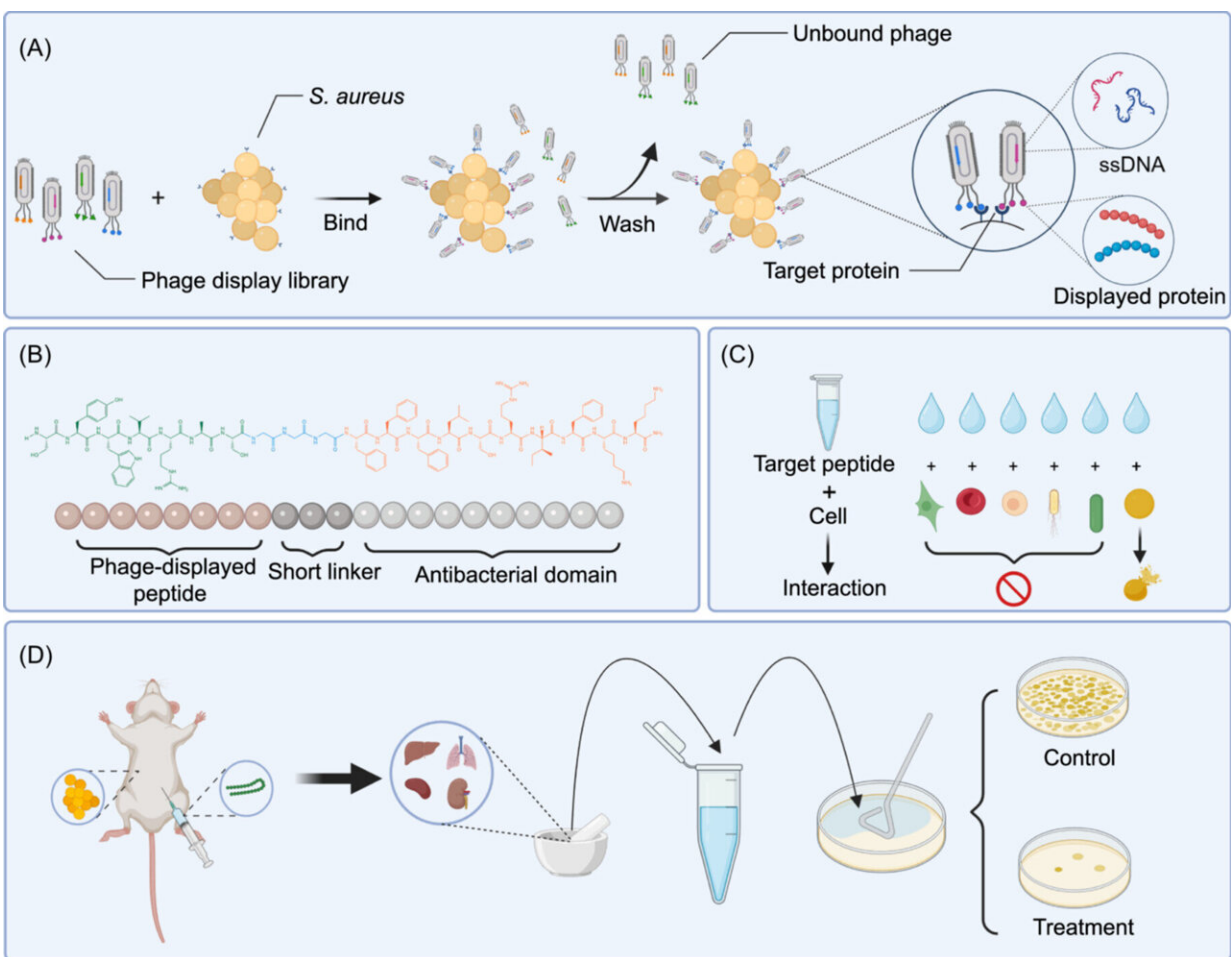


Phage display technology effectively improves specific targeting ability of anti-microbial peptides against *S. aureus*

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A. Phage display technology. B. Chemical structure of peptide. C. Specific interaction of target peptide. D. In vivo antibacterial activity. Credit: Xi Ma, State Key Laboratory of Animal Nutrition and Feeding, College of Animal Science and Technology, China Agricultural University

Antibiotics are widely used in clinical treatment and animal production as an effective means to combat microbial infections. Antimicrobial peptides, as potential alternatives to antibiotics, have shown promising applications. However, owing to their broad-spectrum antimicrobial activity, most antimicrobial peptides tend to cause imbalance in the host's intestinal flora. In addition, the systemic use of broad-spectrum drugs for the treatment of localized infections may cause toxicity, which should not be overlooked.

