Unraveling Nitrogen Homeostasis: The Role of PstA in *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus causes a diverse spectrum of clinical infections. The emergence and spread of *S. aureus* Methicillin-resistant strain (MRSA) have escalated concerns due to its resistance to multiple antibiotics and resilience in the changing environment, posing significant challenges for treatment and infection control strategies. Addressing this urgent healthcare crisis requires a comprehensive understanding of how *S. aureus* modulates its central metabolism to survive nutrition starvation. In this study, we focus on elucidating the regulatory network governing glutamine metabolism in *S. aureus*, a fundamental process essential for bacterial growth. We used EMSA, BLI, and qPCR to study how the transcriptional repressor GlnR regulates glutamine metabolism. We found that glutamine synthetase GlnA helps GlnR bind to DNA, suppressing glutamine metabolism genes when glutamine is present. However, the regulation of GlnR in the absence of glutamine is unknown. We hypothesize that PstA abolishes the GlnA-DNA interaction in the absence of glutamine, leading to the de-repression of glutamine metabolism genes. Understanding these regulatory mechanisms can improve our grasp of bacterial metabolism regulation. Targeting the identified regulatory proteins in future research could uncover novel antimicrobial agents to address MRSA-related issues, thus advancing infectious disease therapeutics.

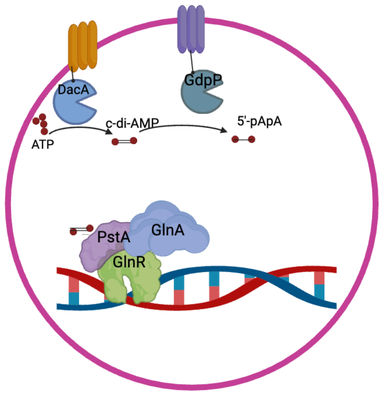
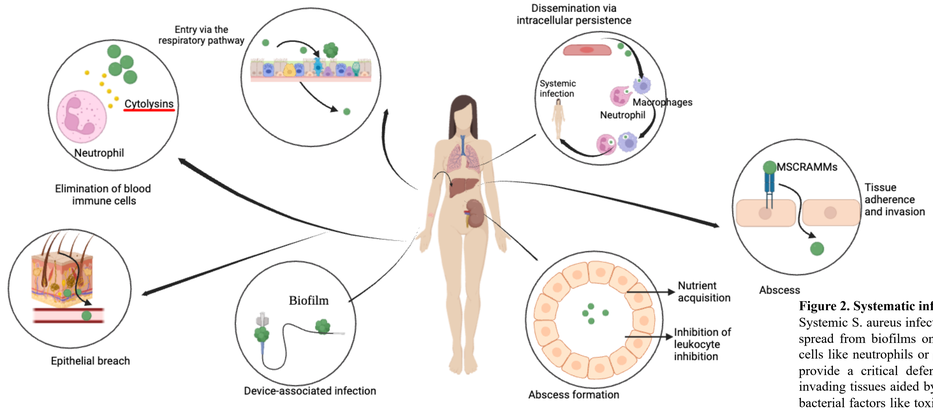


Figure 1. Schematics of c-di-AMP metabolism and nitrogen homeostasis

INTRODUCTION



S. aureus demonstrates resilience during infections and adapts to nutrient-deficient environments.

We aim to comprehensively understand this adaptation process, potentially facilitating the development of targeted therapeutics for control.

