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 ΔgshA 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

Day Mono Poly
0 Pa transposon mutant library
4 Pa Pa + Sa
8 Pa gshA Pa + Sa
12 Pa gshA Pa + Sa

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

Results
Co-infection Fitness Determinants

<table>
<thead>
<tr>
<th>Gene</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>desB</td>
<td>Lipid metabolism enzyme</td>
</tr>
<tr>
<td>gshA</td>
<td>Glutathione biosynthesis enzyme</td>
</tr>
<tr>
<td>mexCD-oprF</td>
<td>Multidrug efflux pump</td>
</tr>
<tr>
<td>mexT</td>
<td>Multidrug efflux pump</td>
</tr>
<tr>
<td>mexEF-oprN</td>
<td>Multidrug efflux pump</td>
</tr>
</tbody>
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Several genes are co-infection fitness determinants, but gshA is the most required

ΔgshA 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?

References


