Pseudomonas aeruginosa chronic wound co-infection fitness determinants

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Abstract

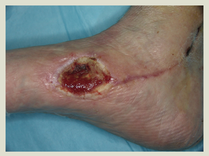
More than 5 million Americans suffer from chronic wounds contributing to over $20 billion of health care costs per year in the United States alone. Chronic wounds are typically infected with multispecies communities of bacteria that interact, resulting in enhanced antimicrobial resistance and more severe host tissue damage. Despite the prevalence and severity of polymicrobial infections, we still do not fully understand the interactions occurring between species infecting chronic wounds. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the two most commonly isolated bacterial species from chronic wounds. Therefore, we determined the *P. aeruginosa* genetic requirements for co-infection fitness with *S. aureus* during a murine chronic wound infection using transposon sequencing (Tn-seq). Our Tn-seq data revealed several genes that were required during co-infection with *S. aureus*, especially the glutamate-cysteine ligase *gshA*. We have shown that this gene, which catalyzes the reaction to produce the precursor to glutathione, is required for protection from hydrogen peroxide stress and for wild-type growth rates in minimal media. Glutathione recovers the *gshA* mutant to wild-type growth rates. During growth *in vitro* with *S. aureus,* the *gshA* mutant demonstrates equal fitness to the wild-type; therefore, this gene plays a crucial role exclusively *in vivo* during co-infection with *S. aureus*. Because *gshA* produces the precursor to glutathione, the major cellular antioxidant, we hypothesize *P. aeruginosa* requires glutathione for protection from neutrophil respiratory burst during co-infection. Future directions are focused on determining if glutathione can recover Δ*gshA* co-infection fitness and if respiratory burst is more robust during co-infection than *P. aeruginosa* mono-infection.

Introduction

What are chronic wounds?
Wounds that fail to heal in a timely manner

How many people have them?
More than 5 million Americans

How much do they cost?
$20 billion in health care in the US

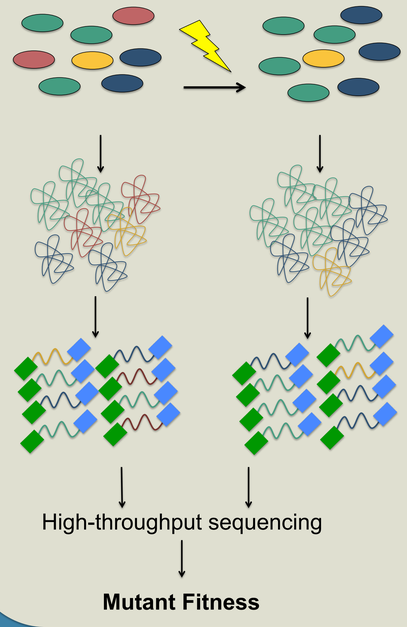


Biofilms are a major problem in chronic wound infections
"Microbial populations that have attached to a biological or non-biological surface"

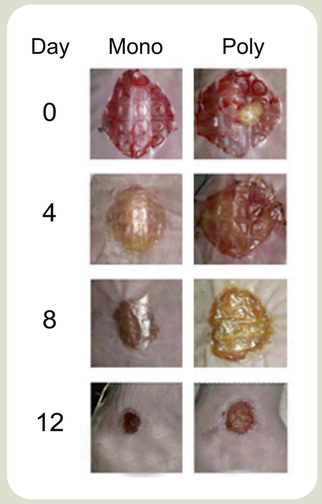
Microbial Community
Pseudomonas aeruginosa, *Staphylococcus aureus, Enterococcus faecalis and Finegoldia magna, Proteus sp.*