Figure 2. Systematic infection of *S. aureus*.
Systemic S. aureus infection often begins when the bacteria breach the skin barrier or spread from biofilms on medical devices. In the bloodstream, they attack immune cells like neutrophils or persist within them. Passing through the liver, Kupffer cells provide a critical defense. Surviving this, the bacteria spread, attaching to and invading tissues aided by surface proteins. Abscess formation follows, influenced by bacterial factors like toxins and enzymes. This figure is adapted from Cheung et all, 2021. (PMID: 33522395)

Result 1. GlnR binds to the promoter region of *glnR-glnA* operon

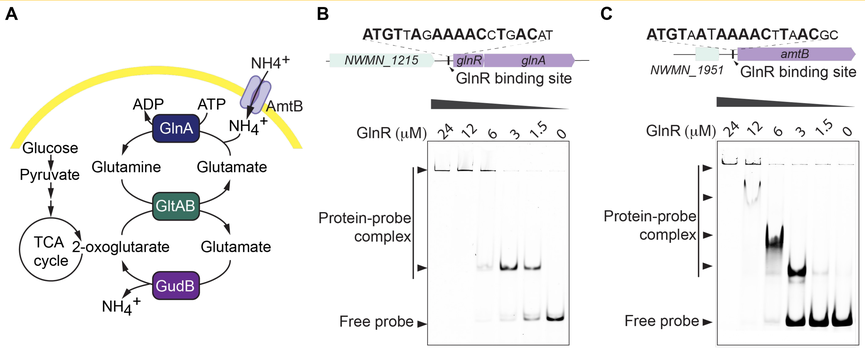


Figure 3. GlnR binds to the promoter region of *glnR-glnA* operon
(A) Schematic figure of the glutamine metabolism. (B) GlnR binding site on the promoter region of the glnR-glnA operon (Top). GlnR binds to the promoter region of *glnR* using electrophoretic mobility shift assay (EMSA) (bottom). (C) GlnR binding site on the promoter region of the amtB gene (Top). GlnR binding to the promoter region of the *amtB* gene (bottom). The DNA probe is labeled by FAM.

Result 4. Δ*pstA* exhibited increased expression of glutamine metabolism genes

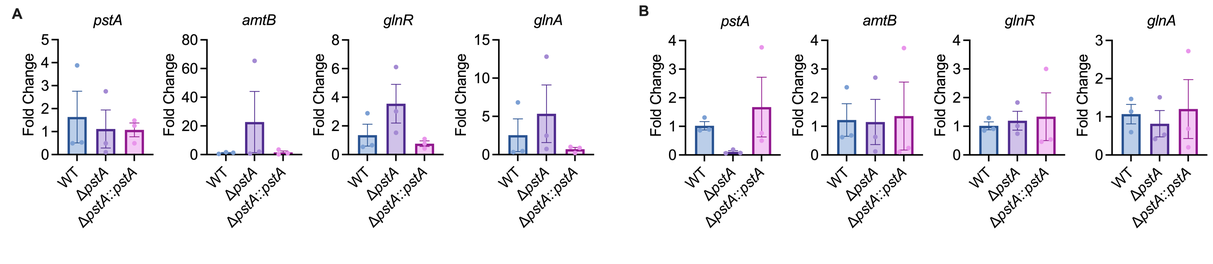


Figure 4. Real-time quantitative reverse transcription PCR (RT-qPCR) to quantify the transcription levels of *glnA*, *glnR*, and amtB genes in wild-type (WT), *ΔpstA*, and *ΔpstA* complementation strains (*ΔpstA::pstA*). Bacteria were cultured overnight in chemical-defined media (A) glutamine-defined media, (GDM) , and (B) glutamine-defined media supplemented with Glutamine (20μM), followed by RNA extraction for RT-qPCR analysis. Fold changes relative to the WT strain were calculated using the 2–ΔΔCt method, utilizing the 16S rRNA gene as the internal control.

Result 2. PstA promotes GlnR binding to DNA

(A)

Protein	Function
TopB	DNA topoisomerase
GlnR	**Glutamine synthetase repressor**
GdpP	C-di-AMP phosphodiesterase
dUTPase	DUTP pyrophosphatase
GlnA	**Glutamine synthetase**
PstA	**P II family protein**
FmtC	Phosphatidylglycerol lysylfraansferase
RuvV	Holliday junction DNA helicase RuvA

