Research Article

Reservoir of Bacterial Exotoxin Genes in the Environment

Veronica Casas, Joseph Magbanua, Gerico Sobrepeña, Scott T. Kelley, and Stanley R. Maloy

Department of Biology, Center for Microbial Sciences, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182-1010, USA

Correspondence should be addressed to Stanley R. Maloy, smaloy@sciences.sdsu.edu

Many bacteria produce secreted virulence factors called exotoxins. Exotoxins are often encoded by mobile genetic elements, including bacteriophage (phage). Phage can transfer genetic information to the bacteria they infect. When a phage transfers virulence genes to an avirulent bacterium, the bacterium can acquire the ability to cause disease. It is important to understand the role played by the phage that carry these genes in the evolution of pathogens. This is the first report of an environmental reservoir of a bacterial exotoxin gene in an atypical host. Screening bacterial isolates from the environment via PCR identified an isolate with a DNA sequence >95% identical to the *Staphylococcus aureus* enterotoxin A gene (*sea*). 16S DNA sequence comparisons and growth studies identified the environmental isolate as a psychrophilic *Pseudomonas* spp. The results indicate that the *sea* gene is present in an alternative bacterial host, providing the first evidence for an environmental pool of exotoxin genes in bacteria.

1. Introduction

Exotoxins are secreted polypeptides produced by certain bacterial pathogens. Many exotoxin genes are carried on mobile genetic elements, including bacterial viruses (bacteriophage or phage). These virulence genes are responsible for many of the symptoms associated with the human disease [1–3]. As highly mobile genetic elements, phage can readily move between different environments, and are generally more resistant to environmental stress than their bacterial counterparts [4–11]. As a result, phage may survive in the environment in reservoirs not yet characterized. Living in these environmental reservoirs, the phage can influence evolution of the bacteria within these environments in several different manners. Horizontal gene transfer between the phage and the bacterium can result in the rapid evolution of new pathogens and may have serious implications in public health [12].

When a phage infects a bacterium, two alternative possibilities may result. One possibility is that the phage can replicate itself using phage and host factors, resulting in lysis of the bacterial host and release of new phage (the lytic lifestyle). Alternatively, the phage can integrate into the bacterial genome and the bacterial host can utilize certain genes the phage carries in its genome for its own benefit (the lysogenic lifestyle) [13]. If a phage encodes virulence genes, such as exotoxin genes, the phage could facilitate the transfer of these genes to nontoxigenic bacterial hosts, thereby increasing the exotoxin gene pool.

The toxins of *Vibrio cholera* (cholera toxin), *Escherichia coli* (shiga toxin), *Corynebacterium diphtheria* (diphtheria toxin), and *Staphylococcus aureus* (enterotoxin A) are encoded by phage [14–17]. The *Staphylococcus* enterotoxin A (*sea*) gene carried by virulent strains of *S. aureus* is encoded by multiple phages, including $\phi11$, $\phi12$, $\phi13$, 80α, and 42D [18–21]. Other toxins are carried by phages isolated from *S. aureus* strains from animals, food, and the environment [20, 22–31]. Given that multiple *S. aureus* toxins are encoded by phage, it is possible that multiple transduction events over time have resulted in the generation of the current virulent *S. aureus* strains. An environmental reservoir of toxin genes would provide novel virulence genes, and the genetic exchange between phage and novel bacterial hosts, could provide the mechanism for evolution of novel human pathogens.

To explore this hypothesis, we cultured environmental bacteria and screened them for a phage-encoded exotoxin gene. In this study, we describe the isolation of bacteria from

environmental ambient air and the screening of the isolates for the phage-encoded *sea* gene using an exotoxin-specific colony PCR assay. One isolate was confirmed positive for the *sea* gene, and the sequence of the *sea* gene was determined. Using 16S rDNA PCR sequencing and comparison to the nonredundant GenBank nucleotide database, we determined that the environmental isolate was a novel host for the *sea* exotoxin. This is the first report of an alternative bacterial host from the environment that carries a phage-encoded exotoxin gene that is commonly associated with a different bacterial host.

2. Results

2.1. Cultivation and Exotoxin-Specific (sea) PCR Screening of Environmental Isolates.
Bacterial isolates were cultivated from the ambient environment by exposing Luria Bertani (LB) plates to air and then incubated at room temperature for 48–72 hr. Eighty nine isolates were subcultured into sterile 96-well plates containing LB with 15% glycerol, and grown with aeration for 48–72 hr. Using colony PCR specific for the *Staphylococcus* enterotoxin A (*sea*) gene, each of the cultivated environmental isolates was screened for the presence of the *sea* gene. Of 89 isolates screened, one putative *sea* positive isolate was identified. The isolate was single colony purified, and the *sea* PCR was repeated on this purified isolate to confirm that the isolate (SEAB3C070426 lab-designated identification) was positive for the *sea* gene. The resulting *sea*-specific PCR product was gel-purified and sequenced.

2.2. Characterization of Environmental Isolate.
The cultivated and purified environmental isolate "SEAB3C070426" was microbiologically characterized by Gram staining and microscopic evaluation. The environmental isolate was identified as a Gram negative rod. Furthermore, the purified isolate's growth characteristics were evaluated against a *S. aureus* known to carry the *sea* exotoxin gene (*S. aureus* Food Research Institute 913 strain). The environmental isolate did not grow on *S. aureus* enrichment media at 35°C [32], but grew on LB at room temperature after 48–72 hr. In contrast, the *S. aureus* FRI913 control grew on both the enrichment media and LB at both temperatures.

To molecularly identify the cultivated environmental isolate carrying the *sea* sequence, 16S rDNA colony PCR was performed and the resulting PCR product was sequenced. The resulting 16S rDNA sequence was imported into the ARB bacterial 16S rDNA database to identify its nearest relatives for downstream phylogenetic analyses [33]. The 16S rDNA PCR product sequence grouped with *Pseudomonas* spp. using the ARB alignment. The nearest relatives identified by ARB were exported and used to generate a phylogenetic tree. The phylogenetic analyses of the ambient air isolate, its nearest relatives, and select outgroups (including *S. aureus*) were performed using the PAUP* program (Figure 1) [33]. The consensus tree generated from Maximum Likelihood (ML), Maximum Parsimony (MP), and Neighbor Joining (NJ) analyses grouped the ambient

air isolate with *Pseudomonas* spp., not with *S. aureus*. The ML, MP, and NJ bootsrap values for the main branches of the consensus tree separating the ambient air isolate from *S. aureus* and grouping it with *Pseudomonas* spp. were ≥94. The GenBank Accession number of environmental isolate "SEAB3C070426" is FJ979636.

2.3. Characterization of the sea PCR Product.
Sequencing of the *sea* PCR product obtained from the environmental isolate generated a 280 bp DNA sequence. The nucleotide composition of the amplified sequence was analyzed using BioEdit [34]. The sequence of the amplified PCR product contained a G + C content of 30% over 92 predicted amino acids. A ClustalW [35] alignment of the translated partial sequence of the *sea*-related gene with known *sea* genes is presented in Figure S1 in Supplemental Material available online at doi: doi:10.1155/2010/754368. A BLASTN alignment of the sequence against the nonredundant GenBank database confirmed that the amplified PCR product shared 95–96% nucleotide sequence identity with known *sea* genes. Most notably, the amplified PCR product was 96% similar to a known *S. aureus* phage, ϕNM3. A multiple alignment of the partial sequence of the *sea*-related gene and guide tree of the top BLASTN hits were produced using ClustalX2 [36] (Figures 2 and 3). BLASTN alignment of the *sea*-related gene against annotated *S. aureus* genomes from The SEED database was also performed [37]. This alignment indicated the *sea*-related gene was related to other *S. aureus* phage-associated enterotoxin genes (supplementary Figure S2). The *sea*-related gene was uploaded to the GenBank database and the Accession number is HQ698309.

