Analysis of the ability of *Staphylococcus aureus* to use glycerol-3-phosphate as a phosphate source

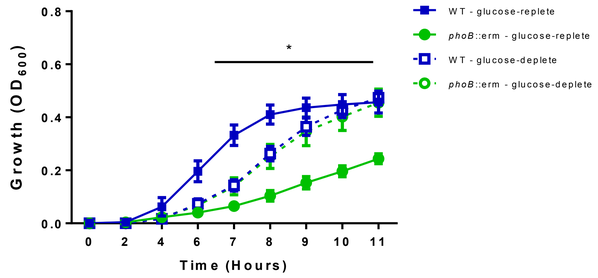
Kevin Grudzinski*, Jessica Kelliher and Thomas Kehl-Fie

Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, IL

1. Abstract:

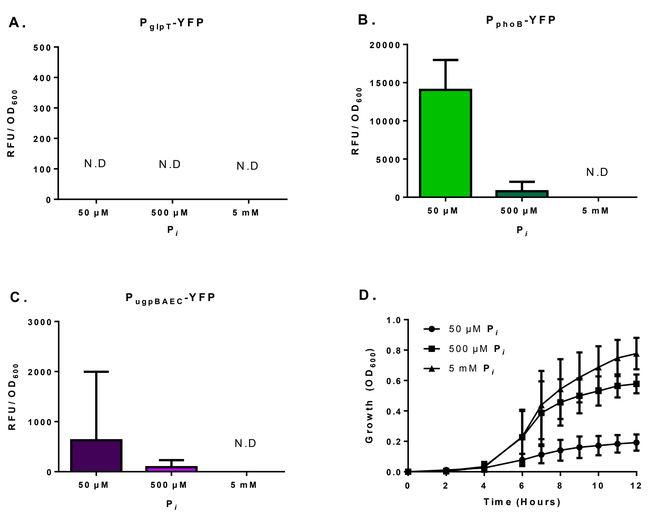
Staphylococcus aureus is a devastating pathogen that colonizes a third of the population. Multidrug-resistant strains continue spreading, leading the CDC to state that *S. aureus* is a serious threat to public health. During infection, pathogens must obtain all of their nutrients from the host. Transporters dedicated to the acquisition of phosphate, an essential nutrient, have been implicated in the virulence of other pathogens. However, how *S. aureus* obtains phosphate is unknown. Answering this question will enhance our understanding of staphylococcal disease. In the host, phosphate can be found as inorganic phosphate and organophosphates, such as glycerol-3-phosphate (G3P). Analysis of the staphylococcal genome identified a putative G3P transporter. Subsequently, we found that G3P can be used as a phosphate source by *S. aureus*. A strain lacking a putative alkaline phosphatase, PhoB, grows similarly to wild type in the absence of glucose when G3P is the only phosphate source, suggesting this molecule can be imported whole into the cell. However, transcriptional analysis revealed that the importer, GlpT, is not induced by phosphate starvation, suggesting phosphate acquisition may not be its primary role. In the presence or absence of glucose, a *glpT* mutant grew similar to wild type when G3P was the sole phosphate source. However, a *phoB* mutant was severely attenuated in the presence of glucose with G3P as the sole phosphate source. Cumulatively, our data suggests *S. aureus* preferentially utilizes PhoB to cleave G3P extracellularly, importing glycerol and phosphate separately under phosphate or carbon starvation.

2. The alkaline phosphatase (PhoB) is critical for growth in the presence on glycerol-3-phosphate when glucose is present.



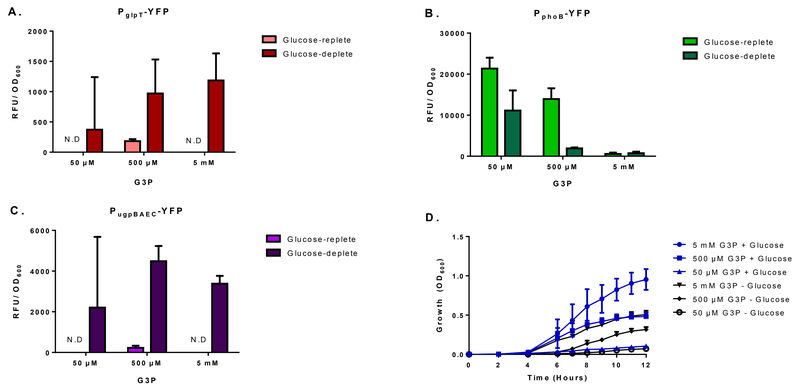
Wild type *S. aureus* (USA300 LAC JE2) or *phoB*::*erm* was grown in PFM9 unbuffered media supplemented with 5 mM G3P, as the sole phosphate source, ±glucose. Growth was measured by OD_{600} over 11 hours. Error bars indicate ±SEM (n=3). Two-Way Anova with Tukey Post Test of WT – glucose-deplete v. *phoB*::*erm* – glucose-deplete. * = p<0.05

