

Mechanisms of Rapid Bactericidal and Anti-Biofilm Alpha-Mangostin *In Vitro* Activity against *Staphylococcus aureus*

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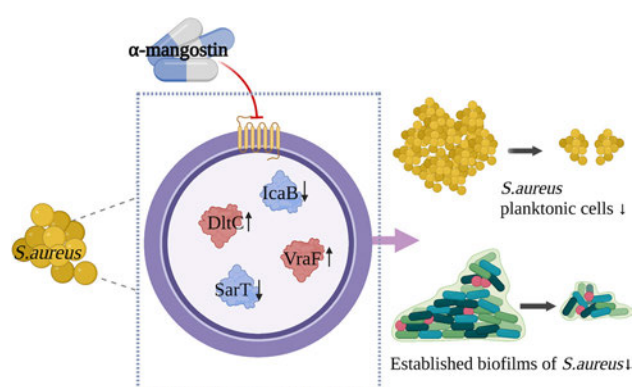
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Abstract

Alpha-mangostin (α -mangostin) was discovered as a potent natural product against Gram-positive bacteria, whereas the underlying molecular mechanisms are still unclear. This study indicated that α -mangostin (at $4\times$ MIC) rapidly killed *Staphylococcus aureus* planktonic cells more effectively (at least 2-log_{10} CFU/ml) than daptomycin, vancomycin and linezolid at 1 and 3 h in the time-killing test. Interestingly, this study also found that a high concentration of α -mangostin ($\geq 4\times$ MIC) significantly reduced established biofilms of *S. aureus*. There were 58 single nucleotide polymorphisms (SNPs) in α -mangostin nonsensitive *S. aureus* isolates by whole-genome sequencing, of which 35 SNPs were located on both sides of the *sarT* gene and 10 SNPs in the *sarT* gene. A total of 147 proteins with a different abundance were determined by proteomics analysis, of which 91 proteins increased, whereas 56 proteins decreased. The abundance of regulatory proteins SarX and SarZ increased. In contrast, the abundance of SarT and IcaB was significantly reduced (they belonged to SarA family and *ica* system, associated with the biofilm formation of *S. aureus*). The abundance of cell membrane proteins VraF and DltC was augmented, but the abundance of cell membrane protein UgtP remarkably decreased. Propidium iodide and



DiBaC₄(3) staining assay revealed that the fluorescence intensities of DNA and the cell membrane were elevated in the α -mangostin treated *S. aureus* isolates. In conclusion, this study reveals that α -mangostin was effective against *S. aureus* planktonic cells by targeting cell membranes. The anti-biofilm effect of α -mangostin may be through inhibiting the function of SarT and IcaB.

Key words: alpha-mangostin, *Staphylococcus aureus*, biofilm, cell membrane, SarT

Introduction

Staphylococcus aureus is considered one of the leading pathogens of hospital and community-acquired infections worldwide and causes acute diseases, such

as pneumonia, endocarditis, and bacteremia (Tong et al. 2015). Moreover, *S. aureus* commonly forms biofilms on artificial implants, such as indwelling catheters, orthopaedic implants, and heart valves (Donlan 2001; Tande and Patel 2014; Cyphert and von Recum 2017;

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Hall and Mah 2017; Otto 2018). A systematic review of wound studies found a 78.2% prevalence of biofilms in chronic wounds and established biofilm infection as a significant cause of delayed wound healing (Roy et al. 2020). The most common bacterial strains in these infected wounds were *S. aureus* and *Pseudomonas aeruginosa* (Ghoreishi et al. 2022). Established biofilms are greatly recalcitrant to antibiotic therapy and tremendously increase medical cost and morbidity (Mah and O'Toole 2001; Stewart and Franklin 2008; Kalil et al. 2016). Due to the high incidence rate of biofilm-related *S. aureus* infections, it is urgent to develop novel antibacterial agents, especially anti-biofilm antimicrobials.

Recently, many natural products have been utilized as antimicrobials against *S. aureus* infections (Iinuma et al. 1996; Gibbons 2008; Kali 2015). For example, α -mangostin obtained from pericarps of mangosteen, has bright potential and prospects owing to its remarkable biological activities, such as anticancer, anti-allergy, antiviral, antibacterial, antioxidant, and anti-inflammatory (Chen et al. 2018; Chavan and Muth 2021). α -Mangostin has been demonstrated to exhibit rapid bactericidal and anti-biofilm activities against *S. aureus* (Phuong et al. 2017; Felix et al. 2022).

At present, many researchers have explored that α -mangostin has bactericidal activities against *S. aureus* by increasing the permeability of cell membrane, but it is still unclear which genes or proteins are included in this process (Koh et al. 2013; Lin et al. 2020; Meah et al. 2020; Felix et al. 2022). Many genes are associated with the formation of persister cells and biofilms of *S. aureus*, such as *norA*, *norB*, *dnaK*, *groE*, and *mepR*, which are down-regulated by α -mangostin (Felix et al. 2022). While another study found that the expression of *ebpS* and *fnbB* was regulated by α -mangostin, but noticeable differences were also observed between different *S. aureus* strains (Nguyen et al. 2021). Therefore, the present research intends to investigate mechanisms of α -mangostin against *S. aureus* through genome sequencing and proteomics analysis.