3. Discussion

Transfer of exotoxin genes to new, as yet uncharacterized, bacterial hosts may facilitate the evolution of novel human pathogens. Many of the exotoxins produced by phage-encoded genes target key eukaryotic cellular processes such as protein synthesis [38–43]. These phage encode the genes responsible for many of the symptoms associated with the human disease. Until recently, most epidemiological and ecological studies of infectious diseases have focused on the presence and activity of the bacteria per se, neglecting the potentially significant role of the phage that carry the exotoxin genes and their role in transmitting these virulence traits [44–47].

Sequence analysis of several environmental metagenomes has shown that phage carrying exotoxin genes are common in the environment, however 16S rDNA analysis of metagenomes from the same environmental samples did not identify the cognate bacterial phage host [48]. A potential explanation of this finding is that phage in the environment may propagate in alternate bacterial hosts rather than those commonly associated with the human disease. The classic textbook description of phage-bacteria interactions implies that phage infection is limited to a specific host. However, as the ecology and physiology of phages has been further investigated, it is clear that some phage can infect multiple
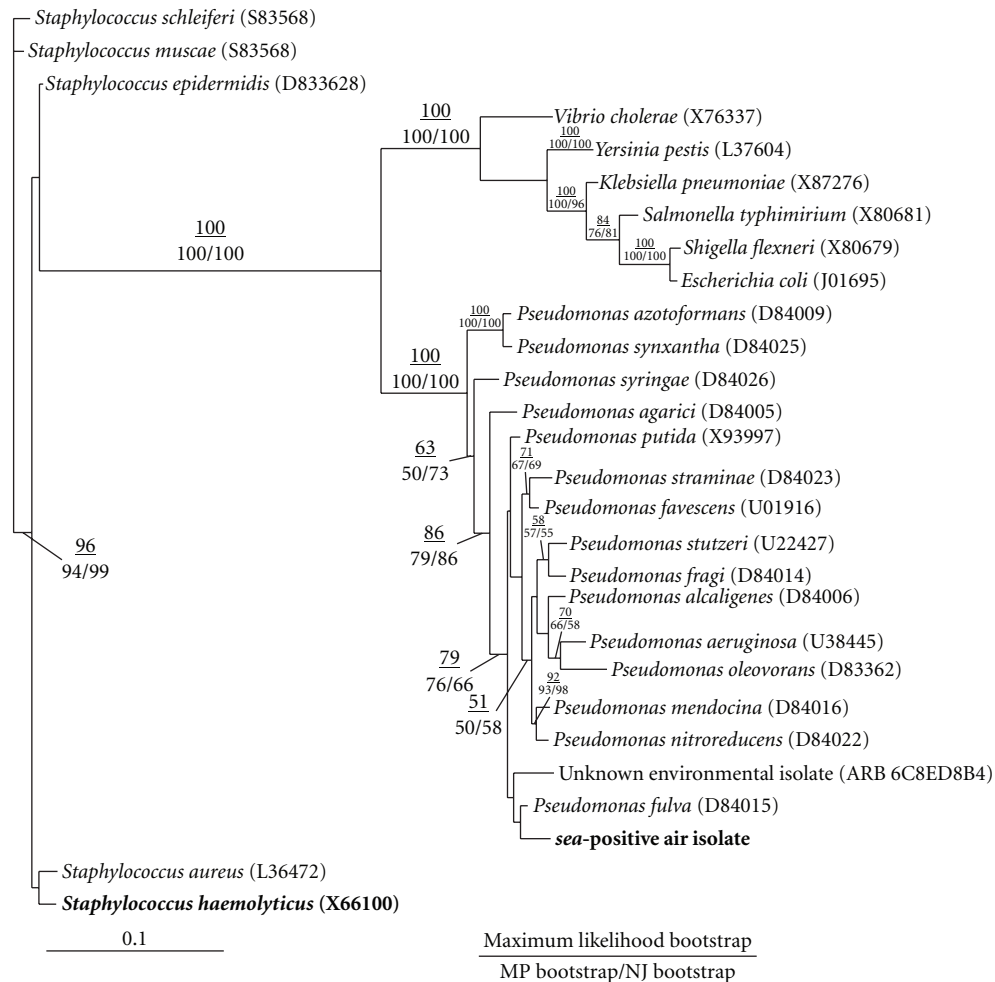
FIGURE 1: Phylogenetic tree of *sea*-positive isolate, its nearest relatives, and select outgroups. PAUP* was used to generate a consensus phylogenetic tree. Maximum Likelihood (ML) analysis was performed using 100 replicates with 10 random addition sequences searches per replicate. Maximum Parsimony (MP) analysis was performed using searches on 100 bootstrap replicated datasets with 10 search replicates. The Neighbor-joining (NJ) bootstrap analysis was performed with 1000 replicates. The (ML bootstrap)/(MP bootstrap/NJ bootstrap) values are as indicated. The *sea*-positive isolate is highlighted in bold and GenBank Accession numbers of each organism are in parentheses.

bacterial hosts [23, 49–73]. Examples of phage that carry exotoxin genes and can infect alternative hosts include *ctxϕ* (infects both *Vibrio cholerae* and *Vibrio mimicus* [74]), *stx-2* phage (infects *E. coli* and *Enterobacter cloacae* [75]), and Botulinum toxin E phage (infects *Clostridium botulinum* and *Clostridium butyricum* [76]).

The mechanisms phage have developed to allow infection of a range of hosts are varied. Phage T2 and phage Mu alter their tail fibers to allow infection of alternative hosts [77, 78]. *Bordetella* phage carry diversity generating retroelements that allow the phage to infect bacteria with different cell surface receptors and physiology [79]. Some phage can inactivate or recombine with endogenous lysogens to alter their host range [45, 80, 81]. Inter- and intraspecies examples of phage showing a larger infection range than previously believed have been demonstrated through experimentation with lab strains [52, 62, 65, 67, 71, 72, 82–87].

Infection of alternative hosts in nature has also been demonstrated. Phage isolated from natural marine environments have been shown to subtly influence the composition of the bacterial community of those same environments [88]. Conversely, phage populations from various natural environments such as soil, lake water, and marine sediments have been shown to replicate when incubated with microbes from a different marine environment [6]. Comparison of the distribution of phage types in particular environments with the types of bacteria found in that same environment suggests that phage with quite broad host ranges must exist in nature [89]. Additionally, analyses of uncultured environmental phage libraries have revealed an abundance of mobile elements and genes involved in the mobilization of DNA [90–92]. Altogether these results suggest that phage are capable of infecting different hosts in the environment, providing a major mechanism for the spread of genes

