8-12-2021

Proline biosynthesis regulates proline transport in Staphylococcus aureus.

Alyssa NL Jarabek

McKenzie K. Lehman
University of Nebraska Medical Center

Paul D. Fey
University of Nebraska Medical Center

UNMC
UNIVERSITY OF NEBRASKA MEDICAL CENTER

Summer Undergraduate Research Program

Proline biosynthesis regulates proline transport in *Staphylococcus aureus*.

Jarabek, ANL, Lehman, MK, Fey, PD
University of Nebraska Medical Center, Omaha, NE

Background and Significance

Staphylococcus aureus is metabolically diverse with the ability to rapidly adapt to a vast array of nutrient sources. This allows the pathogen to colonize a variety of niches in the host. For instance, *S. aureus* is the leading cause of skin and soft tissue infections, a niche that has been shown to become glucose-depleted over the course of an infection. Previous studies have shown that in niches where glucose is deficient, *S. aureus* utilizes peptides and free amino acids as nutrient sources. Primarily, these amino acids include glutamate and amino acids that can serve as substrates for glutamate synthesis. While arginine and histidine serve as substrates in glutamate synthesis, proline is the primary source of glutamate. Indeed, *S. aureus* utilizes proline as a secondary carbon source only when glucose is absent, and it can be synthesized from arginine or acquired via proline transporters from its environment. Although *S. aureus* encodes two putative pathways for proline biosynthesis, it has been shown that pyrroline-5-carboxylate reductase (encoded by *proC)* is the sole proline biosynthetic pathway in *S. aureus* (Figure 1). Studies from our laboratory have revealed that despite encoding five putative proline transporters (*B7H15_03660*, *opuC*, *opuD*, *proP, putP* (Figure 2)), only two of the transporters, PutP and B7H15_03660 are responsible for a majority of proline transport under the laboratory conditions tested (Figure 3). Surprisingly, when we introduced the *proC* mutation into the *B7H15_03660 putP* double mutant, we observed proline-dependent growth, even though the primary proline transporters and proline biosynthetic pathway were knocked-out (Figure 4a). In contrast, the penta Δ*proC* strain was unable to grow (Figure 4b). These data suggest that inhibiting proline biosynthesis alters proline transport, and therefore one or more of the additional transporters, OpuC, OpuD, and/or ProP, are activated under these conditions. With these observations, we sought to better understand the intricate relationship of proline biosynthesis and transport in *S. aureus*.

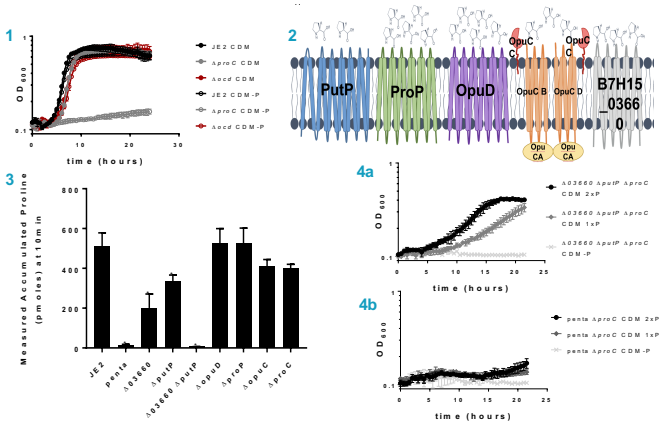
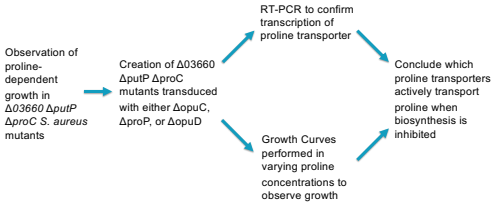


Figure 1: Proline biosynthesis alters proline transport. 1) Growth curve performed in chemically defined media (CDM) and CDM lacking proline (CDM-P). The Δ*ocd* strain demonstrates growth that phenocopies that of the wild-type *S. aureus* strain, JE2, whereas the *proC* mutant cannot grow in CDM-P. This indicates that *proC* is the sole enzyme responsible for proline biosynthesis in *S. aureus*. 2) Schematic of the five putative proline transporters in *S. aureus*, PutP, Prop, OpuD, OpuC, and B7H15_03660. 3) Radiolabeled proline transport assay shows that *opuD*, *prop*, and *opuC* knockouts do not result in significant impairment of proline accumulation, while *03660* and *putP* knockouts result in significantly impaired accumulation. Importantly, the Δ*03660 ΔputP* double mutant does not accumulate proline, similar to the proline transporter null strain (penta). 4) Growth curves of the a) Δ*03660 ΔputP ΔproC* mutant and b) penta Δ*proC* mutant in CDM with a proline gradient. Surprisingly, we observed growth of the Δ*03660 ΔputP ΔproC* that is proline dependent suggesting that one or more of the other proline transporters may be active once proline biosynthesis is inhibited. Data are represented by the mean ± SD, n=3, * represents p<0.05.

Hypothesis

We hypothesize that when the *proC* mutation is introduced into *S. aureus,* and thus proline biosynthesis is inhibited, the regulation of proline transport is altered. To address this hypothesis, we have devised the following research track.



