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Isolation and Structural characterization of Bacteriophages from Staphylococcus *Epidermidis* on Human Skin

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Introduction

Staphylococcus epidermidis is a member of the Podoviridae family, many of which are now assumed to pose a risk to public health due to their links to nosocomial infections, endocarditis, sepsis, pneumonia, and other illnesses. *S. aureus* and *S. epidermidis* are the principal causes of nosocomial infections. Based on virion properties (dimensions, particle weight, and buoyancy) and DNA (molecular weight, G-C%, and genomic sequences), Staphylococcal phages were divided into distinct groups or clusters. This increased interest in *Staphylococcus* phages is mostly owing to the rising resistance of this bacteria to medications. The limited diversity of Staphylococcal phages and their lysine has not yet been defined, although bacteriophages and the peptidoglycan hydrolysis enzymes they produce (lysine) are now being studied as potential alternatives to traditional antibiotics. Gram-positive opportunistic pathogen *S. epidermidis* colonizes all areas of human skin [1]. The prospect of PT in the *in vivo* management of *S. epidermidis* infection. However, no comparable studies have been carried out for *S. epidermidis*. Phage therapies (PT) from infections caused by the closely related bacterium *S. aureus* have been investigated in animal models [2] and people [3].

S. epidermidis has developed very sophisticated defenses against phage infection, which most likely reduced the amount of phage available for PT. The common laboratory strain RP62A has a type III-A clustered regularly spaced short palindromic repeat (CRISPR)-Cas system, a type I restriction-modification system, and a eukaryotic serine/threonine kinase (Stk2) that all function to defend bacteria against infection [4, 5]. Host selection has a variety of effects on the efficacy of phages in phage therapy. It is preferable to restrict the host to a single species since this stops the phage from eradicating other species while keeping the rest of the host's microbiome unharmed. In phage therapy, it would be anticipated that using phages with a wider host range will result in fewer therapeutic failures because of incompatible host-phage pairings. Consequently, a greater variety of hosts is preferred for the target species' strains. These phages must not have virulence or antibiotic resistance genes, be easily and quickly propagated, obligately lytic for the target organism, and be stable [6]. PT is one such tactic and involves the use of bacterial viruses (bacteriophages). Bacteriophages are enticing for treating diseases brought on by bacteria that build biofilms and are resistant to antibiotics. Phages target receptors differently than conventional antibiotics, indicating that there is a low chance that phage strains may develop resistance to antibacterial medications. They can also encapsulate enzymes that disturb bacterial biofilms by depolymerizing polysaccharides. For instance, independent phage transmission between animals during veterinary therapy may offer convenience or financial advantage by minimizing the need for repeated phage usage but may not be essential to the effectiveness of the treatment. In this exploration, we used the human host-specific *S. epidermidis* as a propagation platform to check for lytic phages on the skin of healthy volunteers we isolated novel phages from all known *S. epidermidis* phage families using this method.

Methods and Equipment

Isolation of *S. Epidermidis* with a Human Host Specificity

A dry cotton swab was used to collect microorganisms from healthy participants' foreheads in accordance with Swiss ethical permission (invasive sterile collection swab). Trypticase Soy Broth was used to dissolve the biomass on the swabs. Aliquots of this solution were then spread out on mannitol salt agar (MSA) plates, and the plates were then let to sit overnight at 37 °C. A bacteriophage's use in phage treatment may depend on the variety of hosts it may infect. A phage's host range might vary depending on whether it infects only a few strains, numerous strains of the same species, or many species (multivalent host range). It appears that host region selection is not part of standard isolation techniques. Although some organizations prefer to combine several host strains for phage isolation, the host range is often described after phage isolation, and isolation is typically carried out with a single bacterial strain [7-9]. A major public health issue worldwide is the rise of antibiotic resistance among bacterial infections. The emergence of drug-resistant *Staphylococcus* species, particularly *S. aureus* and *S. epidermidis* in hospitals and the general population,

