark> E	IIGNNQYLSKYDTGTLAI	FLGNTPE
IQLTEKHLTDIMPNAF IQLTEKHLVDIMPTAF		AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E AFLAOVGHESGQL <mark>R</mark> YV <mark>R</mark> E		
IQLTEKHLIDIMPNAF	SQAGVFVSPLNAAMARHRIDTPKRVA	AFLAQVGH <mark>ESGQL</mark> RYV <mark>R</mark> E	TIGDNOYLSKYDTGTLAL	HLGNTPE
	SQAGVFVCALNNAMARRRIDSPKRIA	AFLAQIGHESGQL <mark>R</mark> YV <mark>R</mark> E	ILGNNQYLSKYDTGTLAI	RLGNTPE
		AFLAQIGHESGQL <mark>R</mark> YV <mark>R</mark> E AFLAOVGHESGOLRYVRE	II <mark>GNNQYLS</mark> KYDTGTLAL	FLGNTPE
QITENNLIDIMPNA <mark>F</mark>	SQAGVFVSALNSAMA <mark>RR</mark> HIDSPKRVA	AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E	ILGNNQYLS <mark>K</mark> YDTGTLAL	RLGNTPE
4QITESNLIDIMPNAF 4QITESNLIDIMPNAF		AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E AFLAOVGHESGOL <mark>R</mark> YV <mark>R</mark> E		
MQITENNLIDIMPNAF	SQAGVFVSALNSAMT <mark>RR</mark> HIDSPKRVA	AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E ASFLAQVGHESGQL R YV <mark>R</mark> E	ILGNNQYLS <mark>KYDTGT</mark> LAI	RLGNTPE
	<mark>SQAGVFVSPLNSAMA</mark> RRHIDSPKRVA	AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E	LGNNQYLSKYDTGTLAL	RLGNTPE
4QLTENSLIDIMPNAE 4QITEKSLIDIMPNAE		AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E AFLAQIGHESGQLRYVRE		
MQITENNLIDIMPNAF	SQAGVFVSALNAAMTRRRIDTPKRIA	AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E	ILGNNQYLS <mark>K</mark> YDTGTLAI	RLGNTPE
1QITENNLIDIMPNA 10ITENNLIDIMPNA		AFLAQIGHESGQL <mark>R</mark> YV <mark>R</mark> E AFLAOVGHESGOLRYVRE		
QITENNLIDIMPNAF		AFLAQI <mark>GHESGQL</mark> RYV <mark>R</mark> E AFLAQIGHESGQLRYVRE		RLGNTPE
AQITENNLIDIMPNAP	SQAGVFVSALNSAMA <mark>RR</mark> HI <mark>DSPKR</mark> VA	AFLAQIGHESGQL <mark>R</mark> YV <mark>R</mark> E	ILGNNQYLS <mark>K</mark> YDTGTLAL	FLGNTPE
MQITENNLIDIMPNA MOLTENNLIDIMPNA		AFLAQIGHESGQL <mark>R</mark> YV <mark>R</mark> E AFLAOVGHESGQLRYVRE		
MQLTENNLIDIMPNAF	SQAGVFVSALNSAMA <mark>RR</mark> HI <mark>DSPKR</mark> IA	AFLAQV <mark>GHE</mark> SGQL <mark>R</mark> YV <mark>R</mark> E	II <mark>GNNQYLS<mark>K</mark>YDTGTLAI</mark>	RLGNTPE
4QITENNLIDIMPNA 4QLTENSLIDIMPNA	SQAGVFVSPLNSAMA <mark>RR</mark> HIDSPKRIA	AFLAQIGHESGQLEYVE AFLAQVGHESGQLEYVE	ILGNNQYLS <mark>K</mark> YDTGTLAI	RLGNT PE
4QLTENSLIDIMPNAF 4QITENNLIDIMPNAF		AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E AFLAQVGHESGQL <mark>R</mark> YV <mark>R</mark> E		
1QITENNLIDIMPNA	SQAGVFVSVLNAAMTRRLIDTPKRMA	AFLAQVGHESGQLRYVR	ILGNNQYLSKYDTGTLAI ILGNNQYLSKYDTGTLAI	FLGNTPE
MQITENNLIDIMPNAF MQITENNLIDIMPNAF	SQAGVFVSVLNAAMTRRLIDTPKRMA SQAGVFVSALNSAMARRHIDSLKRVA	IAFLAQVGHESGQLRYVRE IAFLAQV <mark>GHESGQLR</mark> YVRE	ILGNNQYLSKYDIGTLAL ILGNNQYLSKYDIGTLAL	RLGNTPE RLGNTPE
101 TEKDLIDIMPNA	TOAGVEVSALNSAMARRYIDSPKRIA	AFLAQVGHESGQLRYVR	IIGNNQYLSKYDTGTLAL	RLGNTPE
QITENNLTNIMPNAF	TQAGVFVSALNTAMARRRIDTPKRIA	AFLAQVGHESGQLQYVRF	ILGNNQYLSKYDTGTLAL	RLGNTPE
4QITENNLLNIMPNA 4QITENNLLNIMPNA	SQAGVFVSALNSAMANH <mark>RIDTPKR</mark> VA SQAGVFVSALNSAMANHRIDTPKRVA	IAFLAQIGHESGQLQYV <mark>R</mark> E AAFLAQIGHESGOLOYVRE	II	FLGNTPE FLGNTPE
101 TENNLLNIMPNA	SQAGVFVSALNSAMANH <mark>RIDTPKR</mark> VA	AFLAQIGHESGQLQYVRE	ILGNNQYLSKYDTGTLAI	RLGNTPE
IQITENNLSNIMPDAF	SQAGVFVSALMAAMTKKHIDTPKRMA SQAGVFVSPLNDAMARH <mark>R</mark> IDTPKRVA	AFLAQVGHESGQLRYVRF	ILGUNQYLSKIDIGILAL	FLGNTPE
1QITENNLSNIMPNA 10TTENNLSNIMPNA	SQAGVFVSPLNDAMARHRIDTPKRVA	AFLAQVGHESGQLRYVR	ILGNNQYLSKYDTGTLAL	RLGNTPE FLGNTDE
QITENNLSNIMPNAF	SQAGVFVSPLNDAMARHRIDTPKRVA	AFLAQVGHESGQLRYVRF	LGDNQYLSKYDTGTLAL	RIGNTPE
IQITENNLSKIMPNA IQITENNLISIMPNA	SQAGVFVSPLNDAMARHRIDTPKRVA SQAGVFVSPLNDAMARHRIDTPKRVA	AFLAQVGHESGQLRYVRE AFLAQVGHESGOLRYVRE	ILGDNQYLSKYDTGTLAI ILGDNQYLSKYDTGTLAI	FLGNTPE
MQITQDNLLNIMPNA	TQAGVFVSALNTAMARRFINTPKRIA	AFLAQVGHESGQLRYVR	ILGNNQYLSKYDTGALAL	RLGNTPE
IQITEDNLLNIMPNA	ROAGVEVSALINTAMARQLIDIPKRIA ROAGVEVSALINDAMARHRIDIPKRVA	AFLAQI <mark>GHESGQLQ</mark> YV <mark>R</mark> F	ILGNNQYLSRIDIGILAL	FLGNTPE
MQITEDNLLNIMPNAF	ROAGVEVSALNDAMARHRIDTPKRVA	AFLAQIGHESGQLQYV <mark>R</mark> E	ILGNNQYLSKYDTGTLAI	FLGNTPE
MQITEDNLLNIMPNA	ROAGVFVSPLNDAMARHRIDTPKRIA	AFLAQI <mark>GHE</mark> SGQLQYV <mark>R</mark> F	IGNNQYLSKYDTGTLAL	FLGNTE
1Q1 TEDNLLNIMPNA 101 TEDNLLNIMPNA	ROAGVFVSALNDAMARHRIDTPKRIA ROAGVFVSPLNDAMARHRIDTPKRVA	AFLAQIGHESCQLQYVRE AFLAQIGHESCHLOYVRF	ILGNNQYLSKYDTGTLAL ILGNNOYLSKYDTGTLAL	RLGNTPE RLGNTPE
MQITEDNLLNIMPNAF	(RQAGVFVSPLNDAMARHRIDTPKRIA	AFLAQIGHESGQLQYVRE	IIGNNQYLSKYDTGTLAI	ILGNTEE
IQITEDNLLTIMPNAF	ROAGVEVSPENDAMARHRIDTPKRVA ROAGVEFSALNDAMARHRIDTPKRIA	AFLAQI <mark>GHESGQLQYVR</mark> E	LGNNQYLSKIDIGILAL	RLGNTPE
QITEDNLLTIMPNA OTTEDNLLTIMPNA	ROAGVEVSALNDAMARHRIDTPKRIA	AFLAQIGHESGQLQYV <mark>R</mark> E	ILCNNQYLSKYDTGTLAL	HLGNTPE BLGNTPE

