

# Development of natural bacteriophages for skin care

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Disclosures: All authors are BiomX employees

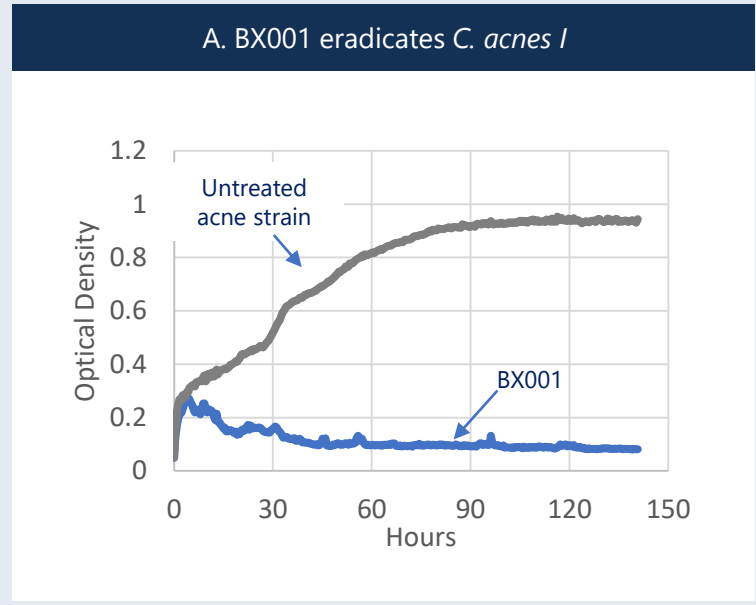
## Background and Rationale

- The importance of a **balanced skin microbiome** for skin appearance is widely recognized.
- **Acne vulgaris** is known to arise from increased sebum production and hyperkeratinization which are thought to stimulate proliferation of the bacteria *C. acnes* (*Cutibacterium acnes*) and lead to inflammation.
- The extensive use of topical and oral antibiotics to address this condition has led to **widespread antibiotic resistance in *C. acnes***. Furthermore, the common growth formation of these bacteria in polysaccharide-rich **biofilm matrices** also protects them from antibiotic activity.
- **Bacteriophages**, bacteria-specific viruses, **are natural components of the skin microbiome** that modulate the bacterial population by a mechanism of action that is distinct from that of antibiotics.
- ***C. acnes*-specific bacteriophages** have been reported to be **more abundant on healthy skin** than on acne skin<sup>1</sup>.
- **BX001, a novel topical gel containing a cocktail of bacteriophages specifically targeting *C. acnes*, is being developed as a skin conditioning agent to improve the appearance of acne-prone skin**