Experimental

Materials and Methods

Bacterial isolates and culture conditions. There were 328 non-duplicate *S. aureus* clinical isolates comprised of 138 methicillin-resistant *S. aureus* (MRSA) and 190 methicillin-sensitive *S. aureus* (MSSA) isolates used in this study. These isolates were obtained from Shenzhen Nanshan People's Hospital, China, from January 2010 to December 2017. All clinical isolates were simultaneously identified by the BD Phoenix™ 100 automated microbiology system (BD, USA) and

mass spectrometer (IVD MALDI Biotyper®, Germany). *S. aureus* isolates were grown in cation-adjusted Mueller-Hinton broth (CAMHB) at 37°C with shaking for antimicrobial susceptibility and time-killing assays. For biofilm assay, *S. aureus* isolates were grown in TSBG (tryptic soy broth with 0.5% glucose) at 37°C. The Ca^{2+} (50 mg/l) was added to CAMHB media in the experiments with daptomycin.

Chemicals and natural products. Vancomycin hydrochloride (catalogue no. HY-17362), linezolid (catalogue no. HY-10394), daptomycin (catalogue no. HY-B0108), and α -mangostin (catalogue no. HY-N0328) were purchased from MedChemExpress (China). Propidium iodide (PI) (catalogue no. P4170) and bis(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC₄(3)) (catalogue no. D8189) were purchased from Sigma-Aldrich (China).

Antimicrobial susceptibility test. The minimum inhibitory concentration (MICs) of α -mangostin against *S. aureus* isolates was determined by the broth macro dilution method in CAMHB according to the Clinical and Laboratory Standards Institute guidelines (CLSI-M100-S30) (CLSI 2020) and the previous study (Song et al. 2020). Briefly, α -mangostin or other antimicrobials were two-fold diluted with CAMHB and mixed with *S. aureus* suspensions (1.5×10^6 CFU/ml). After 18 h incubation at 37°C, the MICs were defined as the lowest concentrations of antibiotics in tubes with no visible growth of bacteria. All experiments were performed in triplicate.

Bacterial growth curve and time-killing assay. The effect of α -mangostin, daptomycin, vancomycin, linezolid, and their synergistic combinations on the growth of *S. aureus* planktonic cells was explored based on a previous study (Wang et al. 2021). *S. aureus* suspension ($\text{OD}_{600} = 0.2$) with α -mangostin or antimicrobials (at $\frac{1}{2} \times \text{MIC}$) was inoculated into 96-well polystyrene microtiter plates (300 μl /well) and cultured for 24 h at 37°C. The optical density at 600 (OD_{600}) was measured with a Bioscreen C system (Lab Systems Helsinki, Finland). All experiments were performed in triplicate. The time-killing assay aimed to measure the rapid bactericidal activity of α -mangostin against *S. aureus* (Zheng et al. 2019). Briefly, this assay was conducted in a final volume of 4 ml CAMHB with α -mangostin or antimicrobials (at $4 \times \text{MIC}$). The samples were incubated at 37°C for 24 h. After 1, 3, and 24 h of time-killing assay, 1 ml aliquots were sampled, and the number of CFU/ml was determined. All experiments were performed in triplicate.

The effect of α -mangostin on *S. aureus* established biofilms. The eradicating effect of α -mangostin on established biofilms of *S. aureus* was measured by crystal violet staining (Zheng et al. 2020). The overnight *S. aureus* cultures were diluted with fresh TSBG (200 μl /well) and inoculated into 96 polystyrene

microtiter plates. After static incubation at 37°C for 24 h (mature biofilms formed), the supernatant was discarded and washed, and then fresh TSBG containing α -mangostin was added. After static incubation at 37°C for 24 h, the supernatant was discarded, and plates were washed three times with 0.9% saline. The biomass of the remaining biofilms of *S. aureus* still attached to the wells was determined by crystal violet staining. All experiments were performed in triplicate.

Induction of α -mangostin non-sensitive clones *in vitro*. The α -mangostin non-sensitive *S. aureus* clones were selected *in vitro* (Zheng et al. 2021). *S. aureus* SA113 (MSSA) and YUSA145 (MRSA) strains were subcultured in CAMHB with α -mangostin. The initial concentration of α -mangostin was equal to $\frac{1}{2} \times \text{MIC}$, then successively increased to a high concentration of $64 \times \text{MIC}$. Individual bacteria clones from the last passage of each concentration were selected and cultured without α -mangostin for two passages. Then the bacterial species were identified by mass spectrometry, and the MIC of α -mangostin was determined again. Finally, the α -mangostin non-sensitive clones (MIC of α -mangostin: $\geq 12.5 \mu\text{M}$) were stored at -20°C for further analysis.

Whole-genome sequencing. The genomic DNA of the parental *S. aureus* isolate YUSA145 and α -mangostin non-sensitive clone YUSA145-L12 was obtained by the DNeasy Blood and Tissue Kit (QIAGEN, Germany). The genomic DNA per sample ($1 \mu\text{g}$) was used, and sequencing libraries were generated with the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs®, Inc., USA). The whole genome sequencing was performed in the Illumina® HiScan™SQ system (Illumina®, USA) based on our previous research (Zheng et al. 2021). Briefly, the coding genes, repetitive sequences, and other sequences were predicted by the following software or tools: GeneMarkS and RepeatMasker (<http://www.repeatmasker.org>) (Smit et al. 2013). Gene functions were analyzed by the following databases: GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes). Genomic alignments and SNPs analysis between the α -mangostin non-sensitive clone YUSA145-L12 genome and the parental YUSA145 genome (subcultured in MHB containing no α -mangostin) and reference genome (USA300 FPR3757, GenBank: CP000255.1) were completed with MUMmer and LASTZ.