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                                          310        320        330        340        350
                                    |    |    |    |    |    |    |    |    |    |
S. aureus strain MSSA476 sea gene        CGCA- - - - - -CATTGATAACCATAATAAGCACCATACAAGTCTACTTTTT
S. aureus subsp. aureus MW2 sea gene     CGCA- - - - - -CATTGATAACCATAATAAGCACCATACAAGTCTACTTTTT
S. aureus subsp. aureus Mu50 sea gene    CGCA- - - - - -CATTGATAACCATAATAAGCACCATACAAGTCTACTTTTT
S. aureus subsp. aureus Mu3 sea gene     CGCA- - - - - -CATTGATAACCATAATAAGCACCATACAAGTCTACTTTTT
S. aureus subsp. aureus Newman strain sea gene  CGCA- - - - - -CATTGATAACCATAATAAGCACCATATAAGTCTACTTTTT
S. aureus subsp. aureus strain MRSA252 sea gene CGCA- - - - - -CATTGATAACCATAATAAGCACCATATAAGTCTACTTTTT
S. aureus strain FRI281 sea gene         CAAAGGATATTGTTGATAAATATAAAGGGAAAAAAGTAGACTTGTATGGT
S. aureus strain ATCC 13565 sea gene     CAAAGGATATTGTTGATAAATATAAAGGGAAAAAAGTAGACTTGTATGGT
S. aureus strain ATCC 25923 sea gene     CAAAGGATATTGTTGATAAATATAAAGGGAAAAAAGTAGACTTGTATGGT
S. aureus phage φNM3 sea gene            CAAAGGATATTGTTGATAAATATAAAGGGAAAAAAGTAGACTTATATGGT
S. aureus strain FRI337 sea gene         CAAAGGATATTGTTGATAAATATAAAGGGAAAAAAGTAGACTTATATGGT
Ambient environment isolate sea gene     CAAAGGATATTGTTGATAAATATAAAGGGAAAAAAGTAGACTTATATGGT

                                          360        370        380        390        400
                                    |    |    |    |    |    |    |    |    |    |
S. aureus strain MSSA476 sea gene        TCCCTTTATATTTATCAACAATATCCTTTGAATCAAAATCTACTAATAAA
S. aureus subsp. aureus MW2 sea gene     TCCCTTTATATTTATCAACAATATCCTTTGAATCAAAATCTACTAATAAA
S. aureus subsp. aureus Mu50 sea gene    TCCCTTTATATTTATCAACAATATCCTTTGAATCAAAATCTACTAATAAA
S. aureus subsp. aureus Mu3 sea gene     TCCCTTTATATTTATCAACAATATCCTTTGAATCAAAATCTACTAATAAA
S. aureus subsp. aureus Newman strain sea gene  TCCCTTTATATTTATCAACAATATCCTTTGAATCAAAATCTACTAATAAA
S. aureus subsp. aureus strain MRSA252 sea gene TCCCTTTATATTTATCAACAATATCCTTTGAATCAAAATCTACTAATAAA
S. aureus strain FRI281 sea gene         GCTTATTATGGTTATCAATG- - - - - -TGCGGGTGGTACACCA- -AACAAA
S. aureus strain ATCC 13565 sea gene     GCTTATTATGGTTATCAATG- - - - - -TGCGGGTGGTACACCA- -AACAAA
S. aureus strain ATCC 25923 sea gene     GCTTATTATGGTTATCAATG- - - - - -TGCGGGTGGTACACCA- -AACAAA
S. aureus phage φNM3 sea gene            GCTTATTATGGTTATCAATG- - - - - -TGCGGGTGGTACACCA- -AACAAA
S. aureus strain FRI337 sea gene         GCTTATTATGGTTATCAATG- - - - - -TGCGGGTGGTACACCA- -AACAAA
Ambient environment isolate sea gene     GCTTATTATGGTTATCAATG- - - - - -TGCGGGTGGTACACCA- -AACAAA

                                          410        420        430        440        450
                                    |    |    |    |    |    |    |    |    |    |
S. aureus strain MSSA476 sea gene        TCGTTATACCACGAATGATC-TGTAAAAAAGCCTTTAAACAATATAGTAT
S. aureus subsp. aureus MW2 sea gene     TCGTTATACCACGAATGATC-TGTAAAAAAGCCTTTAAACAATATAGTAT
S. aureus subsp. aureus Mu50 sea gene    TCGTTATACCACGAATGATC-TGTAAAAAAGCCTTTAAACAATATAGTAT
S. aureus subsp. aureus Mu3 sea gene     TCGTTATACCACGAATGATC-TGTAAAAAAGCCTTTAAACAATATAGTAT
S. aureus subsp. aureus Newman strain sea gene  TCGTTATACCATGAATGATT-TGTAAAAAAGCCTTTAAACAATATAGTAT
S. aureus subsp. aureus strain MRSA252 sea gene TCGTTATACCATGAATGATT-TGTAAAAAAGCCTTTAAACAATATAGTAT
S. aureus strain FRI281 sea gene         ACAGCTTGT-ATGTATGGTGGTGTAACGTTACATGATAATAATCGATTGA
S. aureus strain ATCC 13565 sea gene     ACAGCTTGT-ATGTATGGTGGTGTAACGTTACATGATAATAATCGATTGA
S. aureus strain ATCC 25923 sea gene     ACAGCTTGC-ATGTATGGTGGTGTAACGTTACATGATAATAATCGATTGA
S. aureus phage φNM3 sea gene            ACAGCTTGC-ATGTATGGTGGTGTAACGTTACATGATAATAATCGATTGA
S. aureus strain FRI337 sea gene         ACAGCTTGC-ATGTATGGTGGTGTAACGTTACATGATAATAATCGATTGA
Ambient environment isolate sea gene     ACAGCTTGC-ATGTATGGTGGTGTAACGTTACATGATAATAATCAATTGG
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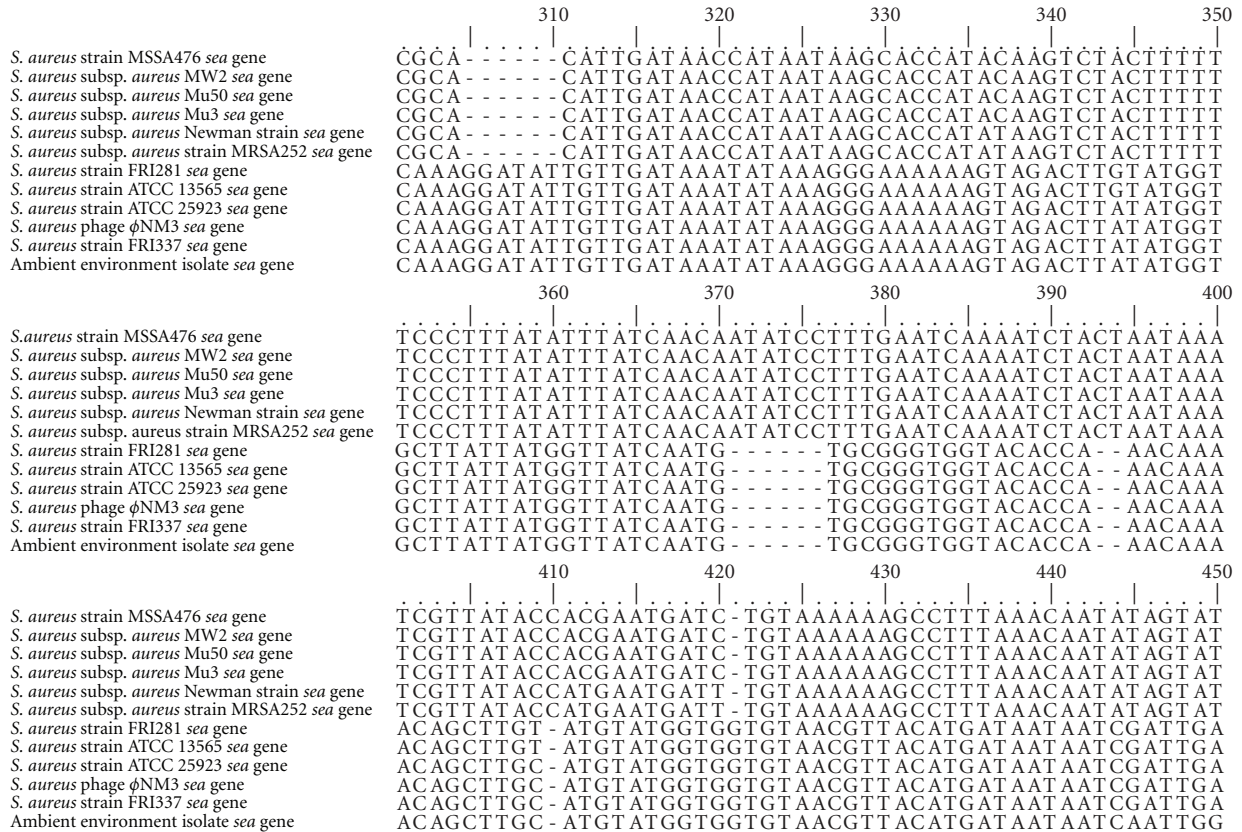
FIGURE 2: ClustalX2 alignment of top BLASTN hits of the *sea* gene from the ambient environmental isolate. The *sea* PCR product amplified from the cultured ambient air isolate was verified against the GenBank nonredundant database. The FASTA files of the top hits were downloaded and aligned using ClustalX2. The Accession number for the *sea*-related gene is HQ698309.
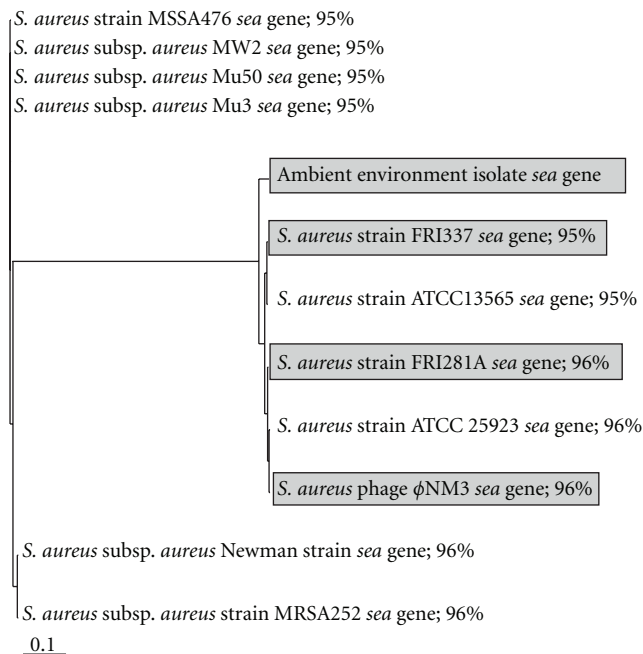

