Supplemental material

TABLE S1 Results of a volumetric viscosity test performed for stain STCH_09 and its phage resistant mutants (BIMs) to assess the difference in the exocellular polysaccharides (free EPS) production.

Strain	**Viscosity time 1 [s]**	**Viscosity time 2 [s]**	**Viscosity time 3 [s]**	**Viscosity time average [s]**	**St. dev.**	**Viscosity**
STCH_09	42	43	42	42	1	normal
STCH_09_BIM	46	46	46	46	0	enhanced
STCH_09_BIM_1	53	50	50	51	2	enhanced
STCH_09_BIM_2	39	37	39	38	1	normal
STCH_09_BIM_3	48	48	43	46	3	enhanced
STCH_09_BIM_4	42	42	37	40	3	normal
STCH_09_BIM_5	33	33	32	33	1	reduced
STCH_09_BIM_6	44	42	38	41	3	normal

TABLE S2 List of mutations detected in phage resistant mutants (BIMs) generated in this study and confirmed by Sanger sequencing.

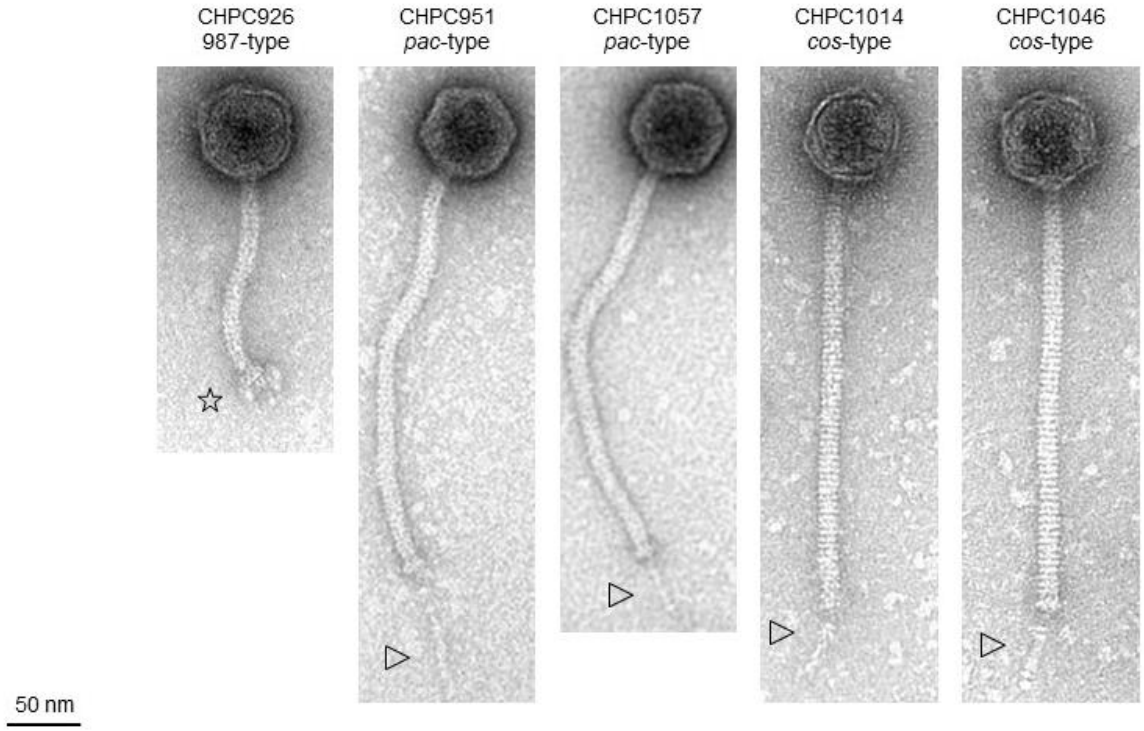
BIM	**Mutated gene**	**Type of mutation**[a]
STCH_09_BIM	Glycosyltransferase *epsH*	AA substitution
	Glycosyltransferase *rgp*	Gene truncation
STCH_13_BIM	Tyrosine-kinase epsD	AA insertion
	Type I R-M system, DNA-methyltransferase subunit M	AA substitution
STCH_14_BIM	Glycosyltransferase *epsE*	Gene truncation
	O-antigen export system permease protein	AA substitution
	Two-component sensor kinase	AA substitution
STCH_15_BIM_1	Glycosyltransferase *epsE*	Gene deletion
	5'-nucleotidase family protein in cluster with NagD-like phosphatase	Gene truncation
	Zinc protease	AA substitution
	Ribose-phosphate pyrophosphokinase	AA substitution
STCH_15_BIM_2	Glycosyltransferase *epsK*	AA substitution
	Guanylate kinase	AA substitution
STCH_15_BIM_3	Glycosyltransferase *epsK*	Gene truncation
STCH_15_BIM_4	Glycosyltransferase *epsK*	Gene truncation
STCH_15_BIM_5	Glycosyltransferase *epsK*	Gene truncation
	Guanylate kinase	AA substitution

[a] AA stands for amino acid

TABLE S3 List of samples collected during purification of cellular fractions with their relevant characteristics.