Proteomics analysis. To illuminate the antibacterial mechanism of α -mangostin against *S. aureus*, the proteomics analysis was conducted in *S. aureus* YUSA145 with the treatment of α -mangostin (at $\frac{1}{2} \times \text{MIC}$) for 4 h. Bacterial cultures were harvested at $\text{OD}_{600} \sim 0.8$ and transferred to a precooled 2 ml screw-cap tube. 1.5 volumes of zirconia/silica beads (Biospec, 0.1 mm) and RIPA lysis buffer (Beyotime Biotechnology, China)

were added to the tube. The cells were lysed with a cell disruption device, and the protein concentrations were measured using a commercial BCA assay. The pretreatment of harvested protein samples ($100 \mu\text{g}$) before LC-MS detection was performed according to our previous study (Wen et al. 2022). Briefly, the harvested protein samples were reduced with 10 mM DTT (Sigma-Aldrich, USA), then alkylated with 50 mM iodoacetamide (IAA; Sigma-Aldrich, USA), avoiding light. The samples were desalted and washed with 0.5 M ammonium bicarbonate with Amicon® Ultra Centrifugal Filters (10 kDa cutoff; Merck Millipore, USA), and digested with trypsin (Promega, USA).

LC-MS/MS detection was conducted in the Ultimate™ 3000 RSLCnano system coupled to a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, USA). Peptides (250 nm) were loaded on 75 μm Acclaim™ PepMap™ C18 reverse-phase analytical column (Thermo Scientific, USA), and eluted with 0.1% acetic acid (containing $\sim 53\%$ acetonitrile). The full scan was performed in the Orbitrap (m/z 300–1500) with a resolution of 70,000. The corresponding MS2 spectra for MS detection were at the resolution of 17,500 (maximally 50 ms). The different abundance proteins were determined with the Proteome Discoverer 2.4 base with the Sequest HT, according to the Uniprot reference proteome of *S. aureus* (strain NCTC 8325/PS47). The minimum unused score of 1.3 (equivalent to 95% confidence) and the false discovery rate (FDR) of less than 1% were used for all reported proteins (decreasing false-positive identification results). The different abundance proteins were analyzed with the OMICSBEAN database (<http://www.omicsbean.com>) for GO annotation, KEGG pathway analysis, and PPI networks.

Membrane integrity assay. Both propidium iodide staining and bis(1,3-dibutylbarbituric acid) trimethine oxonol uptake assay were performed following the protocol of Fan et al. (2019). The PI staining method mainly aimed to evaluate the integrity of the *S. aureus* cell membrane. *S. aureus* suspension ($\text{OD}_{600} = 0.2$) was diluted 100-fold with PBS and pipetted into a 24-well plate. α -Mangostin was added into each well at final concentrations $1 \times \text{MIC}$ and $4 \times \text{MIC}$, respectively. Sodium chloride solution and DMSO were used as the negative control group, and 1% Triton was used as the positive control group. PI solution ($7.5 \mu\text{g}/\text{ml}$) was added to each well and kept the plate at room temperature for 30 min. A microplate reader was used to detect fluorescence intensity at excitation and emission wavelengths of 535 and 615 nm, respectively. DiBAC₄(3) was a fluorescent dye and sensitive to membrane potential. Briefly, *S. aureus* suspension ($\text{OD}_{600} = 0.2$) was added into a black, opaque, flat-bottomed 96-well plate, followed by the incubation with DiBAC₄(3) ($1 \mu\text{M}$) at 37°C in the dark for 30 min. Likewise, α -mangostin

has added into each well with the final concentrations of $1\times\text{MIC}$ and $4\times\text{MIC}$, respectively. Subsequently, the fluorescence intensity was detected at 492 nm and 515 nm. The controls were chosen as described above. Results were expressed in relative fluorescence units. All experiments were performed in triplicate.

Statistical Analysis. The results were shown as mean $\pm$ SD and were compared by Student's *t*-test or one-way analysis of variance (ANOVA). All the data were analyzed by the IBM® SPSS® software package (version 19.0, USA), and the $p < 0.05$ was determined as statistically significant.

Results

The rapid bactericidal activity of α -mangostin on planktonic cells of *S. aureus*. The MICs of α -mangostin against 328 clinical *S. aureus* isolates (190 MSSA and 138 MRSA) ranged from 1.56 μM to 6.25 μM , both with the $\text{MIC}_{50}/\text{MIC}_{90}$ of 3.13/3.13 μM , respectively (Table I). This study found that the low dose of α -mangostin combined with vancomycin ($\frac{1}{2}\times\text{MIC}$) had a significant inhibitory effect on the growth of *S. aureus* SA113 planktonic cells at a logarithmic phase (Fig. 1). Moreover, the growth of *S. aureus* SA113 planktonic cells could be markedly inhibited by the combination of α -mangostin with linezolid (Fig. 1).

Interestingly, this research demonstrated that high-dose of α -mangostin ($4\times\text{MIC}$) had a rapid bactericidal effect on *S. aureus* SA113 planktonic cells and was more effective (at least $2\text{-log}_{10}\text{ CFU/ml}$) than daptomycin,

Table I
Staphylococcus aureus susceptibility to α -mangostin.