Phage display technology involves the fusion of DNA fragments encoding exogenous proteins or small-molecule polypeptides with phage genes encoding surface proteins, which then exist on the surface of phages by means of fusion proteins. Exogenous proteins and small-molecule polypeptides specifically recognize and bind to target molecules. Therefore, this technology is widely used to screen targeted drugs, prepare vaccines and monoclonal antibodies, and monitor and diagnose tumors.

In a new study, researchers have screened nine heptapeptide sequences from a random peptide library using phage display technology, with *S. aureus* 6538 as the target bacterium. The work is [published](#) in the journal *mLife*.

The heptapeptide sequence with the highest affinity (SYWVRAS) was selected as the target domain. The researchers further improved its antimicrobial activity by adding several cationic amino acids (R or K) at the C-terminus after linking the heptapeptide sequence to temporin-SHf, which is rich in hydrophobic amino acids, via GGG as a linker. Compared with temporin-SHf, the designed peptide had higher specific antimicrobial activity against *S. aureus*. This suggests that the screened heptapeptide sequence effectively improves the specific antimicrobial

activity after concatenation with the template peptide.

To further verify the effect of SFK2 treatment on membrane integrity, *S. aureus* 6538 was incubated with a final concentration of 8 μM SFK2, and the fluorescence distribution was observed under a laser scanning confocal microscope. Green fluorescence (SYTO9) and red fluorescence (PI) of *S. aureus* ATCC 6538 induced under 8 μM of SFK2 overlapped, indicating severe disruption of its cell membrane and leading to [cell death](#).

Subsequently, to investigate the targeting ability of SFK2, *S. aureus* ATCC 6538 and *E. coli* ATCC 25922 were mixed 1:1, treated with 8 μM SFK2, and then SYTO9 and PI fluorescent dyes were added for observation using laser scanning confocal microscopy. The [green fluorescence](#) and red fluorescence of *S. aureus* ATCC 6538 were induced to overlap at 8 μM concentration of SFK2, while *E. coli* ATCC 25922 only emitted green fluorescence without red fluorescence, indicating that its membrane integrity was not disrupted.

S. aureus, *P. aeruginosa*, and *E. coli* were incubated with different concentrations of SFK2, and their membrane integrity was quantified using flow cytometry. 8, 4, and 2 μM of SFK2 induced 92.78%, 70.08%, and 28.38% of *S. aureus* mortality, respectively. However, *P. aeruginosa* and *E. coli* maintained more than 90% survival, even at the final concentration of 8 μM SFK2, with negligible differences from the control.

To verify this hypothesis further, a [scanning electron microscope](#) (SEM) and [transmission electron microscopy](#) (TEM) were used to observe the morphological changes in *S. aureus* treated with SFK2. The surface of untreated *S. aureus* was intact and smoothly rounded under SEM. *S. aureus* ruptured after 2 h of treatment with 2 μM SFK2, and there was an outflow of the contents outside the cell. Under the TEM, the cell

membrane structure of *S. aureus* in the control group was complete, and the cytoplasm was dense. After treatment with 2 μ M SFK2 for 2 h, the cell membrane of *S. aureus* was ruptured and the cell contents were discharged.

Researchers constructed and evaluated a mouse model of systemic infection using intraperitoneal and tail vein injections. Both modes of administration significantly reduced the number of colonies in the organs, and symptoms such as hepatocellular rupture and inflammatory cell in-filtration were drastically reduced compared with those in the saline control. Because piglets are similar to humans in terms of anatomy, physiology, and nutritional metabolism, the team further validated the *in vivo* effects of SFK2 using piglets as a model. It was found that SFK2 remained active *in vivo* in a piglet model for treating *S. aureus* infection.

These results indicate that the heptapeptide sequence screened using phage display technology can effectively improve the specific targeting ability of the peptide against *S. aureus*, providing a reference for the subsequent development of narrow-spectrum antibacterial drugs.

More information: Tao Wang et al, Phage-displayed heptapeptide sequence conjugation significantly improves the specific targeting ability of antimicrobial peptides against *Staphylococcus aureus*, *mLife* (2024).

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