OpuC and ProP transport proline in *proC* knockout

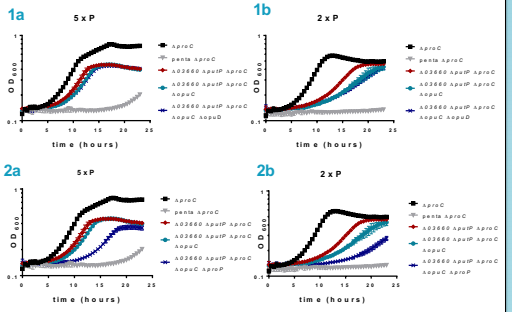


Figure 2: Additional proline transporters are active when proline biosynthesis is inhibited. Growth curves of 1) Δ*proC*, penta Δ*proC*, Δ*03660 ΔputP ΔproC*, Δ*03660 ΔputP ΔproC ΔopuC*, Δ*03660 ΔputP ΔproC ΔopuC ΔopuD*, and 2) Δ*proC*, penta Δ*proC*, Δ*03660 ΔputP ΔproC*, Δ*03660 ΔputP ΔproC ΔopuC*, Δ*03660 ΔputP ΔproC ΔopuC ΔproP* in CDM supplemented with a) five times the standard amount of proline (5xP) or b) two times the standard amount of proline (2xP) in CDM. Notably, there is a slight delay of growth in 5xP when the *opuC* mutation is introduced into the Δ*03660 ΔputP ΔproC*. Moreover, the delay is enhanced, and the growth rate is decreased further in 2xP, suggesting OpuC is capable of transporting proline under these conditions. Additionally, when the *proP* mutation is introduced into the Δ*03660 ΔputP ΔproC ΔopuC* strain, the lag phase is extended and the growth rate is decreased, suggesting ProP can also transport proline when *proC* is knocked out. The *opuD* mutation does not seem to affect growth, indicating it may not be important for proline transport under these conditions. Data are represented by the mean ± SD, n=3.

Is there another proline transporter?

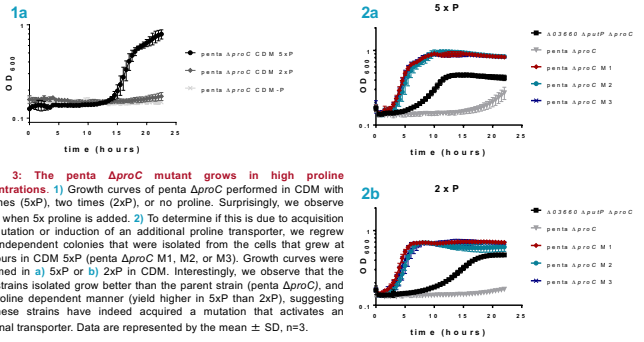


Figure 3: The penta Δ*proC* mutant grows in high proline concentrations. 1) Growth curves of penta Δ*proC* performed in CDM with five times (5xP), two times (2xP), or no proline. Surprisingly, we observe growth when 5x proline is added. 2) To determine if this is due to acquisition of a mutation or induction of an additional proline transporter, we regrew three independent colonies that were isolated from the cells that grew at >15 hours in CDM 5xP (penta Δ*proC* M1, M2, or M3). Growth curves were performed in a) 5xP or b) 2xP in CDM. Interestingly, we observe that the three strains isolated grow better than the parent strain (penta Δ*proC)*, and in a proline dependent manner (yield higher in 5xP than 2xP), suggesting that these strains have indeed acquired a mutation that activates an additional transporter. Data are represented by the mean ± SD, n=3.

Conclusions and future directions

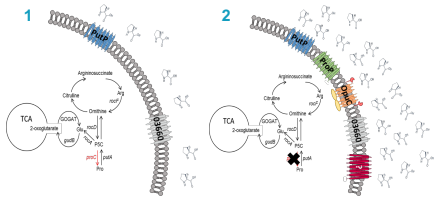


Figure 4: Δ*proC* mutation results in activation of additional proline transporters. Schematic representation of proline transport under conditions of 1) moderate exogenous proline and intact proline biosynthesis and 2) high exogenous proline and proline biosynthesis knocked-out. In a *proC* mutant, OpuC, ProP, and a potential additional proline transporter are important for maximal growth along with PutP and 03660. Future studies will be designed to further characterize the system.

These studies elucidated the regulation of proline transporters in *S. aureus.* We propose the following experiments to better characterize proline transport in *S. aureus:*

- Transcriptomic analysis of proline transporters
- Radiolabeled proline transport assays on Δ*proC* transporter mutants
- Additional proline gradient growth curves on various Δ*proC* proline transporter mutants
- WGS of penta Δ*proC* strains that grow in CDM

Acknowledgements and References

Lehman MK, Nuxoll AS, Yamada KJ, Kielian T, Carson SD, Fey PD. 2019. Protease-mediated growth of Staphylococcus aureus on host proteins is opp3 dependent. mBio 10:e02553-18. https://doi.og/10.1128/mBio.02553-18.

Halsey CR, Lei S, Wax JK, Lehman MK, Nuxoll AS, Steinke L, Sadykov M, Powers R, Fey PD. 2017. Amino acid catabolism in Staphylococcus aureus and the function of carbon catabolite repression. mBio 8:e01434-16. https://doi.org/10.1128/mBio.01434-16.

This project is funded by NIH/NIAID P01AI083211-12 and the UNMC Summer Undergraduate Research Program.