

Biofilm Inhibition Assay The biofilm-forming ability of ATCC 25923 and a clinical MRSA strain, as well as the biofilm-inhibitory ability of surfactin against them, were assessed by the crystal violet staining method.¹⁷ Briefly, the overnight cultures of each strain were diluted with TSB containing serial dilutions of surfactin (1/64×MIC to 2×MIC) to obtain a final concentration of 1×10^6 CFU/mL, and then 200 μ L of each culture were inoculated into a 96-well microtiter plate in triplicates. Then, planktonic cells were removed by washing with 1×phosphate-buffered saline (PBS) three times after incubation at 37°C for 24 h, and each well was stained with 0.5% crystal violet (Solarbio, China) for 20 min. After air-drying, the wells were solubilized with 200 μ L of 95% ethanol for 20 min at room temperature. The absorbance of each well was measured at OD₅₇₀. Moreover, the strength of biofilm formation of the selected strains was classified according to the following criteria: $OD \leq \text{cut-off } OD \text{ (ODC)}$, no biofilm formation; $ODCOD \leq 2 \times ODC$, weak biofilm formation; $2 \times ODCOD \leq 4 \times ODC$, moderate biofilm formation; and $OD > 4 \times ODC$, strong biofilm formation.¹⁸ The ODC was defined as the mean OD of the negative control (TSB only).