antibiotics

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Supplementary Information

Engineering of the CHAPk Staphylococcal Phage Endolysin to Enhance Antibacterial Activity against Stationary-Phase Cells (Supplementary Information)

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 S   Y   D   P   S   F   G   V   M   E   A   G   A   I   D
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 Y   V   L   W   L   T   D   N   K   V   R   T   W   G   N
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 A   K   D   Q   I   K   Q   S   Y   G   T   G   F   K   I
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 H   E   N   K   P   S   T   V   P   K   K   G   W   I   A
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CAC
 H
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Figure S1. Sequence of the chimeric protein CHAPk-SH3blys. Red sequence corresponds to CHAPk, blue sequence corresponds to the SH3b cell-binding domain from lysostaphin, and purple sequence represents the His-tag region.

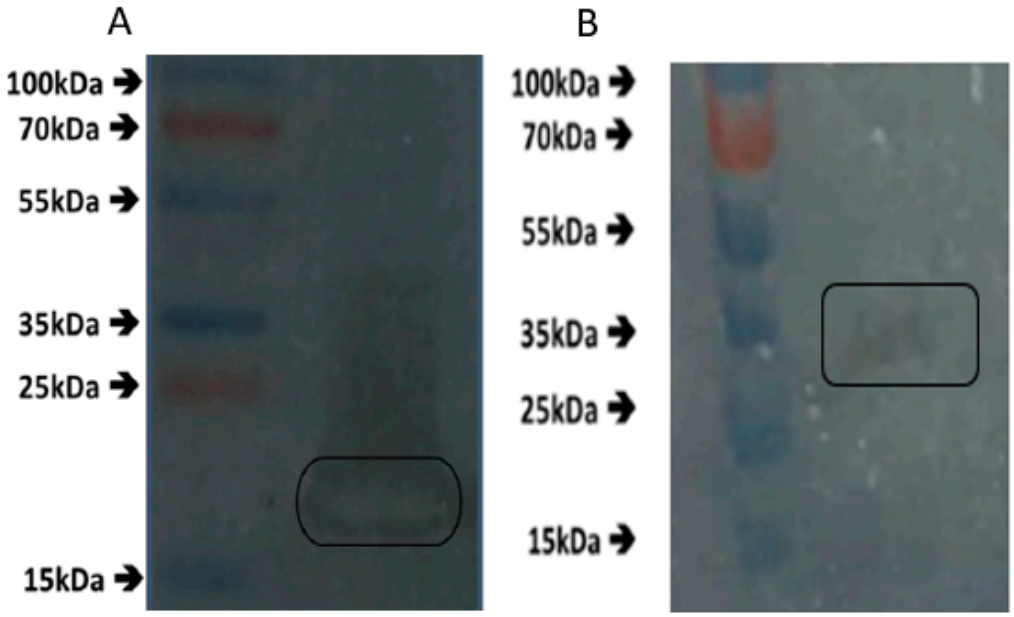

Figure S2. Zymogram using heat inactivated *S. aureus* DPC5246 cells, showing the lytic activity of; (A) CHAPk, having a band of clearing of approximately 20 KDa which corresponds to the predicted molecular mass of 18.6 kDa; (B) CHAPK-SH3blys, having a band of clearing of approximately 35 KDa which corresponds to the predicted molecular mass of 33.6 kDa.

Figure S3. Density of biofilm cultivated from log-phase culture and 7-day-old culture. OD_{595} readings are the average of triplicates plus/minus their standard deviation. Biofilms were cultivated at 37ºC during 16h.