<i>S. aureus</i>	The MICs (μM) of α -mangostin			
	1.56	3.13	6.25	$\text{MIC}_{50}/\text{MIC}_{90}$
MSSA (n = 190)	16	163	11	3.13/3.13
MRSA (n = 138)	13	117	8	3.13/3.13

MIC – minimum inhibitory concentration,
MSSA – methicillin-sensitive *S. aureus*,
MRSA – methicillin-resistant *S. aureus*,
 $\text{MIC}_{50}/\text{MIC}_{90}$ – the MICs for 50% or 90% of bacterial growth inhibition

vancomycin, and linezolid after 1 and 3 h of the time-killing test. However, the combination of α -mangostin with vancomycin, daptomycin or linezolid did not increase the bactericidal activity of α -mangostin against *S. aureus* (Fig. 2).

α -Mangostin significantly reduces established biofilms of *S. aureus*. To evaluate the eradicating effect of α -mangostin on established biofilms of *S. aureus*, the strong biofilm producer *S. aureus* SA113 and YUSA145 isolates were chosen, and the biofilm biomass was determined by crystal violet staining. The present study indicated that established biofilms of *S. aureus* could be notably eradicated by high-dose of α -mangostin in a concentration-dependent manner ($\geq 4\times\text{MIC}$) (Fig. 3).

The genetic mutations in α -mangostin non-sensitive *S. aureus* clones. To further explore the resistance mechanism or potential target gene of α -mangostin in *S. aureus*, the α -mangostin non-sensitive *S. aureus* isolates were finally selected by *in vitro* induction of wild-type strains. Then whole-genome sequencing

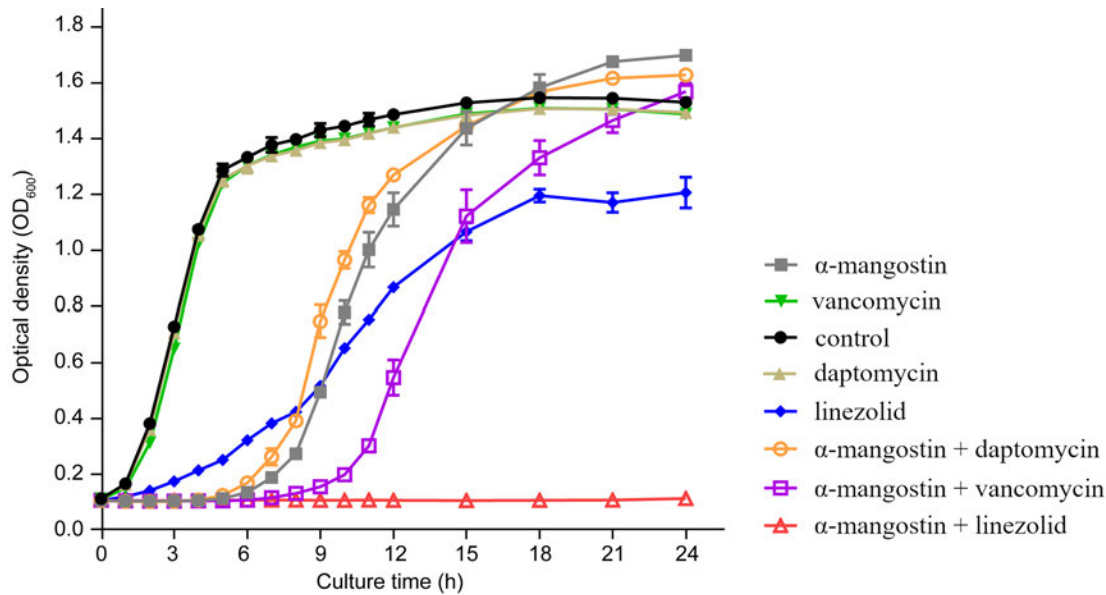


Fig. 1. The effect of sub-MIC of α -mangostin on the growth of *Staphylococcus aureus* planktonic cells. *S. aureus* SA113 was treated with α -mangostin, daptomycin, vancomycin, linezolid, and their synergistic combinations ($\frac{1}{2}\times\text{MIC}$), and the growth of planktonic cells was detected by optical density at 600 nm (OD_{600}). The data presented was the average of three independent experiments (mean $\pm$ SD). MIC, minimum inhibitory concentration.

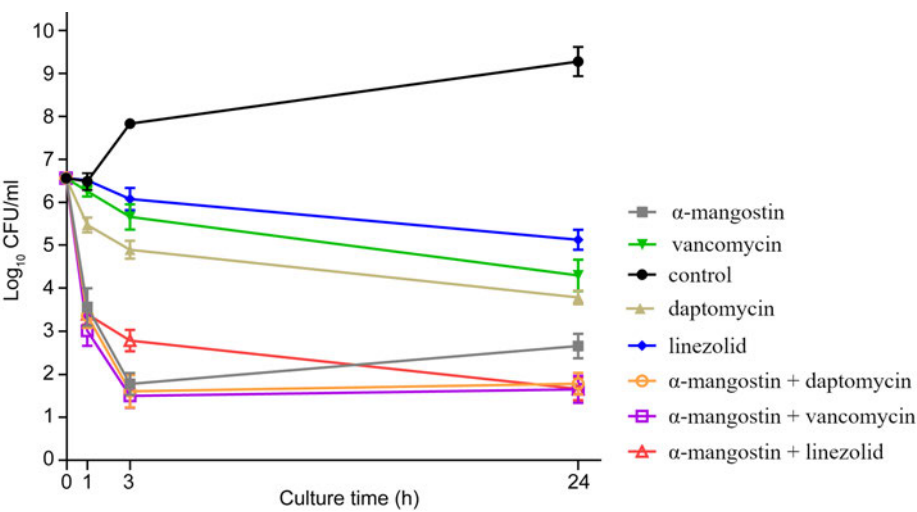


Fig. 2. The antibacterial effect of α -mangostin on the planktonic cells of *Staphylococcus aureus*. *S. aureus* SA113 during logarithmic growth phase was treated with α -mangostin, daptomycin, vancomycin, linezolid, and their synergistic combinations (4 $\times$ MIC), the remaining planktonic cells were enumerated. The data presented was the average of three independent experiments (mean $\pm$ SD). MIC, minimum inhibitory concentration.