FIGURE 3: ClustalX2 guide tree of the top BLASTN hits of the *sea* gene from the ambient environmental isolate. The ambient air isolate is highlighted by a gray box along with *S. aureus* strains FRI337 and FRI281A (Food Research Institute) because the positive control used for *sea* exotoxin PCR comes from *S. aureus* strain FRI913. Percent identities are indicated beside the organism name as listed in GenBank. The GenBank Accession number for the *sea*-related gene is HQ698309.
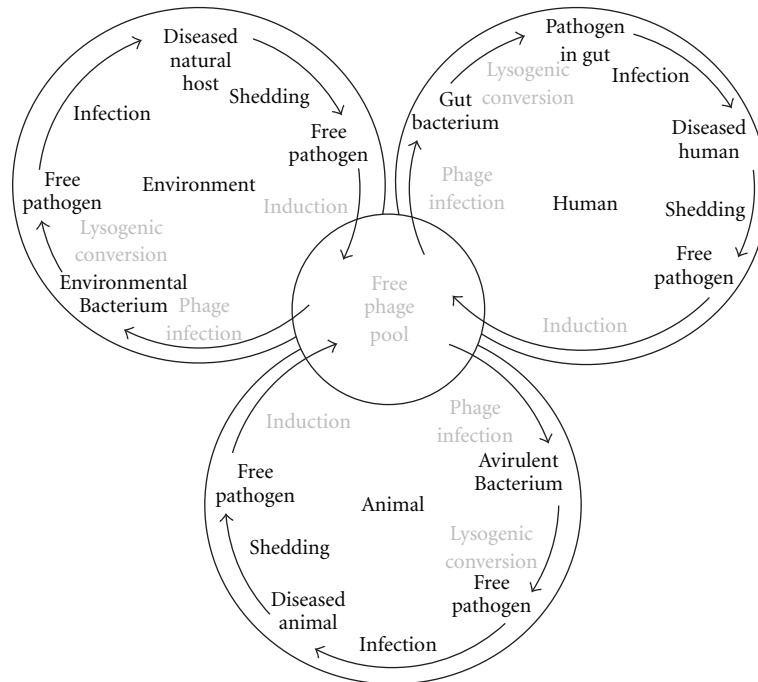
Figure 4: Free phage pool of exotoxin genes. Proposed scenarios for how exotoxin-encoding phage might be maintained in the environment and produce human pathogens through genetic exchange between the free phage pool and the natural, human, and animal environments. Light grey writing indicates phage/host interaction and potential for horizontal gene transfer.

between bacteria. Thus, phage may promote promiscuous horizontal gene transfer in nature.

This report provides the first direct evidence that alternative microbial hosts can carry exotoxin genes. The environmental isolate cultivated from outdoor ambient air was confirmed to carry the *sea* exotoxin gene by repeated exotoxin-specific PCR, and by sequencing and alignment of the *sea*-specific PCR product. The *sea*-specific PCR product was 95-96% identical at the nucleotide level to known *sea* genes in the GenBank nonredundant database.

When characterized microbiologically, the cultivated environmental isolate did not share the same characteristics as the control *S. aureus* FRI913 strain known to carry the *sea* gene. It did not grow on *Staphylococcus* enrichment media, nor did it share the same Gram staining properties as the *S. aureus* FRI913 strain (the environmental isolate was a Gram negative rod versus *Staphylococcus* which are Gram positive cocci). These results suggested that the *sea* gene was present in an alternative environmental host.

Further support for this conclusion was provided by sequencing of the 16S rDNA from the cultivated environmental isolate. Alignment of the 16S rDNA sequence from this isolate with the ARB database indicated the isolate was a *Pseudomonas* spp. and did not group with *S. aureus*. Moreover, phylogenetic analyses using PAUP* indicated that the isolate was related to *Pseudomonas spp*, not *S. aureus*. The ML, MP, and NJ bootstrap values of the main branches of the consensus tree that group the isolate with *Pseudomonas* spp. were ≥94, indicating high probability that these branches are robust. This provides robust statistical evidence that the

isolate is a *Pseudomonas* spp. Further evidence supporting this identification of the ambient air isolate was its relatively high G + C content (52.36%) like many *Pseudomonas* spp., as compared to the low G + C content of *S. aureus* (32.8%).

We were unable to induce a *sea* positive phage by treatment from the environmental isolate via mitomycin C treatment [81]. There are at least two possible explanations why the environmental isolate was carrying the *sea*-related gene, but did not produce phage. First, generalized transduction could have transferred a region of bacterial chromosomal DNA containing the *sea* gene into the *Pseudomonas* spp. without integration of the phage itself [93]. Second, it is possible that the exotoxin was transferred via a phage, but the phage genes were subsequently mutated due to selection against harmful phage genes in the lysogen [94–102].