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highlights the urgent need for fresh tactics to fight Staphylococcal infections. Alternative antimicrobial agents include bacterial viruses (phages) and the lysins they produce to destroy bacterial cell walls, while Staphylococcal phages and their lysates have not yet been discovered. *S. aureus* may cause mild to deadly infections in several body areas [10] and the fact that more than 25% of people have asymptomatic nasal carriage poses a serious threat to public health [11]. Most infections caused by medical devices are caused by the ubiquitous and opportunistic skin bacteria *S. epidermidis* [12]. Staphylococcal infections can benefit from the use of phages and their peptidoglycan hydrolyzing enzymes (lyses) [13, 14]. In comparison to traditional antibiotics, phages and their lysates offer several benefits, including the capacity to kill antibiotic-resistant bacteria, the ability to focus on a small range of bacterial pathogens while sparing helpful species, and the capacity to infiltrate and break biofilms. They were divided into three morphological families with Podoviridae (125 kb) being the largest. The mobilization of virulence factors and pathogenicity islands by temperate phages can result in the formation of prophages that carry pathogenic factors and can enhance fitness and pathogenicity [15].

Bacteriophage Isolation, Reproduction and Spread

The same area where *S. epidermidis* was found also yielded phages. It is ideal for these strains to be devoid of bacteria, molds, detritus, medium, and bacterial endotoxins with a dry and sterile cloth. Forehead was thoroughly wiped from the brows to the hairline. The major pyrogen in phage preparations is the lipid, which is frequently contaminated with macromolecules from host bacteria and medium. A component of the lipopolysaccharide (endotoxin) found in gram-negative bacteria's outer membrane. Endotoxins are amphipathic molecules that may form huge aggregates of more than 1000 kDa in solution because the lipid component is connected to the polysaccharide core. Even a tiny quantity of exposure can result in toxic shock, cell damage, the release of cytokines, and the activation of immunological responses. When analyzing or going to employ phages in eukaryotic systems, endotoxins must be taken out of phage preparations due to this impact. Endotoxin units (EL), which equate to the activity of 100 pg of *Escherichia coli* lipopolysaccharide, are used to measure endotoxin concentrations. An *S. epidermidis* unique to its host. To introduce the phage, the culture was diluted 1:1,000 in sterile TSB, and the combination was then incubated at 37 °C with constant shaking (190 rpm) until it reached an optical density (OD; 600 nm) of 0.2. The phage solution was then used in a 5:1 ratio (bacteriophage) to infect bacterial hosts, and it was incubated at 37 °C with shaking to aid in adsorption. After centrifuging the phage-bacterial solution (3000 x g, 5 - 10 min, 4 °C) to pellet the bacteria, the supernatant was filtered through a 0.22 μm filter. Phages were discovered using the two-layer agar technique [16]. After incubation, the bacterial lawn created cleared areas (plaques), which was a sign that a viable phage was present. Using bilayer agar to simultaneously lyse the host *S. epidermidis* and SM buffer to extract the phage and filtering the suspensions using 0.22 μm filters were the steps used to create the final phage suspensions [17].

Analysis and Sequencing of the Genome

More than 20% of the time, bacterial genomes and plasmids can include both functional and non-functional bacteriophage genes [18]. Indeed, a sizeable fraction of the diversity among bacterial species or clades is likely explained by these non-bacterial prophage sequences. According to earlier instructions, whole phage DNA was extracted. The phage-containing pellets from phage lysates were resuspended in SM buffer after being centrifuged (161,000 x g, 2 hours, 4 °C) in a Centrikon T-1170 ultracentrifuge with a TST 1.1 rotor (Kontron Instruments, U.K.). To get rid of any leftover DNA and RNA, extracts were treated with DNA polymerase I (6 U/ml) and Protease A (0.02 mg/ml) for 1.5 hours at 37 °C. Ethylenediaminetetraacetic acid (EDTA) 0.02 M was then added to inactivate the enzymes. The Proteinase was used to degrade the phage protein capsid, and the materials were then incubated at 56 °C for 1.5 hours. The DNeasy Blood and Tissue Kit were used to purify the phage DNA. Phage 56 was employed as a control in this investigation, and its genome was sequenced. Phage 56 was previously utilized to prevent *S. epidermidis* biofilm development on a catheter *in vitro*. VICTOR was used to derive genome-based phylogeny and the Comprehensive Antibiotic Resistance Database were used to search the phage genome for virulence and antibiotic resistance genes, respectively.

