Development of Bacteriophage Cocktail Targeting *Staphylococcus aureus* to Treat Atopic Dermatitis

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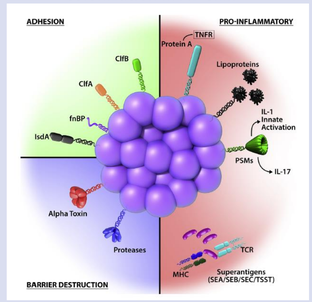
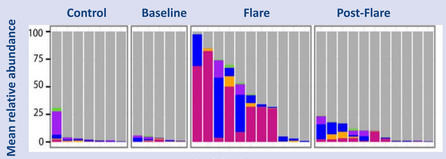
Abstract

Background: Multiple lines of evidence suggest that atopic dermatitis (AD) is associated with increased skin colonization by *Staphylococcus aureus* [1-3].
S. aureus contributes to AD pathogenesis through the release of virulence factors that affect the keratinocytes and immune cells [4].

Mechanisms of *S. aureus* colonization in AD skin



Adapted from Paller et al., 2019



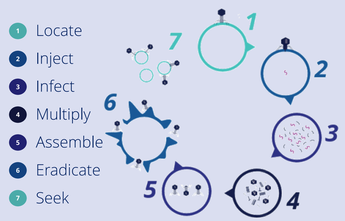
***S. aureus* becomes the dominant bacterial species during AD flares**

Adapted from Byrd et al., 2017

Presently the use of bleach baths as an antibacterial therapy has shown mixed results, possibly due to varying concentrations of bleach used in different studies [5]. Robust targeted and safe modulation of the microbiome may be more beneficial. **The aim of the current study is to develop a bacteriophage (phage) therapy that specifically targets S. aureus without affecting the beneficial bacteria**.

Phages are naturally occurring viruses that kill specific bacteria. Unlike antibiotics, phages are specific to the strain level and therefore have unique advantages in terms of minimizing perturbation of the microbiome. They have no capability to infect mammalian cells and therefore are considered safe.

Phages are self-amplifying therapeutic agents



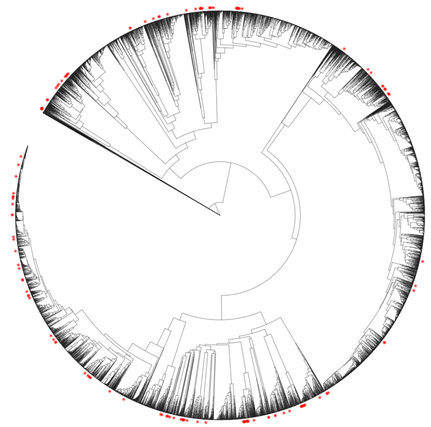
Methods: Natural phages were discovered by screening environmental samples on *S. aureus* strains isolated from human skin samples. The host range was determined by an efficiency of plating (EOP) value above 0.1 and the ability of the cocktail to completely lyse liquid bacterial culture under different growth conditions (e.g., temperature, bacterial stage). Phages with divergent characteristics were combined in a cocktail to address the diversity of *S. aureus* strains in AD and to address the emergence of resistance.

Results

1 *S. aureus* isolates from human skin are phylogenetically variable

- Sequencing analysis demonstrated that the panel of 118 *S. aureus* clinical strains originating in skin were distributed across the phylogenetic tree of all available Refseq *S. aureus* strains (8,337).

Phylogenetic tree of *S. aureus* isolates



S. aureus phylogenetic tree, based on 8,337 known sequences, and a panel of 118 isolates from human skin marked with red asterisks. The isolates are distributed across the phylogenetic tree.