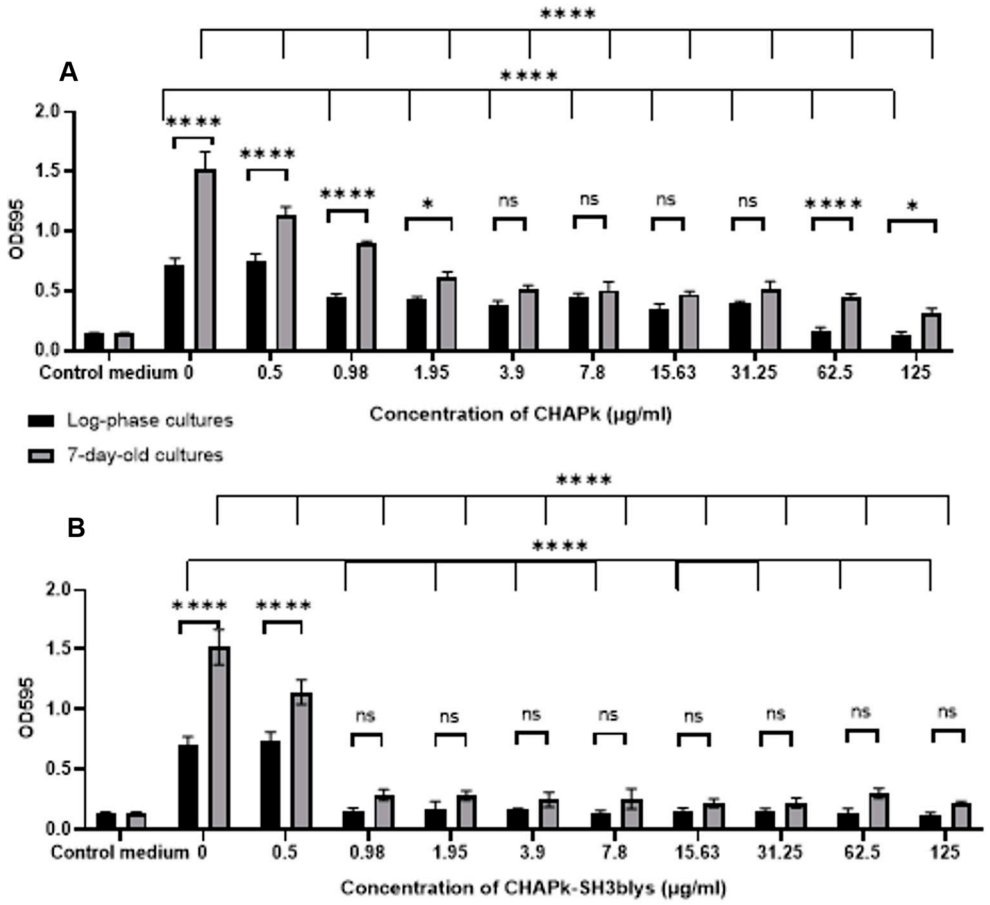

Figure S4. Biofilm prevention assay using concentrations of (A) CHAPk or (B) CHAPk-SH3blys ranging from 0.5 to 125µg/ml. OD_{595} readings are the average of triplicates plus/minus their standard deviation. p-values <0.0001 are represented by ****. No significant statistical differences are represented by ns. p-values comparing concentrations of CHAPk (between biofilms from log-phase and 7-day-old cultures) 1.95 and 125 represented by * were 0.022 and 0.0244, respectively.

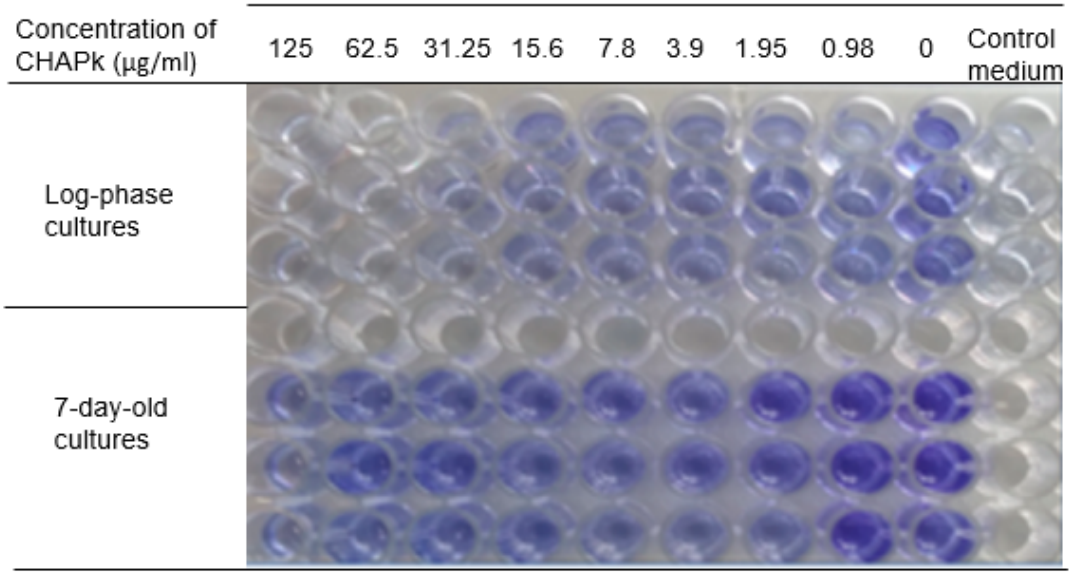

Figure S5. Biofilm prevention assay using concentrations of CHAPk ranging from 125 to 0.98 µg/ml. Visual representation of the 96-well plates after treatment with crystal violet.