Phage Therapy: Phage Characterization and Identification

A culture of susceptible bacteria was combined with a sample of the phage filtrate before being incubated. The phage cell combination was examined for lysis either during or following incubation [19]. The best phage treatment techniques are plaque production and culture lysis. Given that these phage detection tests are carried out in laboratories and not on living subjects, where the phage or host is developing might vary, viruses with false-negative findings (reproducing in the host but not creating visible plaques) would probably not be beneficial in phage therapy due to poor productivity [20]. These assays can be used for quick primary screening since they have a greater risk of false positives than false negatives, and only phages that exhibit activity should be tested using the plaque-forming test [21]. Additionally, by spotting increasingly more diluted phage stocks on the same plate, several organizations combine spot and plate testing. Plaques may typically be identified at various dilutions when just a few sides are visible [22, 23]. Experiments with monophasic growth were conducted. The interval between adsorption and the beginning of phage breakdown was designated as the latent period. PFU/ml after the blast divided by PFU/ml prior to the detonation was used to compute the blast size. As previously mentioned, 10 mM $CaCl_2$ was added to the media for Sipho viruses vB_SepS_BE01 and vB_SepS_BE02 [24].

Tests for Biofilm Degradation Using a 96-Well Plate

Two Staphylococcal species, *S. aureus* and *S. epidermidis*, are the main causes of nosocomial infections because of their ability to colonize and build biofilms on medical devices and human organs [25]. Staphylococcal biofilms are intricate formations in which extracellular bacterial material surrounds bacterial cells. A substance that protects against antibacterial medications (including polysaccharides, teichoic acids, proteins, and extracellular DNA). *S. epidermidis*, one of the organisms that are most common on human skin, quickly colonizes endothelium, catheterization, and contact lenses. Although once thought to be a benign strain of bacteria, today understood to be an exploitative microorganism that frequently causes invasive disease in people with impaired immune systems, premature newborns, and biofilm-related diseases. Bacterial cultures were diluted, 1:100 in TSB that had 0.25% glucose added to them (TSB-glucose, 150 μl per well). For two hours, plates were incubated at 37 °C. Following incubation, the biofilm was washed off the plates by submerging them in sterile water, zooplankton cells were eliminated by flipping the plates.

As a control, SM buffer was employed untreated. Following treatment, biofilms were three-washed and dyed at ambient temperature for 15 min with crystal violet (CV; 0.06%). After removing the extra CV, the plates were submerged in water three more times. The biofilms were then dried at room temperature for an additional hour. OD was then measured at 550 nm using a Minimax 300 Imaging Cytometer (Molecular Devices in San Jose, California, USA), after the addition of 30% acetic acid to each well. Each experiment was run in triplicate and five wells were utilized for each condition. The Kruskal-Walli's test was used to compare biofilms that had not been treated to those that had been treated with phage, with Dunn's multiple comparison test used to compensate for multiple comparisons. If P 0.05, data were deemed to be statistically different.

Results

An effective way to separate *S. epidermidis* phages, our hypothesis was that habitats with high concentrations of *S. epidermidis* would also have lytic *S. epidermidis* phages (Figure 1) [26]. Since the head is a common location for *S. epidermis* [1], it offers a significant surface area that may be investigated non-invasively. We focused on the forehead of healthy volunteers. The utilization of this sample location was confirmed by the presence of *S. epidermidis* on the forehead in 2 out of the total of 3 people. In 11 of the 2 sites from which *S. epidermidis* was isolated (6%), phage-containing plaques of human host-specific bacteria were found in lawns. In one instance, a number of plaques with various shapes were seen and dispersed for analysis. Plaques were not found in 13 instances, although at least one more *S. epidermidis* was identified.

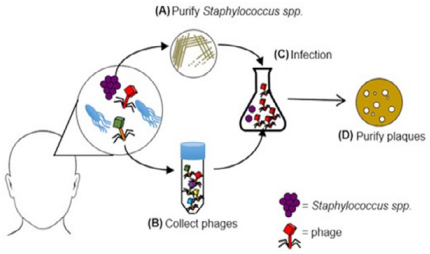

Figure 1: The phage isolation procedure employed in this investigation is shown schematically. Skin samples from healthy participants were collected. **(A)** The species of each bacterium was identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) after commensal Staphylococci were isolated using selective medium. **(B)** A second sample was obtained from the same skin region, filtered, and centrifuged to concentrate the phages. **(C)** Then, *S. epidermidis* was employed as the phage's host. **(D)** In double-layer agar plates, phage plaques were created for further purification and characterization [26].