## BX001 bacteriophages specifically eradicate *C. acnes* *in-vitro* and *ex-vivo*

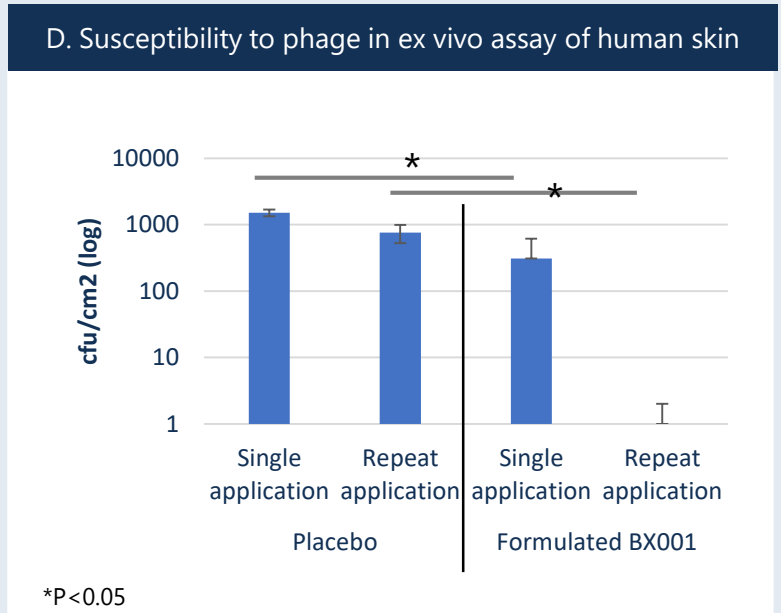
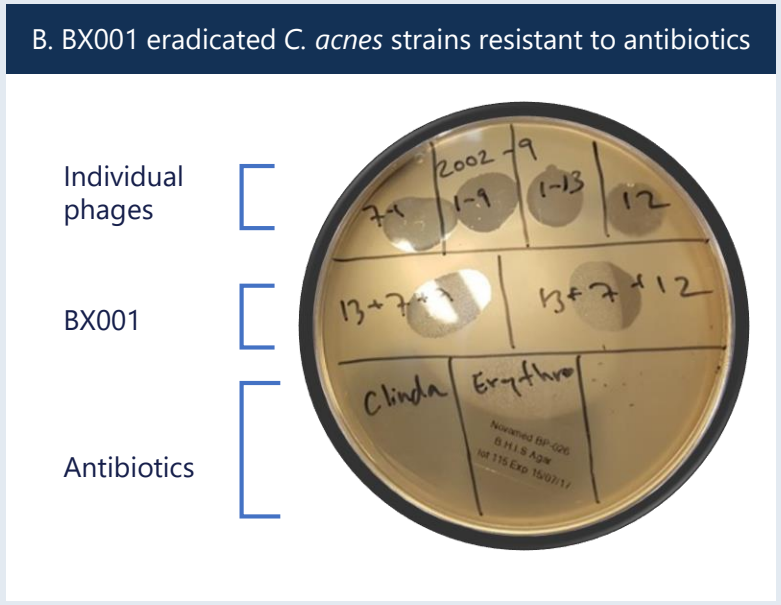
### Methods and results

- A. Susceptibility to phage in liquid** – *C. acnes* strains were grown in BHIS (Brain Heart Infusion Salt Broth) broth under anaerobic conditions in the presence or absence of bacteriophage. Bacterial growth was monitored by optical density. **In the presence of BX001, no growth of *C. acnes* resistant mutant bacteria is observed, even after 150 hours.**
- B. Susceptibility to phage and antibiotics (Abx) in solid matrix** – Bacterial lawns were grown on BHIS agar under anaerobic conditions, Bacteriophage or antibiotics were overlaid on the bacterial lawn. Clear areas show bacterial sensitivity and eradication. **Shown is one bacterial strain that is resistant to multiple antibiotics, and susceptible to phages.**
- C. Specificity of the phages against *C. acnes* was demonstrated in solid matrix assay.** None of the phages comprising BX001 targeted other skin bacteria.
- D. Susceptibility to phage in ex-vivo assay of human skin** - Reconstituted human skin was artificially infected with *C. acnes* and cultured for 1hr. Gel containing bacteriophage was applied either once or twice onto the infected skin. Samples from the skin were evaluated for colony forming units (CFU). Resulting bacterial levels after single or repeat application were compared to placebo (vehicle). **Repeat application (twice) of the phage cocktail completely eradicated *C. acnes*.**



C. Phages are highly specific for *C. acnes*

Bacteria	Origin	Phage susceptibility		
		Phage A	Phage B	Phage C
<i>Cutibacterium acnes</i>	CCUG 6369	+	+	+
<i>Cutibacterium acnes</i>	CCUG 38584	+	+	+
<i>Cutibacterium acnes</i>	DSMZ 16379	+	+	+
<i>Cutibacterium granulosum</i>	CCUG 32987	-	-	-
<i>Cutibacterium avidum</i>	CCUG 36754	-	-	-
<i>Staphylococcus aureus</i>	CCUG 41582	-	-	-
<i>Staphylococcus epidermidis</i>	CCUG 21989	-	-	-