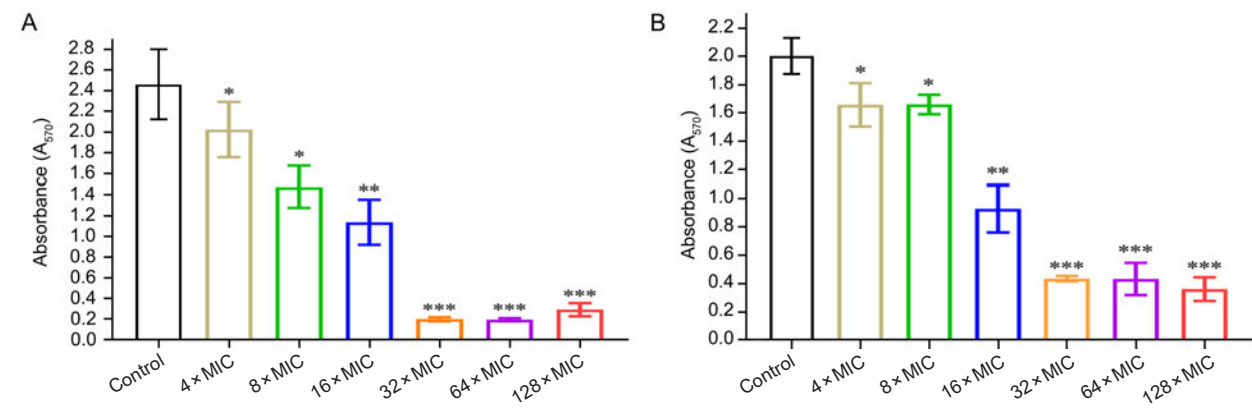


Fig. 3. Effect of different concentrations of α -mangostin on *Staphylococcus aureus* biofilms. A) *S. aureus* SA113 and B) YUSA145 formed mature biofilms, then were treated with different concentrations of α -mangostin for 24 h. The remaining biofilm biomass was determined by crystal violet staining. The data presented was the average of three independent experiments (mean $\pm$ SD). Compared with control: * p < 0.05; ** p < 0.01; *** p < 0.001; Student's t -test. MIC – minimum inhibitory concentration

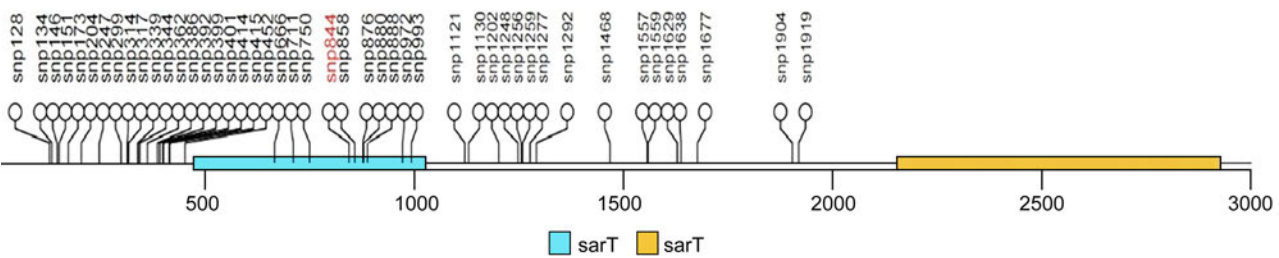


Fig. 4. Schematic illustration of the SNPs in the *sarT* gene of the α -mangostin non-sensitive *Staphylococcus aureus* isolate. There were 35 SNPs located on both sides of the *sarT* gene, 10 SNPs in the *sarT* gene included one non-synonymous mutation (in red) and nine synonymous mutations.

was performed to detect mutations. In the beginning, *S. aureus* SA113 and YUSA145 isolates were induced, but only YUSA145 isolate after about 160 days of arduous induction, resulting in the increased MICs of α -mangostin ($\geq 12.5 \times$ MIC). The genetic mutations in the α -mangostin non-sensitive clone YUSA145-L12

were detected by whole-genome sequencing. This study indicated that there were 58 SNPs in the *S. aureus* YUSA145-L12 clone (Table SI), of which 35 SNPs were located on both sides of the *sarT* gene, and 10 were located on the *sarT* gene, including one non-synonymous mutation and nine synonymous mutations (Fig. 4).

