

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 $\leq$ cut-off OD (ODC), no biofilm formation; ODCOD $\leq$ 2×ODC, weak biofilm formation; 2×ODCOD $\leq$ 4×ODC, moderate biofilm formation; and OD4×ODC, (strong biofilm formation.¹⁸ The ODC was defined as the mean OD of the negative control (TSB only