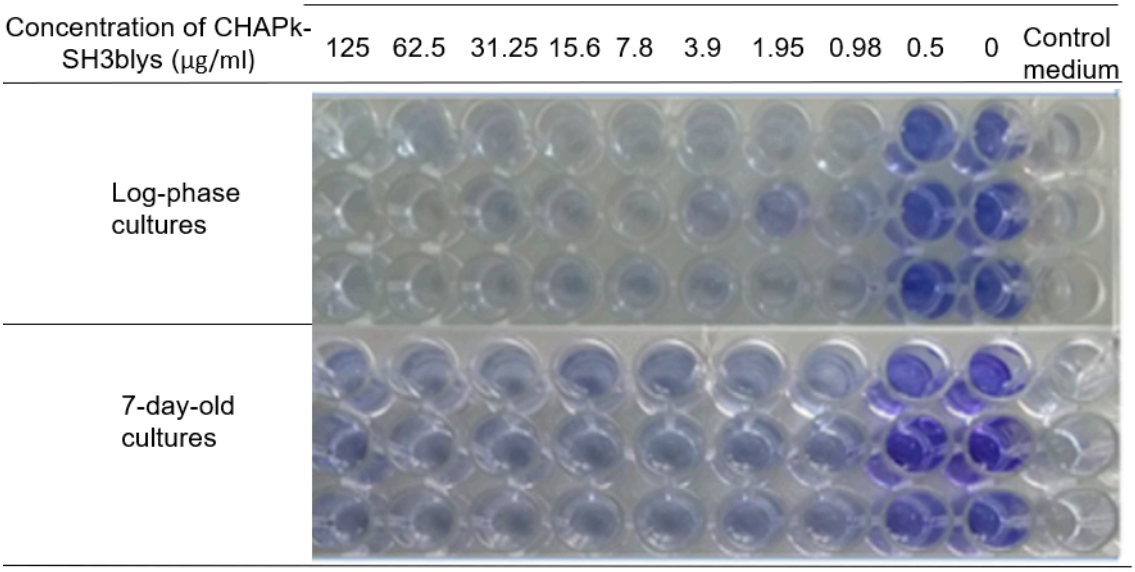

Figure S6. Biofilm prevention assay using concentrations of CHAPk-SH3blys ranging from 0.5 to 125 μg/ml. Visual representation of the 96-well plates after treatment with crystal violet.