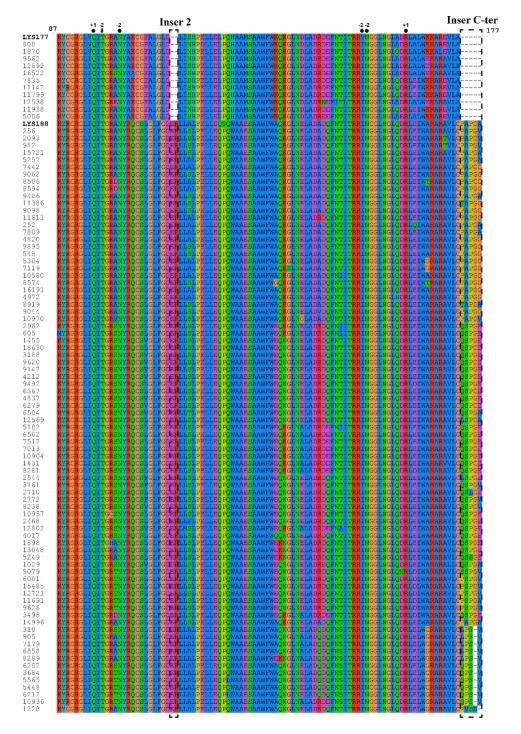


Figure S3. Multiple alignment of LYS177, LYS188 and close homologues of both enzymes. Annotations on functional relevant sites conserved in the alignment were extracted from the GH19 engineering database (GH19ED, accessible at https://gh19ed.biocatnet.de) and reported above each site. Sequence ID is 12,365 for LYS177 and 11,988 for LYS188; other sequences are named according to