Properties of the Study's Phages' Genomes

Five brand-new bacteriophages that were isolated from human skin had circular genomic representations. The inner ring of each panel depicts the GC skew. The anticipated coding sequences are shown in the second ring; the color of each arrow denotes the predicted function. To compare with other Staphylococcal bacteriophages, the remaining outer rings show BLASTn sequence similarities. Phietavirus phages (A) and Sextaecvirus phages (B) have sequence similarities with vB_SepS_BE01 and vB_SepS_BE02, respectively (Table 1). A podovirus, vB_SepP_BE03, is (C). A sequence that is thought to code for an N-acetylmuramoyl-L-alanine amidase is highlighted with an asterisk (see Figure S1, Supporting Information). The two remaining phages, vB_SepM_BE04 (D) and vB_SepM_BE06 (E), are like known myoviruses of the Sepunavirus genus. The CGView server was used to create plots.

Table 1: Properties of phages [26].

Name	Family	Genus	Lifestyle	Length (bp)	%GC	CDS
vB_SepS_BE01	Siphoviridae	*Phietavirus*	Temperate	42 718	34.8	70
456	Siphoviridae	*Phietavirus*	Temperate	43 393	34.7	73
vB_SepS_BE02	Siphoviridae	*Sextaecvirus*	Virulent	95 233	29.4	141
vB_SepP_BE03	Podoviridae	Unclassified Picovirinae	Virulent	18 271	30	20
vB_SepM_BE04	Myoviridae	*Sepunavirus*	Virulent	142 331	27.9	208
vB_SepM_BE06	Myoviridae	*Sepunavirus*	Virulent	140 659	28	200
vB_SepM_BE07	Myoviridae	*Sepunavirus*	Virulent	140 661	28	200
vB_SepM_BE09	Myoviridae	*Sepunavirus*	Virulent	140 668	28	201
vB_SepM_phiIPLA-C1C	Myoviridae	*Sepunavirus*	Virulent	140 961	28	203

Discussion

The technique concentrates on the T-phage but may be used to identify additional phages at high titers (> 109 PFU/ml). T-phage was specifically produced in liquid lysate of *E. coli* bacterial host, purified by centrifugation and 0.22 μm filtration. A T-phage-specific approach that is broadly applicable to other phages that can be recovered at high titers (>109 PFU) and chloroform treatment, concentrated by ultrafiltration centrifugation, and kept in bulk phage banks at 4 °C in the buffer. A single or a small number of host bacterial strains are frequently required for phage replication, which is typically focused on raw water sources. A single or a small number of host bacterial strains are frequently required for phage replication, which is typically focused on raw water sources. Rarely has this method been successful in isolating infectious phages from *S. epidermidis*, and there aren't many *S. epidermidis*-specific phages that can be used therapeutically. Each major Staphylococcal phage family (Siphoviridae, Podoviridae, and Myoviridae) includes at least one distinct representative that has been isolated and functionally defined (Figure 2) [26]. Due to their small genomes and obligatory lytic nature, podoviruses are desirable therapeutic possibilities. 13/20 putative genes in the vB_SepP_BE03 genome can be functionally annotated, which is desirable for therapeutic uses. The restricted host range displayed by vB_SepP_BE03, however, may not be ideal for upcoming therapies. In this work, the comparative genomic analysis found novel phage genes that may help us understand how *S. epidermidis* and phages interact, in addition to the isolation of complete phages that may be helpful in treatment. According to Pirnay et al., "therapeutic phage

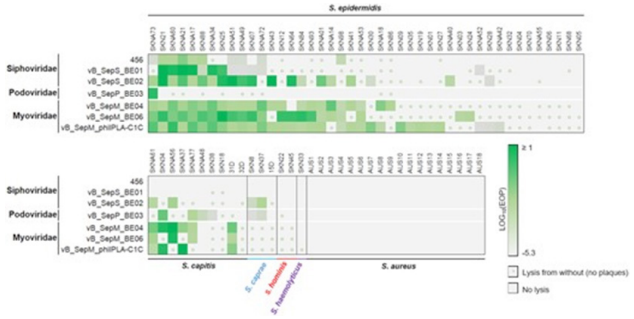

Figure 2: Characterization of staphylococci and staphylococcal phages [2].

products come into two paradigmatic categories: a specialized application or a pre-tax concierge that fits everyone. A product that can infect many clinical isolates of the target pathogen is necessary for the ready-porter strategy. We employed a cocktail of four phages to treat *S. aureus*, and it was successful against >90% of isolates [2]. This method cannot be used with the *S epidermidis* phages found in the current investigation since they only infect around 50% of the strains examined.

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