Based on the examples from the literature and the data generated from this study, we propose the following model for transfer of phage-encoded exotoxin genes to a novel bacterium leading to the creation of a new human pathogen. A "free phage pool" (Figure 4) can potentially lead to new disease outbreaks in three ways: (i) transduction of exotoxin genes to an environmental bacterium that subsequently infects a human; (ii) transduction of exotoxin genes from the environmental reservoir to a bacterium in the normal human microbiota; (iii) transduction of a bacterium associated with a nonhuman animal, with subsequent infection of a human. In all three scenarios, once the phage is propagated within the alternate bacterial hosts it can lyse the host and re-enter the "free-phage pool" ready to transduce the exotoxin genes to other bacteria. In this manner, the genes are maintained in

the environment independent of the bacterial host typically involved in the human disease.

The evidence that there is an environmental reservoir of exotoxin genes in bacteria that are not normally associated with human disease, suggests the possibility that novel diseases may evolve through horizontal transfer of virulence genes via transduction to new microbial hosts (Figure 4).

4. Materials and Methods

4.1. Sampling of Indoor and Outdoor Air. To collect bacterial air isolates, Luria Bertani (LB) agar plates containing 50 mg of cyclohexamide to prevent growth of fungi, were exposed to ambient air. These plates were then incubated at room temperature for 48–72 hr. All isolates that grew on the LB plates conditions were then subcultured into sterile 96-well plates containing LB and 15% glycerol. These subcultured isolates were grown, with aeration, at room temperature for 48–72 hr then stored at 4°C until screened by PCR.

4.2. PCR Assays and Sequencing. A colony PCR assay was used to initially screen for the Staphylococcus enterotoxin A (*sea*) gene. The *sea*-specific PCR primers amplified a 498 bp partial sequence within the coding region of the *sea* gene. The *sea* primers were (forward primer) 5′-GCAGGGAACAGCTTTAGGC-3′ and (reverse primer) 5′-GTTCTGTAGAAGTATGAAACACG-3′. To identify the bacterial isolate carrying the *sea* gene, a 16S rDNA PCR assay was used and the resulting PCR product sequenced. The primers used for the 16S rDNA PCR were (forward) 5′-AGAGTTTGATCMTGGCTCAG-3′ and (reverse) 5′-TACGGYTACCTTGTTACGACTT-3′. Five microliters of the subcultured isolates suspension was used as a template in the *sea*-specific PCR16S rDNA PCR. The PCR thermocycling conditions for the *sea*-specific PCR and 16S rDNA PCR were as described previously [103]. To control against possible PCR contamination of the environmental sample DNAs, all PCRs were performed in a completely separate room and different building with different air handling systems. The PCR products were run on a 1% agarose gel at 150 V. Sequencing of the *sea*-specific PCR and 16S rDNA PCR products was performed at the SDSU Microchemical Core Facility. To confirm the *sea* PCR product was the target gene, a BLASTN alignment of the PCR product was performed against the nonredundant GenBank database [104]. The top 12 hits were then aligned and a tree generated using ClustalX2 [36].

4.3. Microbiological Characterization of Environmental Isolate. The air isolate carrying the *sea*-like gene was characterized by plating onto Staph 110 media [105], a media that enriches for *Staphylococcus aureus*, and LB medium. The plates were incubated at room temperature and at 35°C. A Gram stain was also performed on the isolate identified as *sea*-positive by colony PCR.

4.4. Molecular Characterization of Environmental Isolate. Phylogenetic analyses were performed on the 16S rDNA

PCR product sequence to molecularly identify the ambient air isolate. An alignment with the ARB 16S rDNA database was used to identify the nearest relatives to the ambient air isolate for use in creating a phylogenetic tree in PAUP* [33]. Maximum Likelihood (ML) was implemented in PAUP* to build the highest likelihood tree under an HKY85 model of sequence evolution with estimated nucleotide frequencies, shape parameter, and number of invariant sites. The heuristic search approach to find the best ML tree included 100 random addition sequence searches using TBR branch swapping. The ML bootstrap involved 100 replicates with 10 random addition sequences searches per replicate. The best Maximum Parsimony (MP) tree was found through a random addition sequence heuristic search strategy with 100 replicates. The maximum number of trees kept during each search was capped at 1000. MP bootstrap analyses were performed using searches on 100 bootstrap replicated datasets using the same heuristic search strategy except with 10, rather than 100, search replicates. The Neighbor-joining (NJ) bootstrap analysis was performed with 1000 replicates.

References

[1] D. P. Falcao, "Bacterial enteropathogens: general characteristics," *Revista de Ciencias Farmaceuticas*, vol. 17, pp. 25–55, 1996.

[2] S. Leppla, "The anthrax toxin complex," in *Sourcebook of Bacterial Protein Toxins*, J. Alouf and J. Freer, Eds., pp. 277–298, Academic Press, New York, NY, USA, 1991.

[3] M. Pittman, "The concept of pertussis as a toxin-mediated disease," *Pediatric Infectious Disease*, vol. 3, no. 5, pp. 467–486, 1984.

[4] M. Muniesa, F. Lucena, and J. Jofre, "Comparative survival of free Shiga toxin 2-encoding phages and *Escherichia coli* strains outside the gut," *Applied and Environmental Microbiology*, vol. 65, no. 12, pp. 5615–5618, 1999.

[5] R. Dumke, U. Schröter-Bobsin, E. Jacobs, and I. Röske, "Detection of phages carrying the Shiga toxin 1 and 2 genes in waste water and river water samples," *Letters in Applied Microbiology*, vol. 42, no. 1, pp. 48–53, 2006.

[6] E. Sano, S. Carlson, L. Wegley, and F. Rohwer, "Movement of viruses between biomes," *Applied and Environmental Microbiology*, vol. 70, no. 10, pp. 5842–5846, 2004.

[7] G. S. Johannessen, C. E. James, H. E. Allison, D. L. Smith, J. R. Saunders, and A. J. McCarthy, "Survival of a Shiga toxin-encoding bacteriophage in a compost model," *FEMS Microbiology Letters*, vol. 245, no. 2, pp. 369–375, 2005.

[8] A. Aertsen, D. Faster, and C. W. Michiels, "Induction of Shiga toxin-converting prophage in *Escherichia coli* by high hydrostatic pressure," *Applied and Environmental Microbiology*, vol. 71, no. 3, pp. 1155–1162, 2005.

[9] L. Mocé-Llivina, M. Muniesa, H. Pimenta-Vale, F. Lucena, and J. Jofre, "Survival of bacterial indicator species and bacteriophages after thermal treatment of sludge and sewage," *Applied and Environmental Microbiology*, vol. 69, no. 3, pp. 1452–1456, 2003.

[10] S. C. Jiang and J. H. Paul, "Gene transfer by transduction in the marine environment," *Applied and Environmental Microbiology*, vol. 64, no. 8, pp. 2780–2787, 1998.

[11] T. Hidaka and T. Fujimura, "On the heat- and chloroform-resistance of marine bacteriophages," *Memoirs of the Faculty of Fisheries, Kagoshima University*, vol. 20, pp. 155–158, 1971.

[12] H. Brüssow, C. Canchaya, and W. D. Hardt, "Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion," *Microbiology and Molecular Biology Reviews*, vol. 68, no. 3, pp. 560–602, 2004.