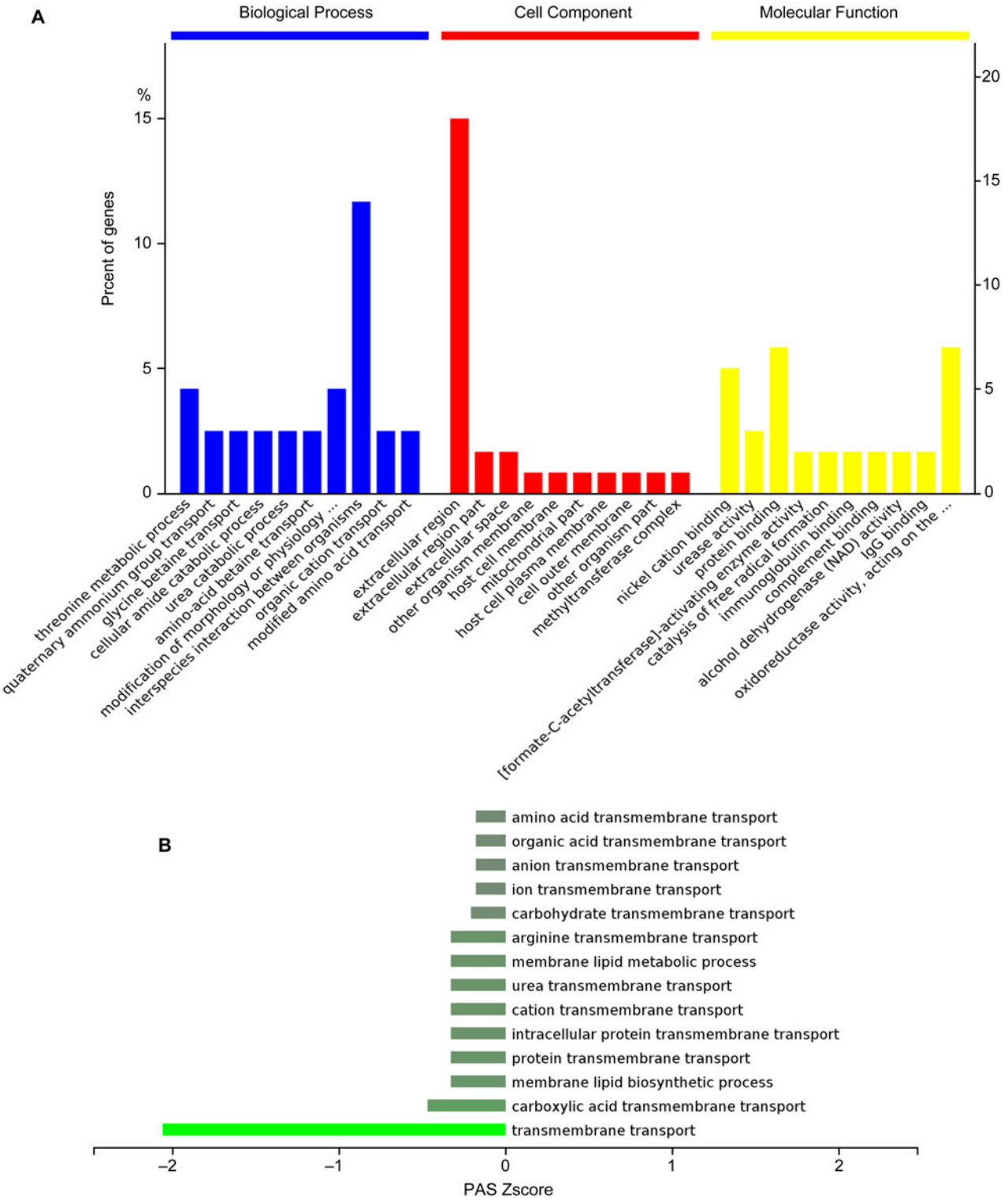


Fig. 5. The different abundance proteins in- α -mangostin-treated *Staphylococcus aureus* isolate. A) The molecular functions of different abundance proteins were classified by the GO analysis; B) different abundance proteins related to cell membrane synthesis and transport of biological process.

**The different abundance of proteins in α -mangos-
tin treated *S. aureus* isolate.** A total of 147 proteins with
different abundances were determined (identified pep-
tides > 2, p -value < 0.05, with fold-change > 2 or < 0.5)
in α -mangostin treated *S. aureus* isolate, and of which
the increase was observed for 91 proteins, whereas

decrease for 56 proteins (Table SII). According to the
GO annotation, the predominant cellular components
of different abundance proteins were involved in oxi-
doreductase activity, nickel cation binding, and protein
binding (Fig. 5A). Interestingly, the abundance of a vari-
ety of proteins in pathways related to the synthesis and