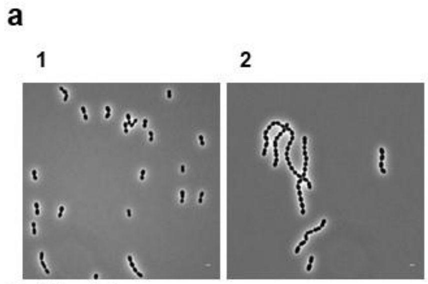
Purification step no.	**Sample name**	**Cell wall components present in the sample**[a]
1	Cells in exponential phase	surface enzymes, membrane, membrane proteins, cell wall proteins, EPS/CPS, LTA, WTA, WPS, PG
2	Cells devoid of surface enzymes, membrane and membrane proteins	cell wall proteins, CPS, LTA, WTA, WPS, PG
3	Purified cell walls	CPS, WTA, WPS, PG
4	Purified peptidoglycan	PG

[a] Cell wall components: exocellular polysaccharides (EPS/CPS), lipoteichoic acids (LTA), wall teichoic acids (WTA), polysaccharides intercalated with cell walls (WPS), peptidoglycan (PG)

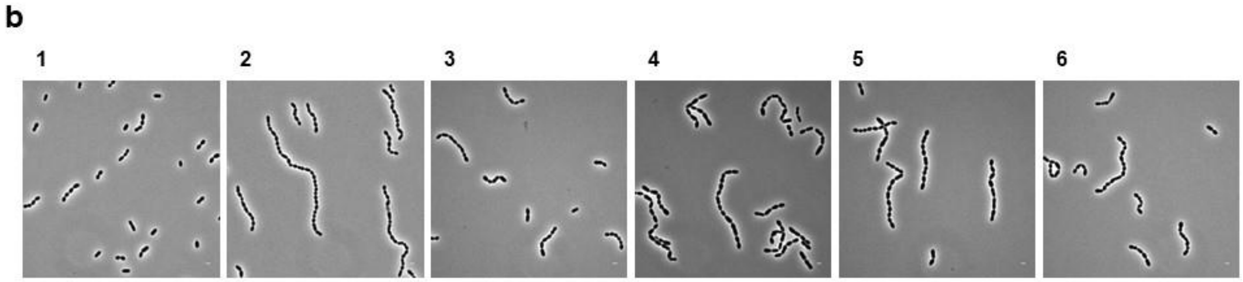


	n = 7	n = 8	n = 8	n = 8	n = 11
Head diameter	62 ± 1	59 ± 2	59 ± 2	58 ± 4	58 ± 2
Tail length	126 ± 3	252 ± 2	247 ± 2	241 ± 9	245 ± 4

FIG S1 Transmission electron micrograph images of *S. thermophilus* phages used in this study. The symbols indicate baseplate structures (asterisk) and a single central tail fiber (triangle). Head and tail sizes are indicated in nm. Images of CHPC926, CHPC1014, CHPC1046 were published previously (Szymczak *et al.* 2017).



# cells in chains		
Average	3 ± 1	9 ± 6
Min	1	2
Max	8	29



# cells in chains						
Average	2 ± 1	13 ± 8	5 ± 3	7 ± 4	9 ± 5	9 ± 5
Min	1	2	2	2	2	2
Max	8	45	20	24	26	22

FIG S2 Changes in chain lengths of *S. thermophilus* wild type strains and their phage-resistant mutants. (a) STCH_14 forms short chains (1) and its phage-resistant mutant STCH_14_BIM forms elongated chains (2). (b) STCH_15 forms short chains (1) and its phage-resistant mutants STCH_15_BIM_1, STCH_15_BIM_2, STCH_15_BIM_3, STCH_15_BIM_4, STCH_15_BIM_5 form elongated chains (panels no. 2-6, respectively). For each strain, cells in 100 chains were counted to calculate the average number of cells per chain. Scale bars: 1 µm.