[13] H.-W. Ackermann and M. S. DuBow, *Viruses of Prokaryotes; Volume I: General Properties of Bacteriophages*, CRC Press, Boca Raton, Fla, USA, 1987.

[14] V. J. Freeman, "Studies on the virulence of bacteriophage-infected strains of *Corynebacterium diphtheriae*," *Journal of bacteriology*, vol. 61, no. 6, pp. 675–688, 1951.

[15] N. B. Groman, "The relation of bacteriophage to the change of *Corynebacterium diphtheriae* from avirulence to virulence," *Science*, vol. 117, no. 3038, pp. 297–299, 1953.

[16] M. K. Waldor and J. J. Mekalanos, "Lysogenic conversion by a filamentous phage encoding cholera toxin," *Science*, vol. 272, no. 5270, pp. 1910–1913, 1996.

[17] D. W. K. Acheson, J. Reidl, X. Zhang, G. T. Keusch, J. J. Mekalanos, and M. K. Waldor, "In vivo transduction with shiga toxin 1-encoding phage," *Infection and Immunity*, vol. 66, no. 9, pp. 4496–4498, 1998.

[18] R. Novick, "Properties of a cryptic high-frequency transducing phage in *Staphylococcus aureus*," *Virology*, vol. 33, no. 1, pp. 155–166, 1967.

[19] M. J. Betley and J. J. Mekalanos, "*Staphylococcal enterotoxin* A is encoded by phage," *Science*, vol. 229, no. 4709, pp. 185–187, 1985.

[20] D. C. Coleman, D. J. Sullivan, R. J. Russell, J. P. Arbuthnott, B. F. Carey, and H. M. Pomeroy, "*Staphylococcus aureus* bacteriophages mediating the simulataneous lysogenic conversion of β-lysin, staphylokinase and enterotoxin A: molecular mechanism of triple conversion," *Journal of General Microbiology*, vol. 135, no. 6, pp. 1679–1697, 1989.

[21] M. J. Betley, S. Lofdahl, and B. N. Kreiswirth, "*Staphylococcal enterotoxin* A gene is associated with a variable genetic element," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 81, no. 16 I, pp. 5179–5183, 1984.

[22] P. Holochová, V. Růžičková, R. Pantůček, P. Petráš, R. Janisch, and J. Doškař, "Genomic diversity of two lineages of exfoliative toxin A-converting phages predominating in *Staphylococcus aureus* strains in the Czech Republic," *Research in Microbiology*, vol. 161, no. 4, pp. 260–267, 2010.

[23] H. Hoshiba, J. Uchiyama, S. I. Kato et al., "Isolation and characterization of a novel *Staphylococcus aureus* bacteriophage, φMR25, and its therapeutic potential," *Archives of Virology*, vol. 155, no. 4, pp. 545–552, 2010.

[24] J. D. Carroll, M. T. Cafferkey, and D. C. Coleman, "Serotype F double- and triple-converting phage insertionally inactivate the *Staphylococcus aureus* β-toxin determinant by a common molecular mechanism," *FEMS Microbiology Letters*, vol. 106, no. 2, pp. 147–155, 1993.

[25] T. Yamaguchi, T. Hayashi, H. Takami et al., "Phage conversion of exfoliative toxin A production in *Staphylococcus aureus*," *Molecular Microbiology*, vol. 38, no. 4, pp. 694–705, 2000.

[26] Y. Endo, T. Yamada, K. Matsunaga, Y. Hayakawa, T. Kaidoh, and S. Takeuchi, "Phage conversion of exfoliative toxin A in *Staphylococcus aureus* isolated from cows with mastitis," *Veterinary Microbiology*, vol. 96, no. 1, pp. 81–90, 2003.

[27] R. M. Dempsey, D. Carroll, H. Kong, L. Higgins, C. T. Keane, and D. C. Coleman, "*Sau*421, a *Bcg*l-like restriction-modification system encoded by the *Staphylococcus aureus* quandruple-converting phage π42," *Microbiology*, vol. 151, no. 4, pp. 1301–1311, 2005.

[28] C. Goerke, J. Koller, and C. Wolz, "Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in *Staphylococcus aureus*," *Antimicrobial Agents and Chemotherapy*, vol. 50, no. 1, pp. 171–177, 2006.

[29] W. J. B. van Wamel, S. H. M. Rooijakkers, M. Ruyken, K. P. M. van Kessel, and J. A. G. van Strijp, "The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on β-hemolysin-converting bacteriophages," *Journal of Bacteriology*, vol. 188, no. 4, pp. 1310–1315, 2006.

[30] R. Kumagai, K. Nakatani, N. Ikeya, Y. Kito, T. Kaidoh, and S. Takeuchi, "Quadruple or quintuple conversion of hlb, sak, sea (or sep), scn, and chp genes by bacteriophages in non-β-hemolysin-producing bovine isolates of *Staphylococcus aureus*," *Veterinary Microbiology*, vol. 122, no. 1-2, pp. 190–195, 2007.

[31] A. J. Synnott, Y. Kuang, M. Kurimoto, K. Yamamichi, H. Iwano, and Y. Tanji, "Isolation from sewage influent and characterization of novel *Staphylococcus aureus* bacteriophages with wide host ranges and potent lytic capabilities," *Applied and Environmental Microbiology*, vol. 75, no. 13, pp. 4483–4490, 2009.

[32] Difco, *Difco Manual*, Difco Laboratories, Detroit, Mich, USA, 10th edition, 1984.

[33] D. Swofford, *PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods) 4.0 Beta*, Sinauer Associates, Sunderland, Mass, USA, 4.0 Beta edition, 2002.

[34] T. A. Hall, "BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT," *Nucleic Acids Symposium Series*, vol. 41, pp. 95–98, 1999.

[35] J. D. Thompson, D. G. Higgins, and T. J. Gibson, "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice," *Nucleic Acids Research*, vol. 22, no. 22, pp. 4673–4680, 1994.

[36] J. D. Thompson, T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins, "The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools," *Nucleic Acids Research*, vol. 25, no. 24, pp. 4876–4882, 1997.

[37] R. Overbeek, T. Begley, R. M. Butler et al., "The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes," *Nucleic Acids Research*, vol. 33, no. 17, pp. 5691–5702, 2005.

[38] K. Sandvig, O Garred, and B. van Deurs, "Intracellular transport and processing of protein toxins produced by enteric bacteria," *Advances in Experimental Medicine and Biology*, vol. 412, pp. 225–232, 1997.

[39] G. M. Vath, C. A. Earhart, J. V. Rago et al., "The structure of the superantigen exfoliative toxin a suggests a novel regulation as a serine protease," *Biochemistry*, vol. 36, no. 7, pp. 1564–1566, 1997.

[40] B. M. Davis and M. K. Waldor, "Mobile genetic elements and bacteria pathogenesis," in *Mobile DNA II*, N. L. Craig, R. Gragie, M. Gellert, and A. M. Lambowitz, Eds., pp. 1040–1055, ASM Press, Washington, DC, USA, 2002.

[41] C. A. Mims, *Mims' Pathogenesis of Infectious Disease*, Academic Press, San Diego, Calif, USA, 4th edition, 1995.

[42] W. Ziebuhr, K. Ohlsen, H. Karch, T. Korhonen, and J. Hacker, "Evolution of bacterial pathogenesis," *Cellular and Molecular Life Sciences*, vol. 56, no. 9-10, pp. 719–728, 1999.