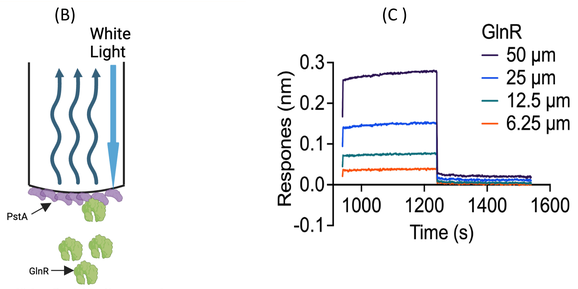


Figure 5 Identifying PstA binding protein
(A) Exploring proteins that may interact with c-di-AMP either directly or indirectly, through a c-di-AMP affinity pull-down assay utilizing c-di-AMP-agarose beads. (B) Assessment of the interaction between PstA and GlnR using biolayer interferometry (BLI) assay, with an equilibrium-binding affinity (Kd) of approximately 15 μM. (C) Binding affinity of PstA to GlnA was assessed by the BLI assay (Kd~ 25 μM).

CONCLUSION

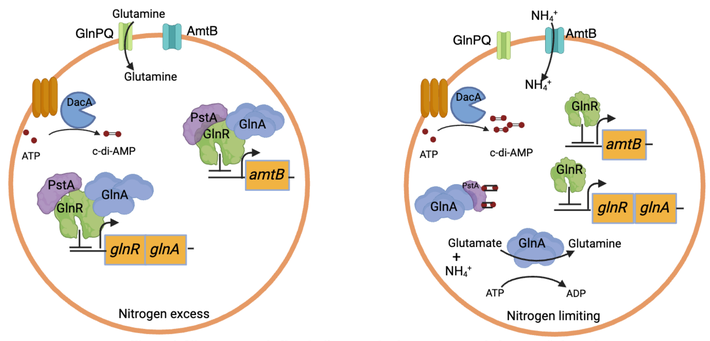


Figure 6. Nitrogen metabolism in *S. aureus* in the presence and absence of glutamine

- We have identified two proteins, GlnR and GlnA, important regulators for glutamine metabolism, which interact with the PII signaling family protein PstA.
- We demonstrated that PstA enhances the binding of GlnR to DNA.

Result 3. PstA promotes GlnR binding to DNA independent of c-di-AMP

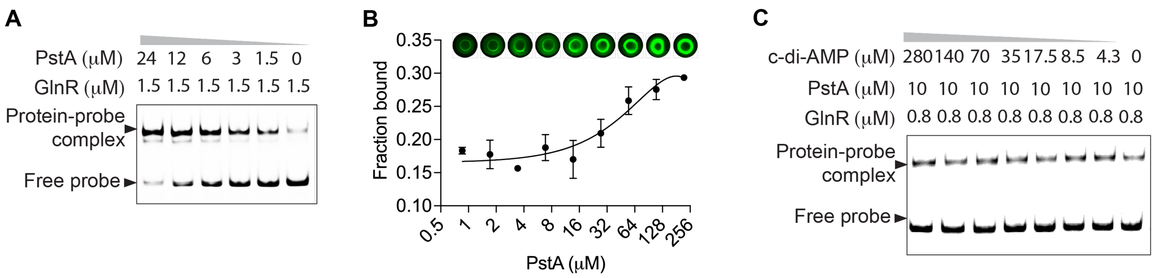


Figure 7. Investigating the interaction of GlnR with DNA in the presence of PstA, and c-di-AMP using EMSA.
(A). PstA facilitates the DNA binding of GlnR by asset by EMSA (B) PstA facilitates DNA binding of GlnR asset by DRACLA (C) PstA-GlnR-DNA interaction is enhanced in the presence of c-di-AMP

FUTURE DIRECTIONS

- Investigate the impact of varying c-di-AMP concentrations on the transcription of genes related to glutamine metabolism.
- Analyze the effects of c-di-AMP on interactions between PstA-GlnR and PstA-GlnA.
- Examine the regulatory role of PstA on the transcription of genes associated with glutamine metabolism under low glutamine conditions.
- Explore the influence of glutamine on c-di-AMP metabolism in *S. aureus.*

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