The Pig Skin Commensal *D. incerta* Exhibits Antimicrobial Activity Against Methicillin-Resistant *Staphylococcus aureus*

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Background

- *Staphylococcus aureus* is a bacterial pathogen that commonly infects the skin
- Methicillin-resistant *S. aureus* (MRSA) strains lead to >300,000 hospitalizations and $1.7B in healthcare cost annually (2017)
- *S. aureus* can colonize the skin, creating an increased risk of subsequent *S. aureus* infection and contributing to community spread of *S. aureus*
- The microbiome contains a diverse ecosystem of microbes in competition with each other and thus is a promising source of novel antibiotics
- Pigs are an established animal model for human skin due to histologic similarities between pig and human skin
- The pig skin microbiome is an unstudied community of that is distinct from the human skin microbiome

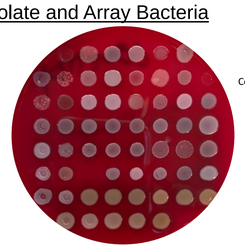
MRSA colonizes skin → **Skin microbiome as a potential source of new antimicrobials against MRSA** ← Microbiome is a source of novel antibiotics

Methods

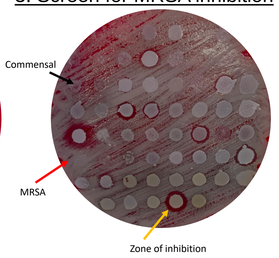
Figure 1. Pig skin microbiome screen for MRSA-inhibiting bacteria

1. Swab Pig Skin

2.Isolate and Array Bacteria



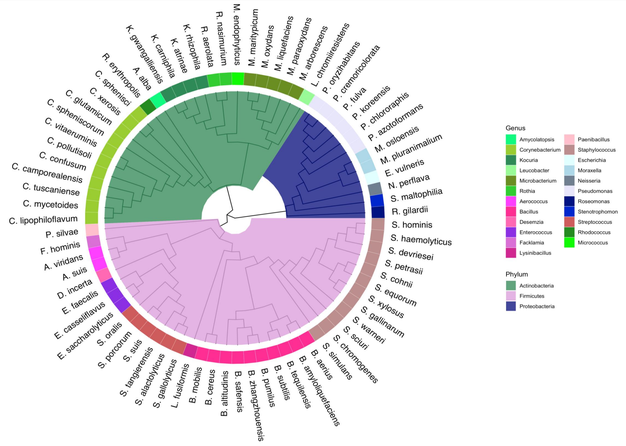
3. Screen for MRSA inhibition



4. Identify species of bacterial isolates

1. PCR 16s rRNA gene
2. MALDI-TOF Mass Spectrometry
3. Whole genome sequencing

Figure 2. Phylogenetic Tree of Cultured Isolates from Pig Skin



Results

Figure 3. Pig inhibitor screen reveals a diverse range of bacterial species

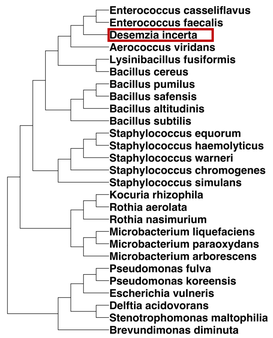


Figure 3: Phylogeny of MRSA-inhibiting bacterial species isolated from pig skin. Tree was generated from multiple sequence alignment of the 16S ribosomal RNA region.

Figure 4. *D. incerta* secretes an antimicrobial protein that can be isolated from cell-free supernatant

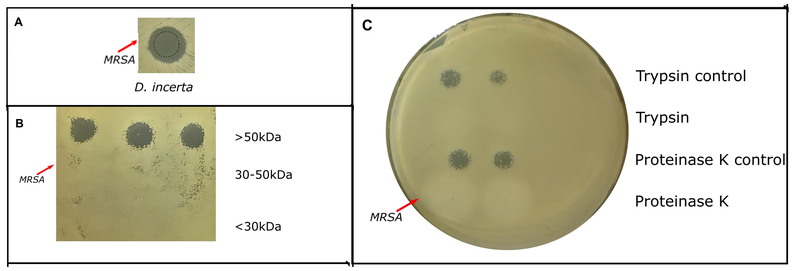


Figure 4: Disk diffusion assay of *D. incerta live cells* (a), cell-free supernatants concentrated through molecular weight cut-off filter (b), and cell-free supernatant treated with protease (c).

Figure 5. Whole genome analysis identifies no known biosynthetic gene clusters

Region	Type	Most Similar Known Cluster (Class)	Similarity (Gene Content)
1	saccharide/fatty acid	capsular polysaccharide (exopolysaccharide)	3%
2	saccharide		
3	saccharide		
4	terpene		
5	saccharide		
6	T3PKS	kijanimicin (polyketide)	4%
7	saccharide		
8	saccharide	chejuenolide A (polyketide)	7%
9	saccharide		
10	saccharide		
11	saccharide/terpene		
12	fatty acid	xantholipin (polyketide)	4%
13	saccharide		
14	saccharide		

Figure 5: antiSMASH homology search for antimicrobial biosynthetic gene clusters. Results are ordered by location on the *D. incerta* draft genome.

Figure 6: Purification scheme of antimicrobial protein from *D. incerta* supernatant

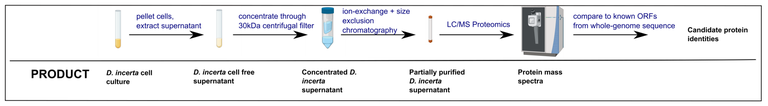


Figure 6: Overview of proposed biochemical approach to identification of the *D. incerta* antimicrobial protein.