3. PhoB, but not the putative G3P importers (UgpBAEC and GlpT), is induced by phosphate limitation.



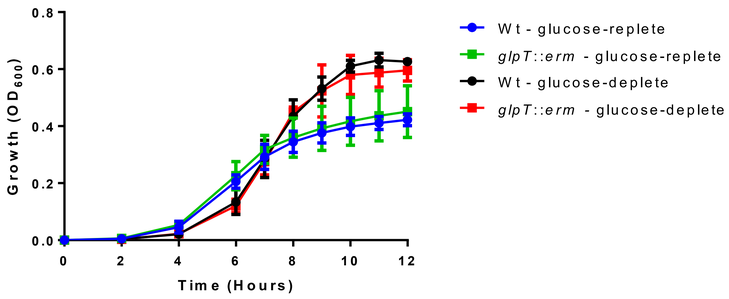
Wild type *S. aureus* (Newman) expressing pAH842E-YFP, p*glpT*-YFP (A), p*phoB-yfp* (B), or p*ugp-yfp* (C) was grown in PFM9 buffered media in a variety of P_i concentrations in glucose-replete media. Flourescence and OD_{600} were measured over 12 hours. The data shown corresponds to relative fluorescence at t=9 hrs. Error bars indicate ±SEM (n=3). (D) Wild type *S. aureus* (Newman) pAH842E-YFP (as a representative of plasmid-bearing Newman) was grown in PFM9 buffered media in a variety of P_i concentrations in glucose-replete conditions. Growth was measured by OD_{600} over 12 hours. Error bars indicate ±SEM (n=3). N.D = none detected

4. UgpBAEC and GlpT are expressed in the absence of glucose.



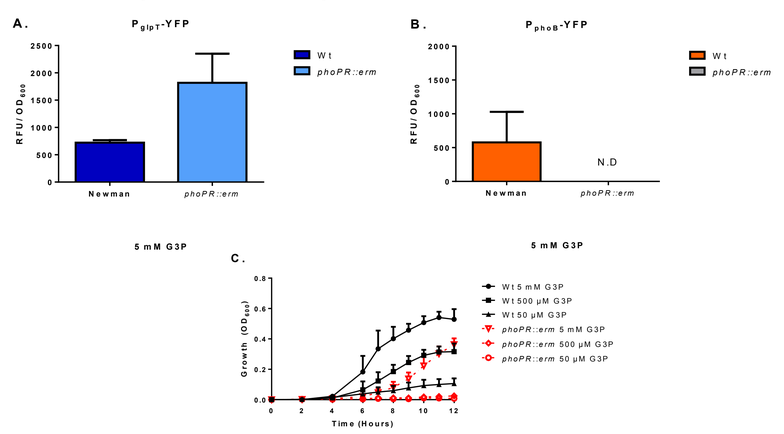
Wild type *S. aureus* (Newman) expressing pAH842E-YFP, p*glpT*-YFP (A), p*phoB*-YFP (B), or p*ugp*-YFP (C) was grown in PFM9 buffered media in a variety of G3P concentrations in glucose-replete or glucose-deplete media. Fluorescence and OD_{600} were measured over 12 hours. The data shown corresponds to relative fluorescence at t=9 hrs. Error bars indicate ±SEM (n=3). (D) Wild type *S. aureus* (Newman) pAH842E-YFP (as a representative of plasmid-bearing Newman) was grown in PFM9 buffered media in a variety of G3P concentrations in glucose-replete or glucose-deplete conditions. Growth was measured by OD_{600} over 12 hours. Error bars indicate ±SEM (n=3). N.D = none detected.

5. Δ*glpT* grows similarly to wild type in the presence of G3P.



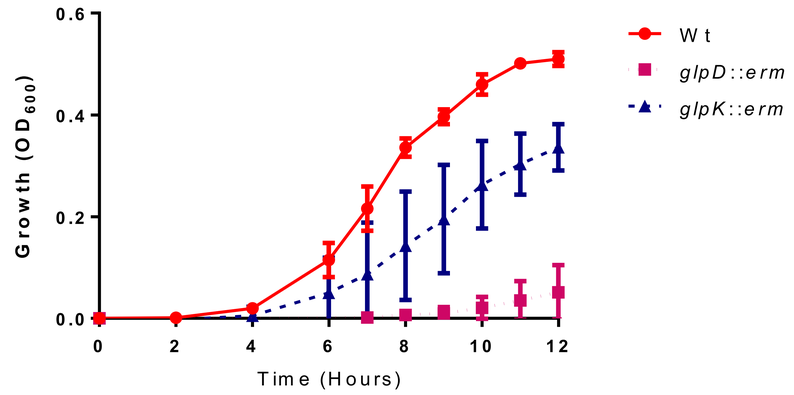
Wild type *S. aureus* (USA300 LAC JE2) or *glpT*::*erm* was grown in PFM9 unbuffered media supplemented with 5 mM G3P ±glucose. Growth was measured by OD_{600} over 12 hours. Error bars indicate ±SEM (n=3).