their ID in GH19ED. Unaligned/insertion regions are highlighted by dashed boxes. Lower case letters are reported above non conserved sites among the two different groups: close homologues of LYS177 (the first 12 sequences) and LYS188 (the other 83). *: catalytic residues; #: water coordination residue; •: substrate binding residues. Numbers indicate the subsite occupied by the sugar moiety predicted to interact with that residue, according to [62]. Unaligned/insertion regions are highlighted by dashed boxes. Inser: insertion.

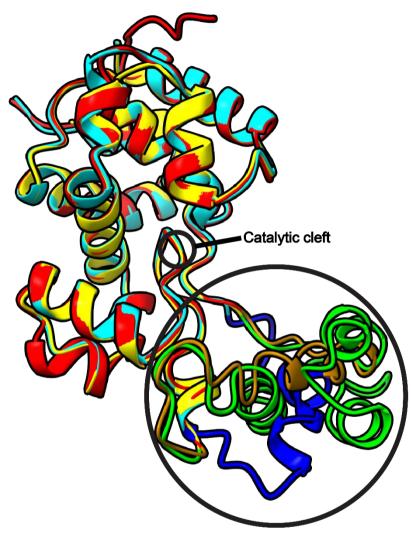


Figure S4. Structural superposition of LYS177 and LYS188 ITASSER models to the 3D structure of *Salmonella Typhimurium*-infecting phage SPN1S endolysin (PDB AN 40k7). SPN1S endolysin is shown in yellow, LYS177 in cyan and LYS188 in red. A single unaligned region of > 5 AA results from the comparison. This region is coloured in blue in LYS177 (from 61 to 83), dark orange in LYS188 (61 to 86) and green in the SPN1S endolysin (59 to 115). Such amino acids stretches were further refined before MD simulation.

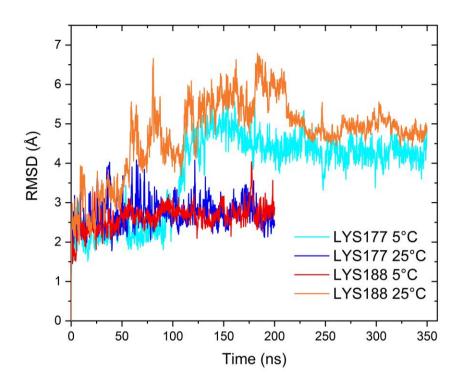


Figure S5. Root mean square deviation of the MD simulations during the production run. The last 100 ns frames of each simulation, collected after stabilization of RMSD values, were used for subsequent analyses.

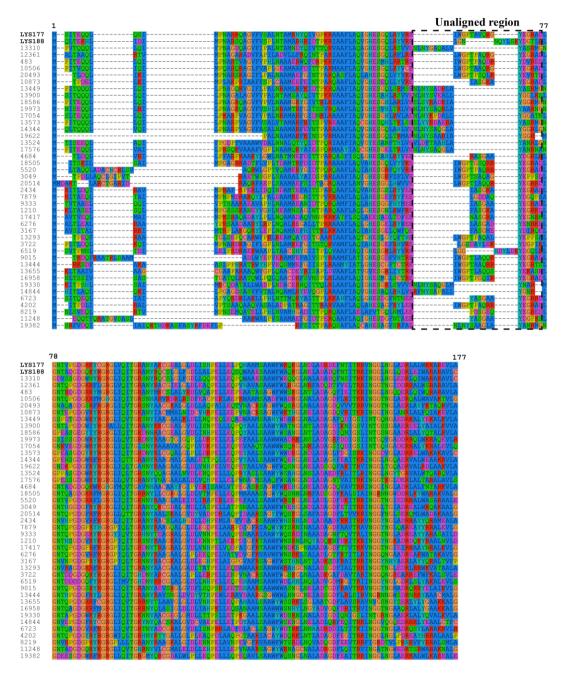


Figure S6. Multiple alignment of LYS177, LYS188 and other phage homologues. 3466 sequences from *Pseudomonas* prophage like homologous group in the GH19ED database (accessible at https://gh19ed.biocatnet.de) were clustered at 60% identity with CD-HIT [60], resulting in 44 centroid sequences; each centroid sequence represents a different portion of the sequence space of the homologous group, avoiding the choice of arbitrary sequences. These centroids sequences, which included LYS177 and LYS188 as representative sequences of their own sequence clusters, were aligned with Mafft 7.313 [54] by using the option 'Leave gappy regions', in order to obtain a global alignment, but avoiding the alignment of locally non-conserved regions.