Transcriptomics Analysis of the Effect of Glabridin on Biofilm Formation in *Staphylococcus aureus*

I. Introduction

Staphylococcus aureus is a pathogen causing common skin and systemic infections in humans and animals. Its ability to form biofilms makes it hard to treat, shielding bacteria from antibiotics and the immune system. The increasing spread of Methicillin-Resistant *S. aureus* (MRSA) has made antibiotic resistance a serious global health problem.

Glabridin (Glb) is a natural flavonoid compound extracted from licorice (*Glycyrrhiza glabra*) with antibacterial properties. Yet, its mechanism for inhibiting biofilms remains unclear. This study uses transcriptomic (RNA-seq) to investigate how Glb affects *S. aureus* biofilm gene expression.

II. Main Content

1. Methods

The Biofilm Check

- **The Congo Red Test**

  Bacteria were grown on Congo red agar plates to identify biofilm-producing strains.

  - **Black**, dry colonies = biofilm-positive bacteria.
  - **Red**, smooth colonies = biofilm-negative bacteria.

The Action Test

A. Crystal Violet Staining

Biofilms were stained purple using crystal violet dye.

- Dark purple = high biofilm formation.
- Light or clear wells = reduced biofilm due to drug effect.

Biofilm biomass was quantified using OD570 measurements.

B. Growth Curve Analysis

Bacterial growth was monitored for 16 hours to see if Glb slows or completely stops growth.

C. Scanning Electron Microscopy (SEM)

SEM was used to see if Glb disrupts biofilm architecture or damages individual bacterial cells.

D. The eDNA Detection

Extracellular DNA (eDNA), which helps biofilm structure stay unbroken, was analyzed using agarose gel electrophoresis to see if Glb reduces its production.

Genetic Mechanism

A. Transcriptome Sequencing (RNA-seq)

Genetic analysis was used to see which genes Glb turned "on" or "off".

B. RT-qPCR Validation

Seven key biofilm genes were re-tested to confirm RNA-seq data was accurate.

2. Results

The "Magic" Numbers

- **Total Kill (MIC): 8 µg/mL** kills the bacteria.
- **The Sweet Spot (Sub-MIC): 2–4 µg/mL** keeps bacteria alive, but destroy their **"slime city" (biofilm).**

Physical Destruction

- **SEM Photos:** Shows the organized bacterial clusters being **shattered** and unable to stick to surfaces.
- **eDNA Reduction:** Glb stops **eDNA** production (the biological glue) that holds the biofilm together.

Glb acts like a “hacker”, disrupting **184 genes** to weaken the bacteria:

- Shuts down the ***Phosphotransferase System***, blocking sugar intake.
- Jams ***Quorum Sensing***, stopping bacterial communication.
- Messes with ***Nitrogen Metabolism***, draining their energy.
- Disrupts the ***Two-component system***, leaving bacteria unable to sense surroundings.

3. Discussion

Glb inhibits biofilms by reducing eDNA “glue” and altering key genes (*icaD*, *icaR*, *SarA*, and *FnbA*). It also starves bacteria by disrupting nutrient and energy metabolism. These findings highlight Glb’s potential as a weapon against resistant strains (MRSA).

III. Conclusion

Glabridin effectively inhibits *S. aureus* biofilm formation by suppressing eDNA production and regulating biofilm-related gene expression. This study provides scientific evidence supporting the development of natural antibiofilm compounds as alternative treatments for bacterial infections and antibiotic resistance.

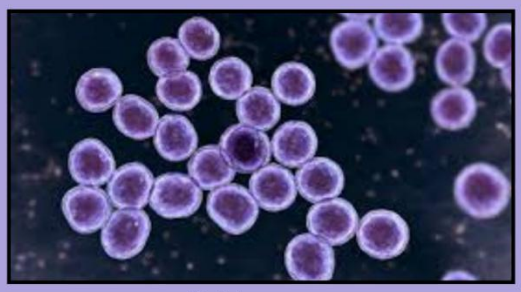
IV. References

Ma, Y., Mao, Y., Kang, X., Zhang, B., Wang, J., Wang, G., & Wang, G. (2025). Transcriptomic analysis of the effect of glabridin on biofilm formation in *Staphylococcus aureus*. *Foodborne Pathogens and Disease, 22*(7), 489–497. https://doi.org/10.1089/fpd.2024.0038

Glabridin Against Biofilms in *Staphylococcus aureus*

🦠 PROBLEM

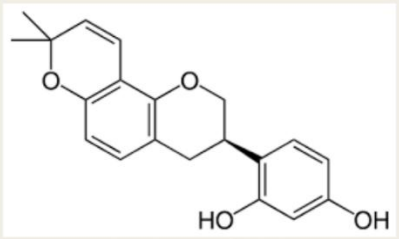
Staphylococcus aureus can form biofilms → resistant to antibiotics
MRSA → a global resistance threat



Staphylococcus aureus

🌿 SOLUTION: GLABRIDIN

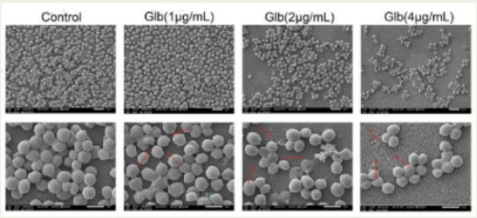
- A flavonoid compound from licorice
- Potential antibacterial & antibiofilm agent



Chemical Structure of Glabridin

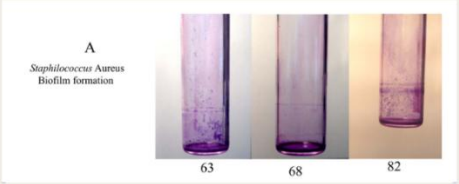
📊 KEY RESULTS

MIC: 8 μg/mL → kills bacteria
Sub-MIC: 2–4 μg/mL → disrupts biofilm
↓ eDNA + disrupts 184 biofilm & metabolism-related genes



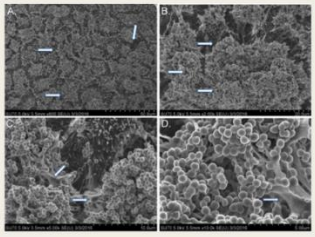
🧪 CORE METHODS

Biofilm test → Congo red, crystal violet
Structure → SEM, eDNA gel
Genetics → RNA-seq + RT-qPCR



(Biofilm test)

Congo red test



Scanning Electron Microscope (SEM)