6. PhoB expression is dependent on PhoPR.



Wild type *S. aureus* (Newman) expressing pAH842E-YFP, p*glpT*-YFP (A) or p*phoB*-YFP (B), and *phoPR*::*erm* expressing pAH842E, p*glpT*-YFP (A) or p*phoB*-YFP (B) was grown in PFM9 buffered media in a variety of G3P concentrations in glucose-deplete media. The data shown corresponds to 5 mM G3P. Lesser concentrations of G3P were not considered because *phoPR*::*erm* has a growth defect. Flourescence and OD_{600} were measured over 12 hours. The data shown corresponds to relative fluorescence at t=12 hrs. Error bars indicate ±SEM (n=3). (D) Wild type *S. aureus* (Newman) pAH842E-YFP (as a representative of plasmid-bearing Newman) and *phoPR*::*erm* pAH842E (as a representative of plasmid-bearing *phoPR*::*erm*) were grown in PFM9 buffered media in a variety of G3P concentrations in glucose-replete or glucose-deplete conditions. Growth was measured by OD_{600} over 12 hours. Error bars indicate ±SEM (n=3). N.D = none detected

7. Glycerol dehydrogenase (GlpD) and glycerol kinase (GlpK) differentially contribute and are import for G3P utilization.

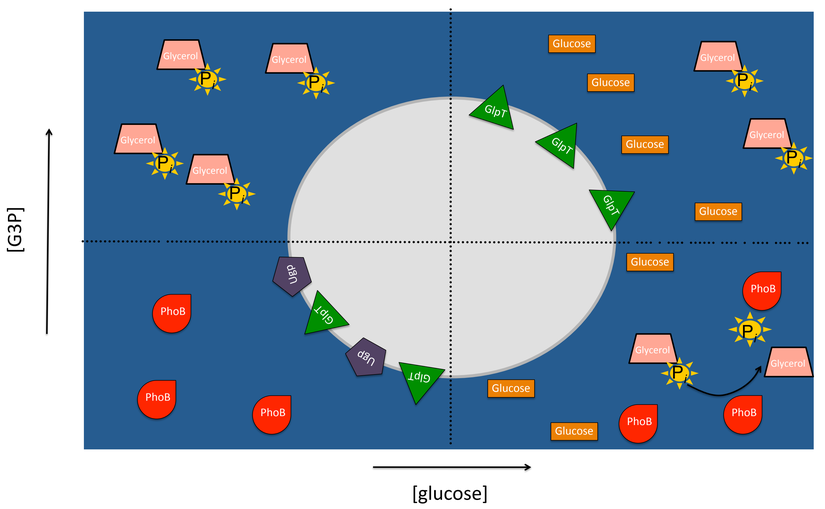


Wild type *S. aureus* (USA300 LAC JE2), *glpD*::*erm*, and *glpK*::*erm* were grown in PFM9 buffered media supplemented with 5 mM G3P. Growth was measured by OD_{600} over 12 hours. Error bars indicate ±SEM (n=4).

8. Conclusions:

- *S. aureus* can utilize G3P as both a phosphate and carbon source.
- While *S. aureus* can import G3P, it prefers to cleave G3P into glycerol and P_i and import these two molecules separately.
- Regulation of PhoB and GlpT are similar to their *E. coli* homologs, while UgpBAEC does not respond to phosphate starvation.
- PhoPR regulates PhoB, but not GlpT.

10. Model of phosphate acquisition by *S. aureus*:



11. Selected References:

1. **Hsieh, Y.J. and B.L. Wanner**. 2010. Global regulation by the seven-component Pi signaling system. Curr Opin Microbiol. **13**(2): p. 198-203.

2. **Lemieux, M.J., Huang, Y. and Wang, D.N.** 2004. Glycerol-3-phosphate transporter of *Escherichia coli* Structure, function and regulation. Res in Microbiol. **155**(2004) 623-629.

3. **Su, T.Z., Schweizer, H.P., and Oxender D.L.** (1991). Carbon-starvation induction of the ugp operon, encoding the binding protein-dependent sn-glycerol-3-phosphate transport system in Escherichia coli. Mol Gen Genet. **230**(1-2): p. 28-32.

12. Acknowledgements:

I would like to thank all of the remaining members of the Kehl-Fie lab for their support in and outside of the lab. This work was supported by a NIH K22 (AI 104805) and a March of Dimes Basil O'Connor Starter Scholar Research Award to TKF. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH or March of Dimes.