Results

Figure 7: Purification of antimicrobial protein from *D. incerta* supernatant

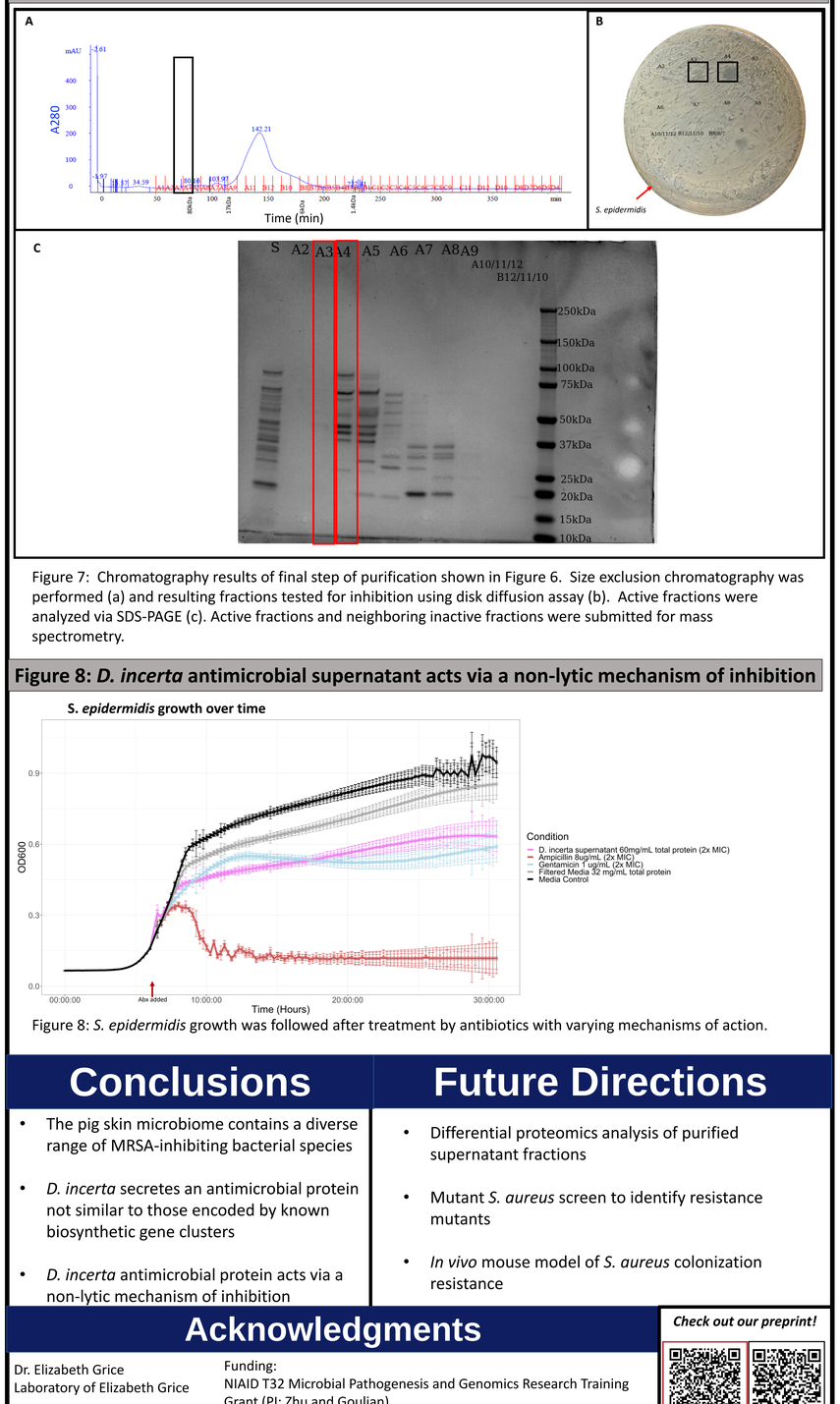


Figure 7: Chromatography results of final step of purification shown in Figure 6. Size exclusion chromatography was performed (a) and resulting fractions tested for inhibition using disk diffusion assay (b). Active fractions were analyzed via SDS-PAGE (c). Active fractions and neighboring inactive fractions were submitted for mass spectrometry.

Figure 8: *D. incerta* antimicrobial supernatant acts via a non-lytic mechanism of inhibition

Figure 8: *S. epidermidis* growth was followed after treatment by antibiotics with varying mechanisms of action.

Conclusions

- The pig skin microbiome contains a diverse range of MRSA-inhibiting bacterial species
- *D. incerta* secretes an antimicrobial protein not similar to those encoded by known biosynthetic gene clusters
- *D. incerta* antimicrobial protein acts via a non-lytic mechanism of inhibition

Future Directions

- Differential proteomics analysis of purified supernatant fractions
- Mutant *S. aureus* screen to identify resistance mutants
- *In vivo* mouse model of *S. aureus* colonization resistance

Acknowledgments

Dr. Elizabeth Grice
Laboratory of Elizabeth Grice

CHOP Proteomics Core Facility

Funding:
NIAID T32 Microbial Pathogenesis and Genomics Research Training Grant (PI: Zhu and Goulian)

Burroughs Wellcome Fund Investigators in the Pathogenesis of Infectious Disease Award (PI: Grice)

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