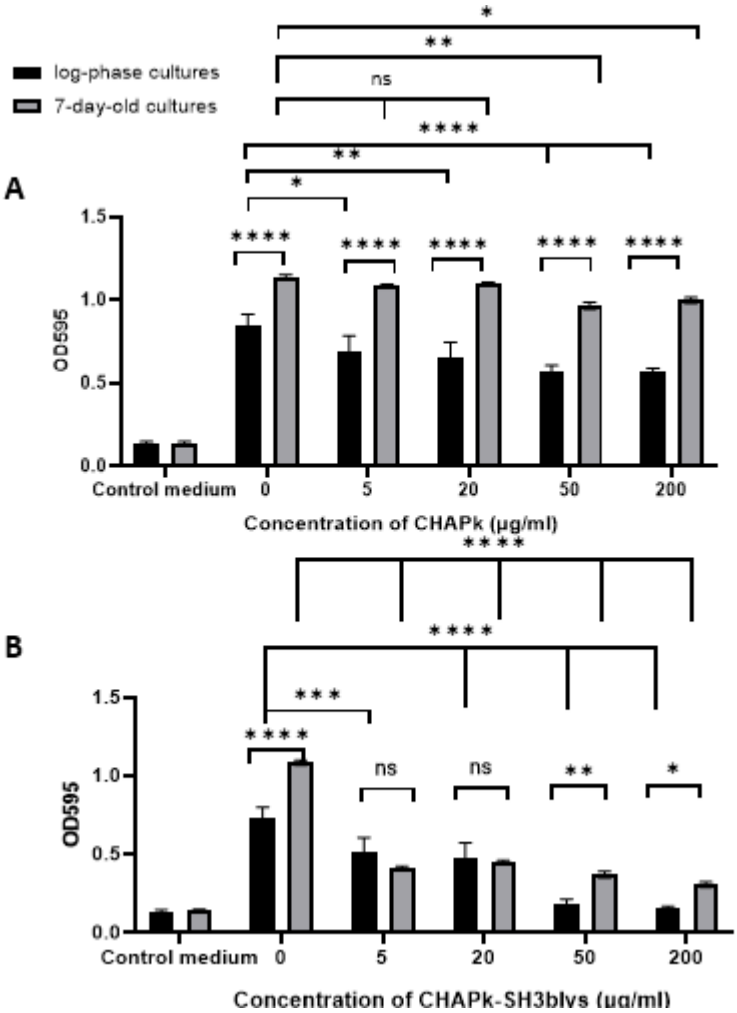

Figure S7. Biofilm disruption assay using *S. aureus* biofilms formed by cells in stationary and exponential phase. Concentrations of (A) CHAPk or (B) CHAPk-SH3blys, from 5 to 200 μg/ml, were used to disrupt biofilms. The disruption was determined by OD readings at 595nm. All readings are the average of triplicates plus/minus their standard deviation. P-values <0.0001 are represented by ****. No significant statistical differences are represented by ns. P-values comparing of untreated biofilm (formed from log-phase cultures) with concentrations of CHAPk of 5 and 20 μg/ml were 0.0136 and 0.011, respectively. P-values comparing untreated biofilm (formed from 7-day-old cultures) with concentrations of CHAPk of 50 and 200 μg/ml were 0.0060 and 0.043, respectively. P-value comparing untreated biofilm (formed from log-phase cultures) with a concentration of CHAPk-SH3blys of 5 μg/ml was 0.0002. P-values comparing CHAPk concentrations of 50 and 200 μg/ml (between biofilms from log-phase and 7-day-old cultures) were 0.006 and 0.043, respectively. P-values CHAPk-SH3blys concentrations of 50 and 200 were (between biofilms from log-phase and 7-day-old cultures) 0.0015 and 0.018, respectively.

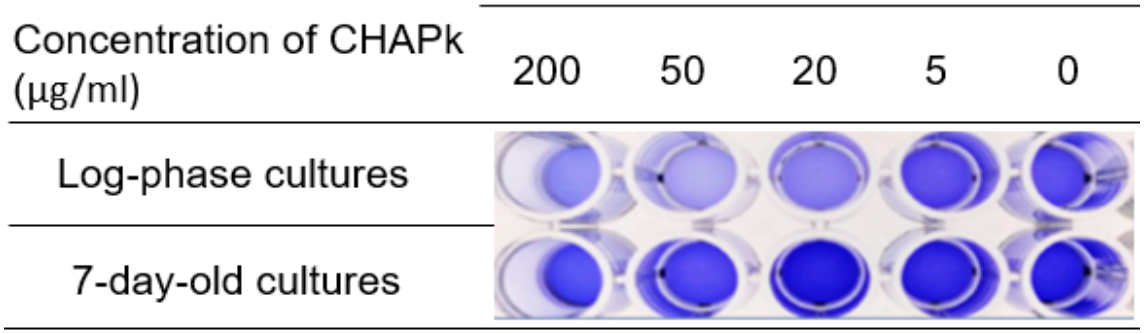

Figure S8. Biofilm disruption assay using *S. aureus* biofilms formed by cells in log-phase and 7-day-old cultures. Concentrations of CHAPk from 5 to 200 µg/ml, were used to disrupt biofilms. Visual representation of the 96-well plates after treatment with crystal violet.

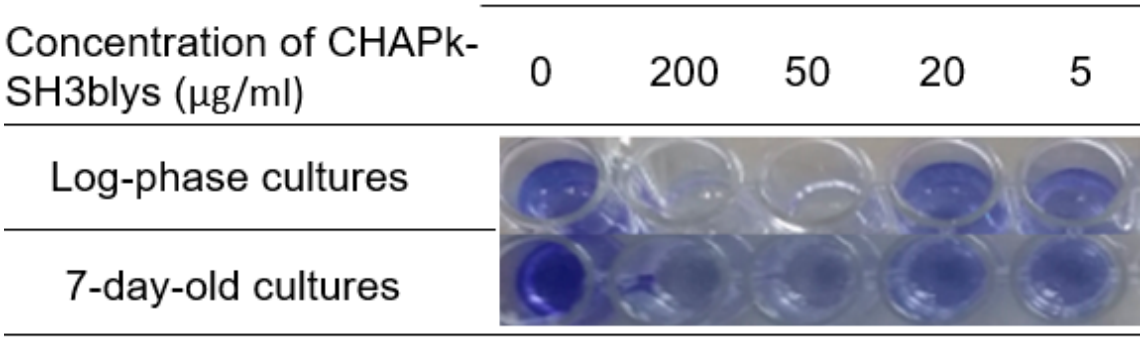

Figure S9. Biofilm disruption assay using *S. aureus* biofilms formed by cells in log-phase and 7-day-old cultures. Concentrations of CHAPk-SH3blys from 5 to 200 µg/ml, were used to disrupt biofilms. Visual representation of the 96-well plates after treatment with crystal violet.