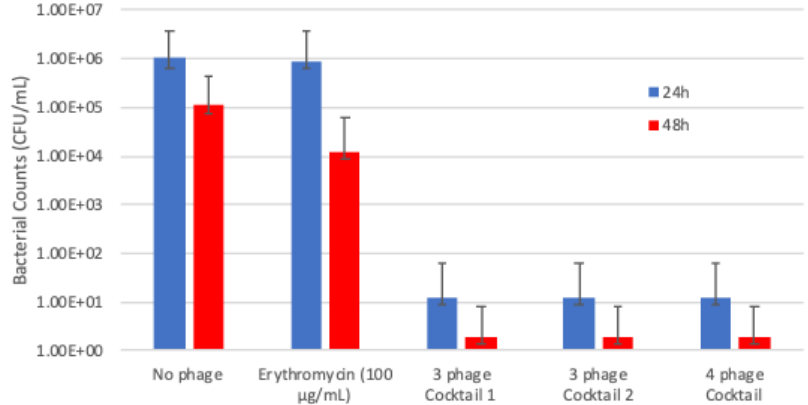
## BX001 phages can kill *C. acnes* in biofilm and penetrate sebum *in-vitro*

### Methods and results

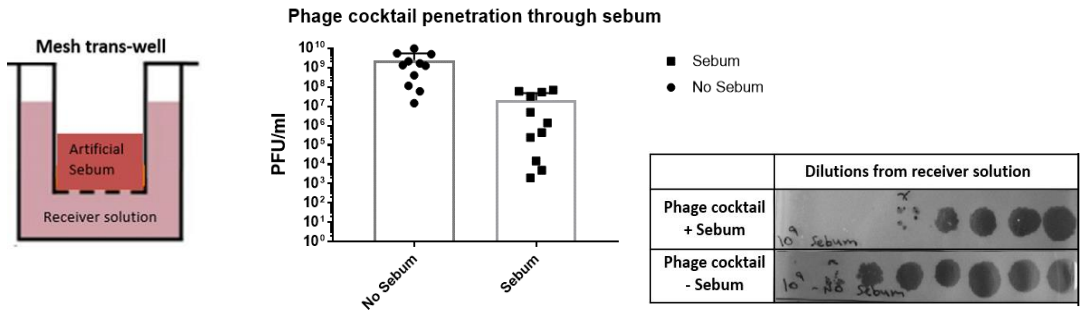
**A. Biofilm assay in-vitro** - *C. acnes* (erythromycin sensitive strain) was induced to form biofilm by culturing in the presence of glucose. *C. acnes* biofilms were exposed to erythromycin or phage cocktail for 24 and 48hrs. Viability of embedded *C. acnes* was tested by bacteria extraction from biofilm and quantitation of colony forming units (CFU). **BX001 phage cocktail was able to eradicate *C. acnes* in biofilm, while antibiotics had no effect after 24 hours and very little effect even after 48 hours.**

**B. Sebum penetration model** - Artificial sebum was generated on a mesh in transwell inserts. BX001 phage cocktail was overlaid on inserts with and without sebum and incubated for 24 hours at 32°C. The concentration of phage in the receiver compartment was determined by plaque forming assay (PFU) using serial dilutions (bottom picture). The graph shows a summary of 11 different experiments. The results demonstrate that **phages targeted for *C. acnes* manage to cross the sebum in this *in vitro* model system and stay viable.**

### A. Phages targeted for *C. acnes* penetrate biofilm



### B. Phages targeted for *C. acnes* penetrate the artificial sebum *in-vitro*



# Bacteriophage safety for topical application

## Methods and results

### Intrinsic safety

- Based on full sequencing and in silico safety evaluation according to the FDA guidance 40CFR Ch. 1
- Natural phage
- Strictly lytic – No integration into host bacteria DNA
- No virulent sequences
- No antibiotic resistant genes