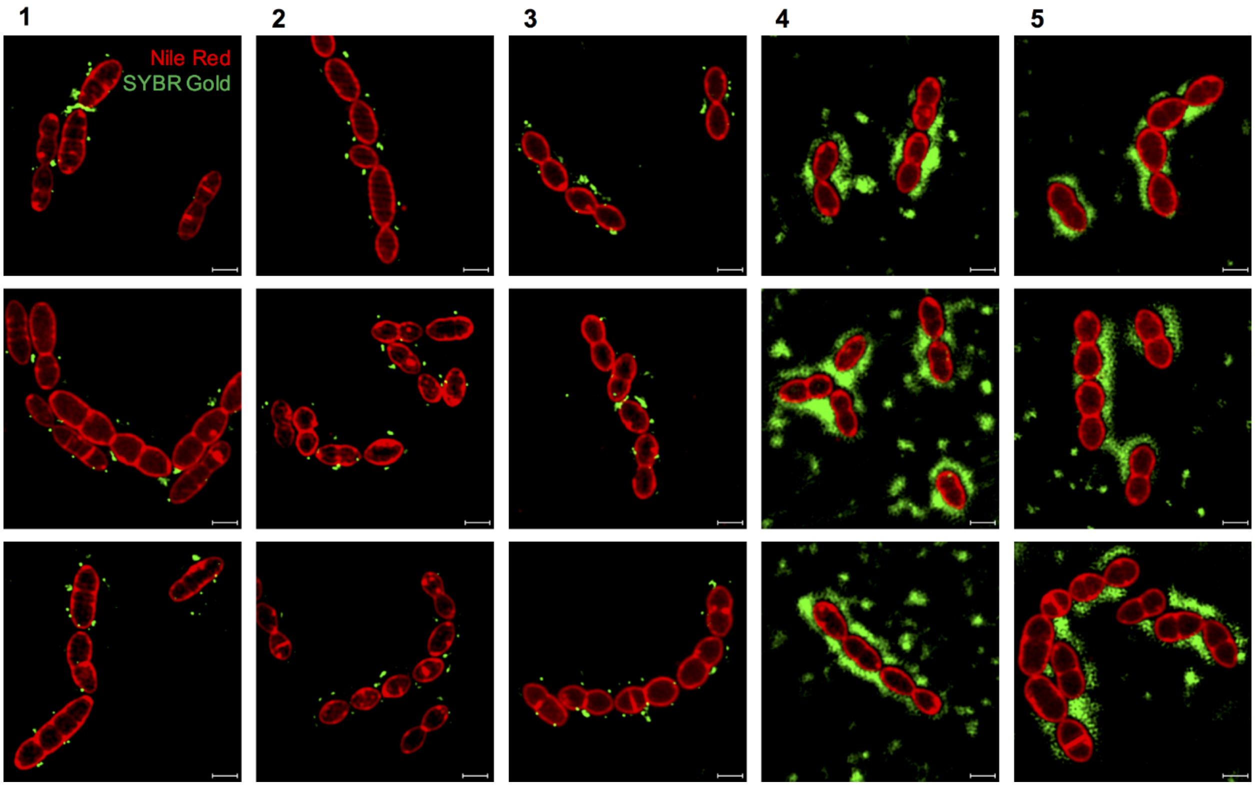


FIG S3 Super-resolution structured illumination microscopy (SR-SIM) images of phage binding to *S. thermophilus* strains. Bacterial cells were stained with Nile Red (red) and mixed with SYBR Gold DNA-labeled phages (green). Panels with phages and their host strains: (1) CHPC926 and STCH_15, (2) CHPC951 and STCH_12, (3) CHPC1057 and STCH_09, (4) CHPC1014 and STCH_13, (5) CHPC1046 and STCH_14. Two binding patterns are observed: spotty (panels no. 1, 2, 3) or diffused (panels no. 4, 5). Scale bars: 1 µm.