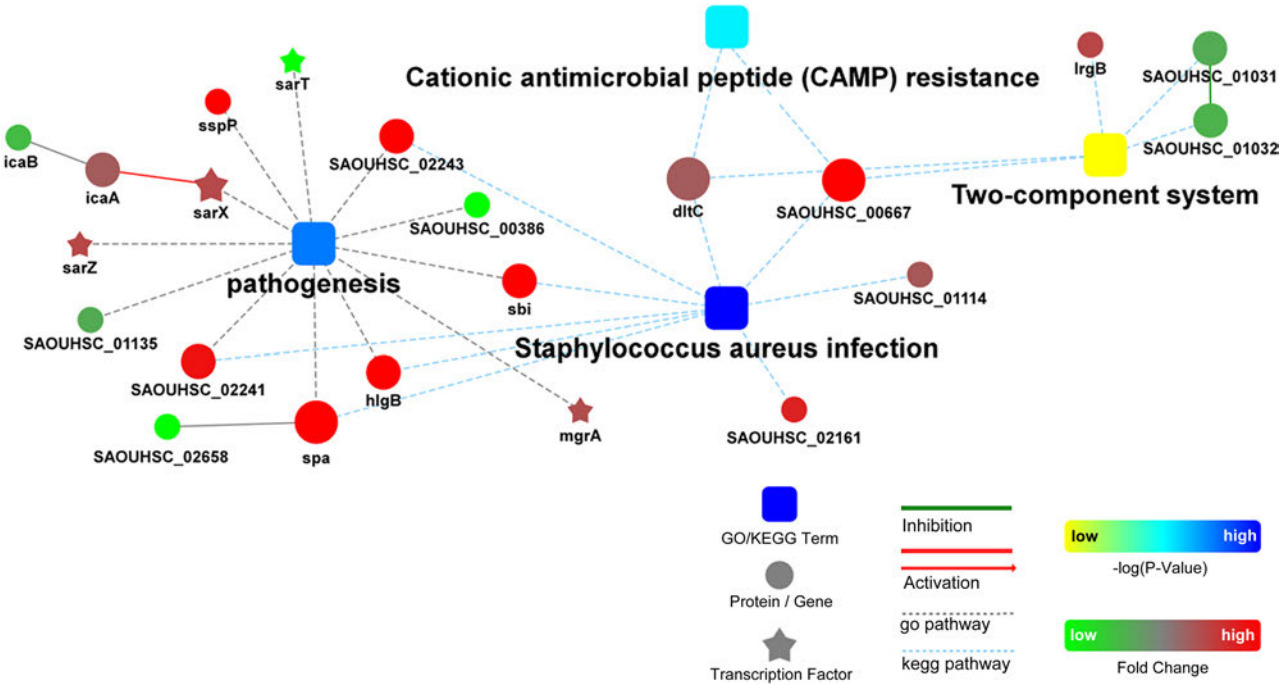


Fig. 6. Protein-protein interaction network of different abundance proteins in α -mangostin-treated *Staphylococcus aureus* isolate. The protein-protein interaction network of different abundance proteins was analyzed through STRING database.

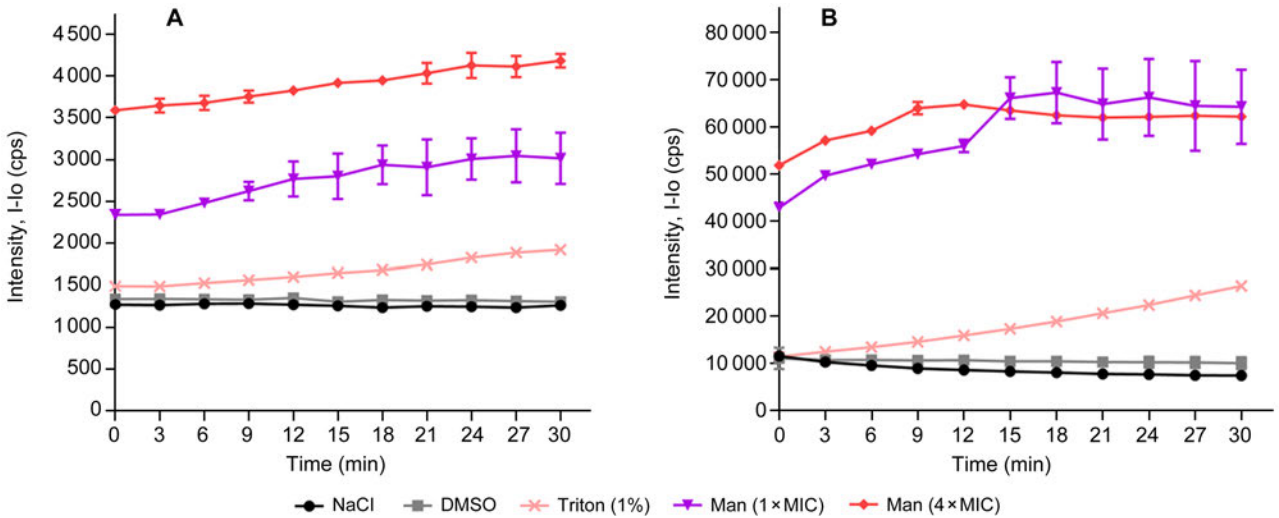


Fig. 7. The fluorescence intensity was significantly increased in α -mangostin-treated *Staphylococcus aureus* isolate. *S. aureus* SA113 was treated with α -mangostin, and staining with A) propidium iodide or B) bis(1,3-dibutylbarbituric acid) trimethine oxonol to evaluate the integrity of *S. aureus* cell membrane. The results were expressed in a relative fluorescence units. The data presented was the average of three independent experiments (mean $\pm$ SD).

transportation of cell membrane was reduced, which might result in the injury to the integrity of bacterial cell membranes (Fig. 5B). The protein-protein interaction network of different abundance proteins in this study was analyzed through STRING database (Fig. 6). Interestingly, the abundance of regulatory proteins SarX and SarZ was elevated, while the abundance of SarT and IcaB was significantly reduced. The abundance of cell membrane proteins VraF and DltC in the cationic antimicrobial peptide (CAMP) pathway of

S. aureus was augmented. However, the abundance of cell membrane proteins UgtP was detected a remarkably decreased (100-fold). **The disruption of membrane integrity in *S. aureus* treated with α -mangostin.** To further evaluate the impact of α -mangostin on the membrane integrity of *S. aureus*, PI (Fig. 7A) and DiBaC₄(3) uptake assays (Fig. 7B) were performed. The results indicated that fluorescence intensity recorded in these assays was significantly increased in the α -mangostin treated group.