Methods

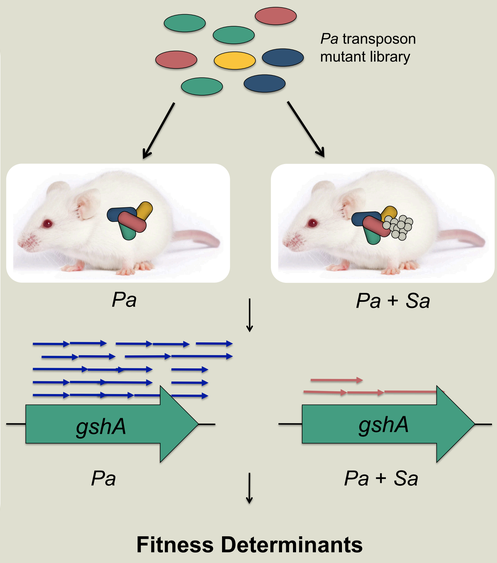
Transposon-sequencing (Tn-seq)



chronic wound murine model for infection



in vivo co-infection Tn-seq



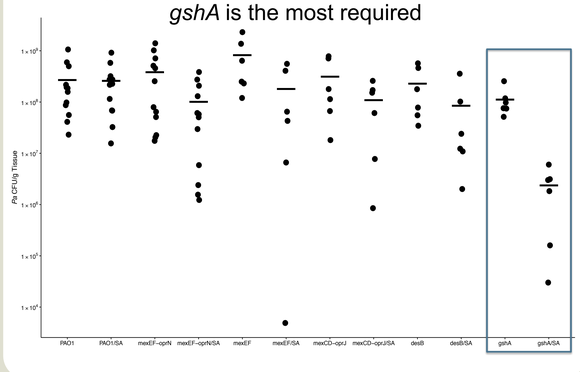
How does *S. aureus* impact *P. aeruginosa* genetic requirements in chronic wound infections?

Results

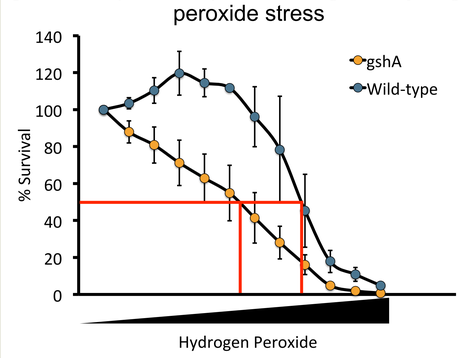
Co-infection Fitness Determinants	
Gene	**Product**
desB	Lipid metabolism enzyme
gshA	Glutathione biosynthesis enzyme
mexCD-oprJ	Multidrug efflux pump
mexT	Multidrug efflux pump
mexEF-oprN	

Glutamate + Cysteine
↓ *gshA*
Glutamyl-cysteine
↓ *gshB*
Glutathione
main antioxidant in cells
protects from ROS's

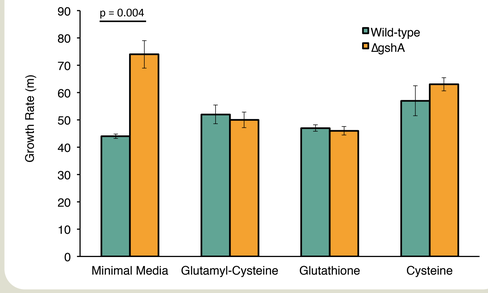
Several genes are co-infection fitness determinants, but *gshA* is the most required



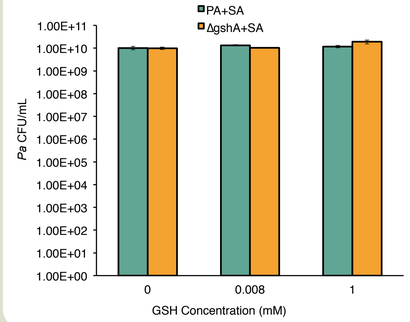
gshA is required for protection against hydrogen peroxide stress



Δ*gshA* grows slower than the wild-type in minimal media, but glutamyl-cysteine, glutathione, and cysteine restore it to wild-type growth rates



gshA is not required for *in vitro* fitness with *S. aureus*



Conclusions and Future Directions

In vivo genetic requirements for *P. aeruginosa* co-infection with *S. aureus* can be determined using Tn-seq
The glutathione biosynthesis gene *gshA* is required for *P. aeruginosa* fitness with *S. aureus* in chronic wounds
gshA is required for protection from peroxide stress and wild-type growth rates in minimal media, but not for *in vitro P. aeruginosa* fitness with *S. aureus*
Does *P. aeruginosa* require *gshA in vivo* with *S. aureus* for protection against neutrophil respiratory burst?

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