[43] A. A. Salyers and D. D. Whitt, *Bacterial Pathogenesis: A Molecular Approach*, ASM Press, Washington, DC, USA, 2002.

[44] S. M. Faruque, I. B. Naser, M. J. Islam et al., "Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 5, pp. 1702–1707, 2005.

[45] R. K. Aziz, R. A. Edwards, W. W. Taylor, D. E. Low, A. McGeer, and M. Kotb, "Mosaic prophages with horizontally acquired genes account for the emergence and diversification of the globally disseminated M1t1 clone of *Streptococcus pyogenes*," *Journal of Bacteriology*, vol. 187, no. 10, pp. 3311–3318, 2005.

[46] R. L. Shapiro, C. Hatheway, and D. L. Swerdlow, "Botulism in the United States: a clinical and epidemiologic review," *Annals of Internal Medicine*, vol. 129, no. 3, pp. 221–228, 1998.

[47] S. M. Faruque, S. K. Roy, A. R. M. A. Alim, A. K. Siddique, and M. J. Albert, "Molecular epidemiology of toxigenic *Vibrio cholerae* in Bangladesh studied by numerical analysis of rRNA gene restriction patterns," *Journal of Clinical Microbiology*, vol. 33, no. 11, pp. 2833–2838, 1995.

[48] V. Casas and F. Rohwer, "Phage metagenomics," *Methods in Enzymology*, vol. 421, pp. 259–268, 2007.

[49] J. A. Baross, J. Liston, and R. Y. Morita, "Ecological relationship between *Vibrio parahaemolyticus* and agar-digesting vibrios as evidenced by bacteriophage susceptibility patterns," *Applied and Environmental Microbiology*, vol. 36, no. 3, pp. 500–505, 1978.

[50] J. P. Bollback and J. P. Huelsenbeck, "Phylogeny, genome evolution, and host specificity of single-stranded RNA bacteriophage (family Leviviridae)," *Journal of Molecular Evolution*, vol. 52, no. 2, pp. 117–128, 2001.

[51] C. Dini and P. J. De Urraza, "Isolation and selection of coliphages as potential biocontrol agents of enterohemorrhagic and Shiga toxin-producing *E. coli*(EHEC and STEC) in cattle," *Journal of Applied Microbiology*, vol. 109, no. 3, pp. 873–887, 2010.

[52] T. Koga, S. Toyoshima, and T. Kawata, "Morphological varieties and host ranges of *Vibrio parahaemolyticus* bacteriophages isolated from seawater," *Applied and Environmental Microbiology*, vol. 44, no. 2, pp. 466–470, 1982.

[53] M. L. Saxelin, E. L. Nurmiaho, M. P. Korhola, and V. Sundman, "Partial characterization of a new C3-type capsule-dissolving phage of *Streptococcus cremoris*," *Canadian Journal of Microbiology*, vol. 25, no. 10, pp. 1182–1187, 1979.

[54] R. H. Olsen, J. S. Siak, and R. H. Gray, "Characteristics of PRD1, a plasmid dependent broad host range DNA bacteriophage," *Journal of Virology*, vol. 14, no. 3, pp. 689–699, 1974.

[55] J. T. Douglas and S. S. Elberg, "Isolation of *Brucella melitensis* phage of broad biotype and species specificity," *Infection and Immunity*, vol. 14, no. 1, pp. 306–308, 1976.

[56] D. E. Bradley, J. N. Coetzee, T. Bothma, and R. W. Hedges, "Phage X: a plasmid-dependent, broad host range, filamen-

[57] J. Anne, W. Wohlleben, and H. J. Burkardt, "Morphological and molecular characterization of several actinophages isolated from soil which lyse *Streptomyces cattleya* or *S. Venezuelae*," *Journal of General Microbiology*, vol. 130, no. 10, pp. 2639–2649, 1984.

[58] J. Hantula, A. Kurki, P. Vuoriranta, and D. H. Bamford, "Ecology of bacteriophages infecting activated sludge bacteria," *Applied and Environmental Microbiology*, vol. 57, no. 8, pp. 2147–2151, 1991.

[59] S. Matsuzaki, S. Tanaka, T. Koga, and T. Kawata, "A broad-host-range vibriophage, KVP40, isolated from sea water," *Microbiology and Immunology*, vol. 36, no. 1, pp. 93–97, 1992.

[60] J. Nolling, A. Groffen, and W. M. De Vos, "ΦF1 and ΦF3, two novel virulent, archaeal phages infecting different thermophilic strains of the genus Methanobacterium," *Journal of General Microbiology*, vol. 139, no. 10, pp. 2511–2516, 1993.

[61] D. H. Bamford, J. Caldentey, and J. K. Bamford, "Bacteriophage PRD1: a broad host range dsDNA tectivirus with an internal membrane," *Advances in Virus Research*, vol. 45, pp. 281–319, 1995.

[62] E. C. Jensen, H. S. Schrader, B. Rieland et al., "Prevalence of broad-host-range lytic bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa*," *Applied and Environmental Microbiology*, vol. 64, no. 2, pp. 575–580, 1998.

[63] A. O. Kiliç, S. I. Pavlova, S. Alpay, S. S. Kiliç, and L. Tao, "Comparative study of vaginal Lactobacillus phages isolated from women in the United States and Turkey: prevalence, morphology, host range, and DNA homology," *Clinical and Diagnostic Laboratory Immunology*, vol. 8, no. 1, pp. 31–39, 2001.

[64] M. A. Khan, H. Satoh, H. Katayama, F. Kurisu, and T. Mino, "Bacteriophages isolated from activated sludge processes and their polyvalency," *Water Research*, vol. 36, no. 13, pp. 3364–3370, 2002.

[65] A. Beumer and J. B. Robinson, "A broad-host-range, generalized transducing phage (SN-T) acquires 16S rRNA genes from different genera of bacteria," *Applied and Environmental Microbiology*, vol. 71, no. 12, pp. 8301–8304, 2005.

[66] Y. Xu, D. R. Peng, H. Y. Xiong et al., "Study on the nucleic acid of *E. coli* bacteriophage with broad host range and its sterilization effect to sewage samples from the environment," *Zhonghua Liu Xing Bing Xue Za Zhi*, vol. 26, no. 5, pp. 356–360, 2005.

[67] T. M. Ruokoranta, A. M. Grahn, J. J. Ravantti, M. M. Poranen, and D. H. Bamford, "Complete genome sequence of the broad host range single-stranded RNA phage PRR1 places it in the levivirus genus with characteristics shared with alloleviviruses," *Journal of Virology*, vol. 80, no. 18, pp. 9326–9330, 2006.

[68] L. Deng and P. K. Hayes, "Evidence for cyanophages active against bloom-forming freshwater cyanobacteria," *Freshwater Biology*, vol. 53, no. 6, pp. 1240–1252, 2008.

[69] D. Schwudke, A. Ergin, K. Michael et al., "Broad-host-range Yersinia phage PY100: genome sequence, proteome analysis of virions, and DNA packaging strategy," *Journal of Bacteriology*, vol. 190, no. 1, pp. 332–342, 2008.

[70] J. Uchiyama, M. Rashel, Y. Maeda et al., "Isolation and characterization of a novel *Enterococcus faecalis* bacteriophage φEF24C as a therapeutic candidate," *FEMS Microbiology Letters*, vol. 278, no. 2, pp. 200–206, 2008.

tous bacterial virus," *Journal of General Microbiology*, vol. 126, no. 2, pp. 389–396, 1981.