Discussion

S. aureus is considered the most frequent cause of infections on indwelling medical devices and tends to develop biofilm, facilitating resistance to antibiotics and host immune defenses (Otto 2018). This study revealed a remarkable reduction of established *S. aureus* biofilms was observed in the groups given high-dose of α -mangostin. To explore the mechanism of α -mangostin against *S. aureus* biofilms, the genetic mutations in the α -mangostin non-sensitive isolates were detected by whole-genome sequencing. This study showed that multiple SNPs occurred located on or near the genes *sarT* and *sarU* of *S. aureus* exposed to high-dose ($\geq 12.5 \times \text{MIC}$) of α -mangostin. When exposed to a low dose ($\frac{1}{2} \times \text{MIC}$) of α -mangostin, the abundance of SarT protein decreased significantly. SarT is a regulator and is involved in the *hla* gene expression. It also down-regulates RNAPIII via SarU (indirect) in *S. aureus* participating in regulating *S. aureus* virulence and biofilm formation (Manna and Cheung 2003; Ciulla et al. 2018; Schilcher and Horswill 2020). Therefore, it is speculated that α -mangostin reduced established biofilms of *S. aureus* by inhibiting the function of SarT.

In this study, a total of 147 proteins with different abundances were identified by proteomics analysis. Significantly, many proteins in pathways related to cell membrane synthesis and transport were down-regulated by α -mangostin through GO annotation. It has been noted that the abundance of UgtP protein is extremely low. The protein UgtP is responsible for anchoring lipoteichoic acid (LTA) on the cell membrane. Deleting the gene for UgtP protein impacts cell growth and division (Gründling and Schneewind 2007; Hesser et al. 2020).

The protein-protein interaction analysis showed that the abundance of IcaB was significantly reduced. The *icaADBC* system regulates the synthesis of polysaccharide intercellular adhesion (PIA), a dominant component of *S. aureus* biofilm (Arciola et al. 2015; Schilcher and Horswill 2020). As reported by Phuong et al. (2017), the MSSA strains were more sensitive to α -mangostin than MRSA. The possible reason was that in their study, the dominant component of MSSA biofilm was tested as PIA, while proteins comprised the central part of the MRSA biofilms. However, more evidence is still needed to confirm the relationship between the biofilm eradication activity of α -mangostin and the regulation of *icaADBC*.

The protein-protein interaction analysis also indicated that the abundance of cell membrane protein DltC and VraF, the two important members in the cationic antimicrobial peptide (CAMP) pathway, was remarkably increased. CAMP is a class of peptides with antimicrobial activity, which interacts with negatively

charged cell membranes and destroys the permeability and integrity of cell membranes (Falord et al. 2012; Yang et al. 2012). In *S. aureus*, the GraSR/VraFG two-component system (TCS) controls CAMP resistance through d-alanylation of teichoic acids, mediated by the DltABCD enzymes, as well as MprF-dependent lysination of phosphatidylglycerol to increases the positive surface charge and prevents CAMP binding by electrostatic repulsion (Falord et al. 2012; Cho et al. 2021). Therefore, α -mangostin might be a membrane-target agent that exhibits a bactericidal action similar to CAMP, causing the up-regulation of DltC and VraF after treatment with α -mangostin.

Propidium iodide (PI) staining and DiBaC₄(3) uptake assay were performed to evaluate the membrane integrity in the present research. It is well known that PI is a membrane-impermeable dye and DNA intercalating agent. When the integrity of the inner membrane is disrupted, PI may bind to DNA in the cell and emit more intense fluorescence. DiBaC₄(3) is a cell membrane potential dye. When the membrane potential is disturbed and depolarized, the fluorescence intensity increases. PI staining assay and DiBaC₄(3) uptake assay results confirmed that α -mangostin is an excellent membrane target bactericide. Koh's research also verified the direct membrane targeting mechanism through molecular dynamic simulations (Koh et al. 2013). However, the proteomics results revealed that the upstream GraS and GraR were not differentially expressed in the α -mangostin treatment group. Whether the mechanism of α -mangostin is related to the CAMP and GraSR TCS requires more experiments to clarify further.

Conclusion

In conclusion, this study reveals that α -mangostin is an effective antibacterial agent against *S. aureus* by targeting the cell membrane. The anti-biofilm effect of α -mangostin may be related to the inhibition of SarT and IcaB.

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Availability of data and material

The raw whole-genome sequencing data was posted in the Sequence Read Archive (SRA) database under BioProject accession number PRJNA587170 in NCBI.

The mass spectrometry proteomics data have been deposited to the Proteome X change Consortium (<http://proteomecentral.proteomexchange.org>) via the iProX partner repository (Ma et al. 2019) with the dataset identifier PXD037518.

Ethical statement

All procedures involving human participants were performed following the ethical standards of Shenzhen Nanshan People's Hospital and the 6th Affiliated Hospital of Shenzhen University

Medical School, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. As this study only involves the detection of patients' *S. aureus* samples and does not carry out drug treatment, patients do not need to sign informed consent according to the requirements of the Ethics Committee of Shenzhen Nanshan People's Hospital and the 6th Affiliated Hospital of Shenzhen University Medical School.

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Author contributions

XD performed MIC detection, biofilm assay, protein extraction and interpreted the proteomics data, and drafted the manuscript. HX conducted the biofilm assay and induction of α -mangostin non-sensitive clones *in vitro*. DL collected *S. aureus* strains, participated in the MIC detection and biofilm assay. JC conducted MIC detection, a time-killing test, and a membrane integrity assay. ZY, QD and PL participated in the analyzed and interpreted the whole-genome sequencing data and proteomics data. JZ and HZ guided the planning of this study, analyzed the data, and carefully revised the manuscript.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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