2 Novel lytic phages for *S. aureus*

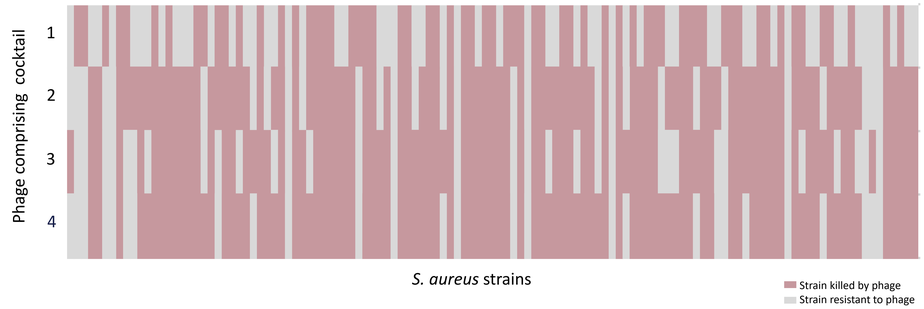
- Screening of environmental samples resulted in the isolation of about 50 lytic phages targeting *S. aureus* isolates.
- Sequence analysis of the phages revealed that they belong to different genera (Kayvirus and Rosenblumvirus) in different families (Herelleviridae and Podoviridae) and contain linear double-stranded deoxyribonucleic acid (dsDNA) genomes between ~17-145 kbp in length.
- Results of host range analysis of individual phages in solid medium ranged from 41% (48/118) to 79% (93/118).

Single phage host range

Phage	Host range coverage in solid medium
Phage #1	79%
Phage #2	41%
Phage #3	41%
Phage #4	52%
Phage #5	76%
Phage #6	73%
Phage #7	73%

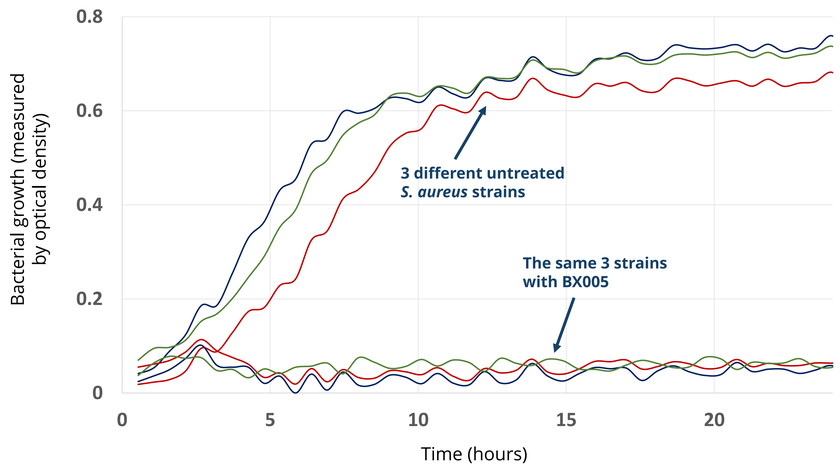
3 Host range of a 4-phage cocktail targeting *S. aureus*

- Phages were combined in different 3- and 4-phage cocktails from which the optimal combination was chosen.
- Combination of 4 phages resulted in a broad host range cocktail, BX005, which resulted in coverage of 92% (109/118) of the strains in solid medium (an efficiency of plating (EOP) value above 0.1).



4 *In vitro* activity of BX005 - no phage resistance

- Complete clearance of bacterial cultures in an *in-vitro* liquid infection assay (37°C, OD=0.05), with no evidence of phage resistance after 24 hours, was achieved.



Untreated strains showed normal growth curve while the same strains in the presence of the BX005 phage cocktail were eradicated with no mutants arising after 24 hours

Discussion and Conclusions

A broad range cocktail of natural phages targeting *S. aureus* was effective in reducing bacterial burden *in-vitro* and holds the potential to offer a novel therapeutic approach for atopic dermatitis.

References

1. Kong et al., 2021
2. Byrd et al., 2017
3. Totté et al., 2016
4. Paller et al., 2019
5. Chopra et al., 2017

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