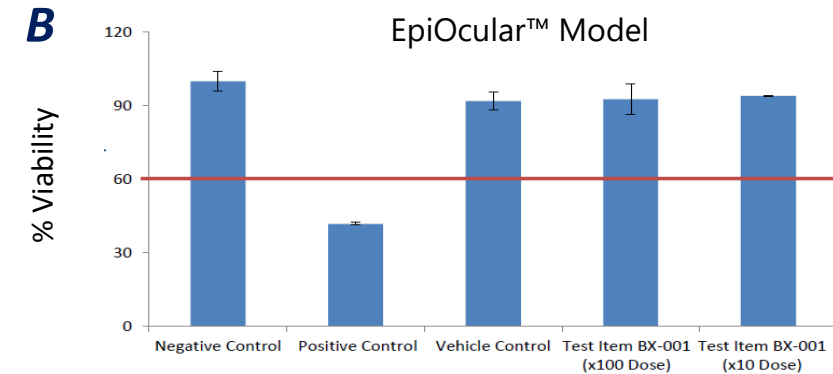
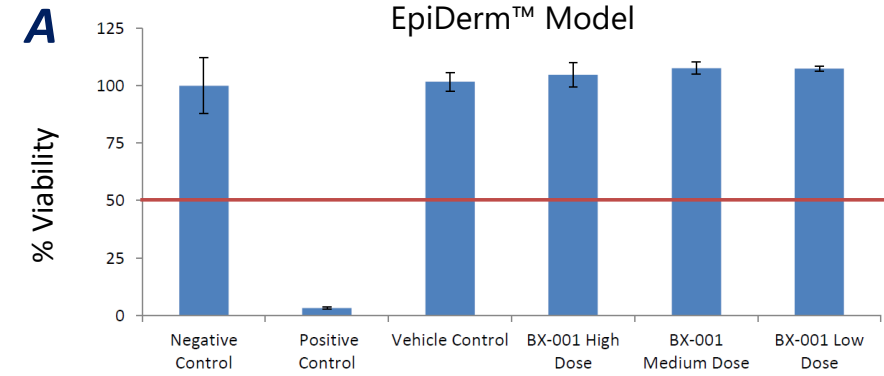
### No irritation observed

- GLP, OECD validated, ex-vivo, non-animal models
- Reconstituted human skin and ocular epithelia models
  - A. EpiDerm™ model (MatTek)
  - B. EpiOcular™ Eye Irritation Test (EIT) (MatTek)
    - Irritation was measured by relative viability of tissues following exposure to BX001 phages as measured with MTT.

### Minimal permeation demonstrated

- Permeation assay using Franz cells system
- GLP assay
- Tested with human epidermal sheet
  - < 0.0039% permeation through epidermis
- Tested with synthetic membrane
  - No permeation through synthetic membrane

BX001 phages do not cause irritation ex-vivo



- **BX001 overcomes antibiotic resistance** - BX001 is active against more than 96% of clinical *C. acnes* isolates tested in-vitro including antibiotic resistant strains.
- **BX001 is successful in penetrating biofilm** –In in-vitro experiments, phage reduced the number of viable bacteria within biofilm by 100,000 fold within 24 hours resulting in undetectable levels after 48 hours as compared to erythromycin, a common antibiotic, that only reduced bacterial levels by approximately 100 fold after 48 hours.
- **BX001 is bacteria specific** - BX001 does not affect other (potentially beneficial) bacteria on the skin. The phage cocktail is comprised of natural phage which are strictly lytic and are specific to *C. acnes*.
- **Phages for *C. acnes* penetrate sebum** – phages penetrated through artificial sebum suggesting potential to reach target in the pilosebaceous unit
- **Safety** – BX001 phages are intrinsically safe, selective to *C. acnes* and considered inert to mammalian cells. BX001 phage have been shown to be non-irritant in validated ex-vivo non-animal models and displayed minimal penetration through epidermis
- A **clinical study** is being conducted to evaluate the safety and tolerability of BX001 with an exploratory evaluation of reduction of *C. acnes* in individuals with mild to moderate acne.

**BX001**  
**A topical gel containing natural phage against *C. acnes* to modulate skin microbiome**



## References

1. Barnard, E. et al. The balance of metagenomic elements shapes the skin microbiome in acne and health. Sci. Rep. 6, 39491; doi: 10.1038/srep39491 (2016).