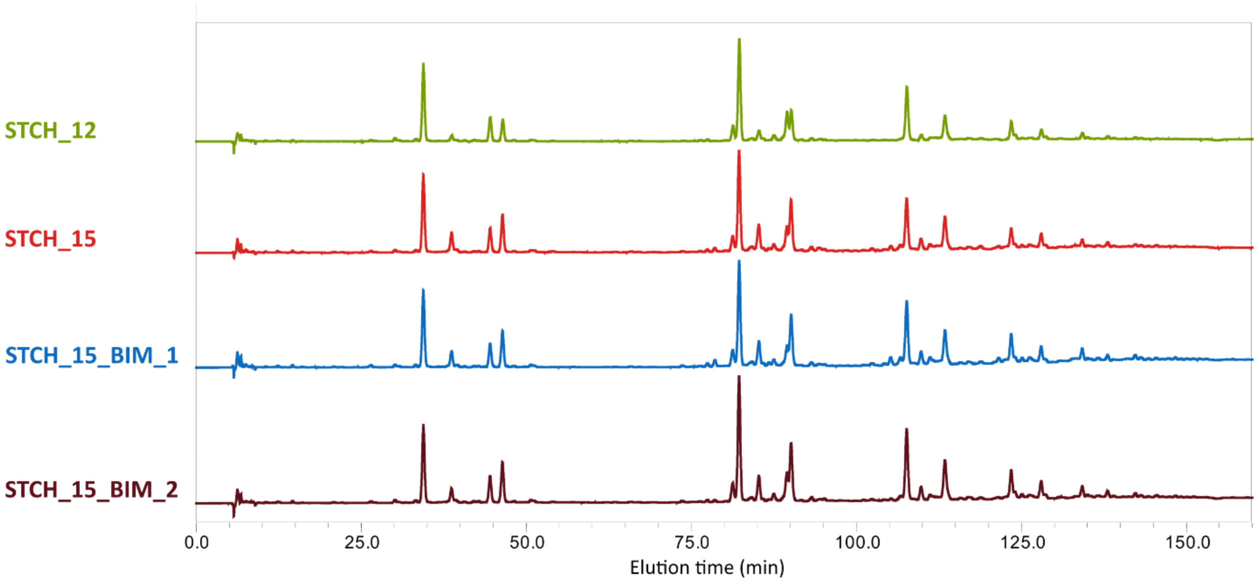


FIG S4 HPLC profiles of muropeptides in peptidoglycan isolated from *S. thermophilus* strains. Analysis was performed for two wild type strains (STCH_12 and STCH_15) and two phage-resistant mutants (STCH_15_BIM_1 and STCH_15_BIM_2).

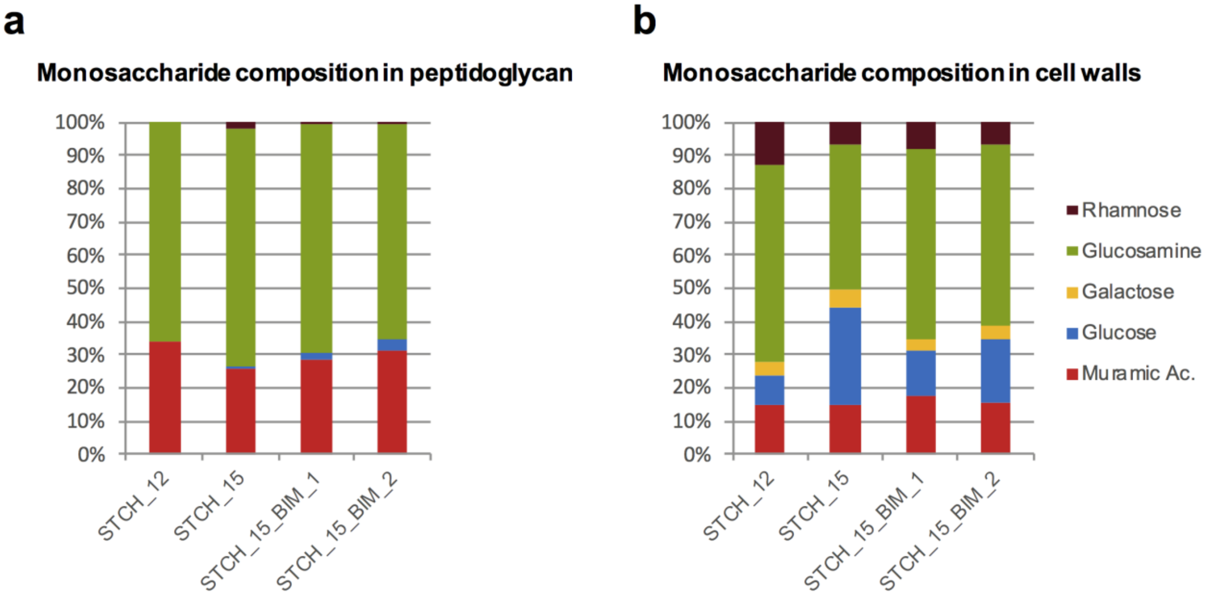


FIG S5 Monosaccharide compositions in peptidoglycan and cell walls isolated from *S. thermophilus* strains. Cellular fractions were purified from two wild type strains (STCH_12 and STCH_15) and two phage-resistant mutants (STCH_15_BIM_1 and STCH_15_BIM_2). Values were calculated based on individual peak areas compared to the total peak area from HPAEC-PAD analyzes preformed for: (a) purified peptidoglycan, (b) purified cell walls. Standards for different saccharides were eluted under the same conditions to allow peak identification.

References

Szymczak P, Janzen T, Neves AR, Kot W, Hansen LH, Lametsch R, Neve H, Franz CMAP, Vogensen FK. 2017. Novel variants of *Streptococcus thermophilus* bacteriophages are indicative of genetic recombination among phages from different bacterial species. Appl Environ Microbiol 83:1–16.