[71] J. Dorscht, J. Klumpp, R. Bielmann et al., "Comparative genome analysis of Listeria bacteriophages reveals extensive mosaicism, programmed translational frameshifting, and a novel prophage insertion site," *Journal of Bacteriology*, vol. 191, no. 23, pp. 7206–7215, 2009.

[72] S. M. Lehman, A. M. Kropinski, A. J. Castle, and A. M. Svircev, "Complete genome of the broad-host-range *Erwinia amylovora* phage ΦEa21-4 and its relationship to *Salmonella* phage Felix O1," *Applied and Environmental Microbiology*, vol. 75, no. 7, pp. 2139–2147, 2009.

[73] X. Qiao, Y. Sun, J. Qiao, F. Di Sanzo, and L. Mindich, "Characterization of Φ2954, a newly isolated bacteriophage containing three dsRNA genomic segments," *BMC Microbiology*, vol. 10, article 55, 2010.

[74] S. M. Faruque, M. M. Rahman, . Asadulghani, K. M. N. Islam, and J. J. Mekalanos, "Lysogenic conversion of environmental *Vibrio mimicus* strains by CTXΦ," *Infection and Immunity*, vol. 67, no. 11, pp. 5723–5729, 1999.

[75] M. Mehrotra, G. Wang, and W. M. Johnson, "Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance," *Journal of Clinical Microbiology*, vol. 38, no. 3, pp. 1032–1035, 2000.

[76] Y. Zhou, H. Sugiyama, and E. A. Johnson, "Transfer of neurotoxigenicity from *Clostridium butyricum* to a nontoxigenic *Clostridium botulinum* type E-like strain," *Applied and Environmental Microbiology*, vol. 59, no. 11, pp. 3825–3831, 1993.

[77] M. Yoichi, M. Abe, K. Miyanaga, H. Unno, and Y. Tanji, "Alteration of tail fiber protein gp38 enables T2 phage to infect *Escherichia coli* O157:H7," *Journal of Biotechnology*, vol. 115, no. 1, pp. 101–107, 2005.

[78] C. Koch, G. Mertens, F. Rudt et al., "The invertible G segment," in *Phage Mu*, N. Symonds, A. Toussaint, P. van de Putte, and M. Howe, Eds., pp. 75–91, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 1987.

[79] S. Doulatov, A. Hodes, L. Dal et al., "Tropism switching in *Bordetella* bacteriophage defines a family of diversity-generating retroelements," *Nature*, vol. 431, no. 7007, pp. 476–481, 2004.

[80] M. M. Susskind, A. Wright, and D. Botstein, "Superinfection exclusion by P22 prophage in lysogens of *Salmonella typhimurium*. II. Genetic evidence for two exclusion systems," *Virology*, vol. 45, no. 3, pp. 638–652, 1971.

[81] S. Maloy, V. Stewart, and R. Taylor, *Genetic Analysis of Pathogenic Bacteria: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 1996.

[82] M. L. Saxelin, E. L. Nurmiaholassila, V. T. Merilainen, and R. I. Forsen, "Ultrastructure and host specificity of bacteriophages of *streptococcus-cremoris, streptococcus-lactis* subsp diacetylactis, and *leuconostoc-cremoris* from Finnish fermented Milk Viili," *Applied and Environmental Microbiology*, vol. 52, pp. 771–777, 1986.

[83] C. M. Carvalho, B. W. Gannon, D. E. Halfhide et al., "The in vivo efficacy of two administration routes of a phage cocktail to reduce numbers of *Campylobacter coli* and *Campylobacter jejuni* in chickens," *BMC Microbiology*, vol. 10, article 232, 2010.

[84] T. Kenzaka, K. Tani, and M. Nasu, "High-frequency phage-mediated gene transfer in freshwater environments determined at single-cell level," *ISME Journal*, vol. 4, no. 5, pp. 648–659, 2010.

[85] T. J. Evans, M. A. Crow, N. R. Williamson et al., "Characterization of a broad-host-range flagellum-dependent phage that mediates high-efficiency generalized transduction in, and between, *Serratia* and *Pantoea*," *Microbiology*, vol. 156, no. 1, pp. 240–247, 2010.

[86] K. Sau, S. K. Gupta, S. Sau, S. C. Mandal, and T. C. Ghosh, "Studies on synonymous codon and amino acid usage biases in the broad-host range bacteriophage KVP40," *Journal of Microbiology*, vol. 45, no. 1, pp. 58–63, 2007.

[87] M. Namura, T. Hijikata, K. Miyanaga, and Y. Tanji, "Detection of *Escherichia coli* with fluorescent labeled phages that have a broad host range to *E. coli* in sewage water," *Biotechnology Progress*, vol. 24, no. 2, pp. 481–486, 2008.

[88] J. A. Fuhrman and M. Schwalbach, "Viral influence on aquatic bacterial communities," *Biological Bulletin*, vol. 204, no. 2, pp. 192–195, 2003.

[89] M. Breitbart, J. H. Miyake, and F. Rohwer, "Global distribution of nearly identical phage-encoded DNA sequences," *FEMS Microbiology Letters*, vol. 236, no. 2, pp. 249–256, 2004.

[90] M. Breitbart, P. Salamon, B. Andresen et al., "Genomic analysis of uncultured marine viral communities," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 22, pp. 14250–14255, 2002.

[91] M. Breitbart, I. Hewson, B. Felts et al., "Metagenomic analyses of an uncultured viral community from human feces," *Journal of Bacteriology*, vol. 185, no. 20, pp. 6220–6223, 2003.

[92] M. Breitbart, L. Wegley, S. Leeds, T. Schoenfeld, and F. Rohwer, "Phage community dynamics in hot springs," *Applied and Environmental Microbiology*, vol. 70, no. 3, pp. 1633–1640, 2004.

[93] A. Thierauf, G. Perez, and A. S. Maloy, "Generalized transduction," *Methods in Molecular Biology*, vol. 501, pp. 267–286, 2009.

[94] R. E. Lenski and B. R. Levin, "Constraints on the coevolution of bacteria and virulent phage: a model, some experiments, and predictions for natural communities," *American Naturalist*, vol. 125, no. 4, pp. 585–602, 1985.

[95] M. T. Horne, "Coevolution of *Escherichia coli* and bacteriophages in chemostat culture," *Science*, vol. 168, no. 3934, pp. 992–993, 1970.

[96] Y. Husimi, "Selection and evolution of bacteriophages in cellstat," *Advances in Biophysics*, vol. 25, no. C, pp. 1–43, 1989.

[97] E. A. Miao and S. I. Miller, "Bacteriophages in the evolution of pathogen-host interactions," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 17, pp. 9452–9454, 1999.

[98] H. Neve, K. I. Zenz, F. Desiere, A. Koch, K. J. Heller, and H. Brüssow, "Comparison of the lysogeny modules from the temperate *Streptococcus thermophilus* bacteriophages TP-J34 and Sfi21: implications for the modular theory of phage evolution," *Virology*, vol. 241, no. 1, pp. 61–72, 1998.

[99] R. W. Hendrix, M. C. M. Smith, R. N. Burns, M. E. Ford, and G. F. Hatfull, "Evolutionary relationships among diverse bacteriophages and prophages: all the world's a phage," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 5, pp. 2192–2197, 1999.

[100] M. A. Tormo-Más, I. Mir, A. Shrestha et al., "Moonlighting bacteriophage proteins derepress staphylococcal pathogenicity islands," *Nature*, vol. 465, no. 7299, pp. 779–782, 2010.

[101] L. A. Marraffini and E. J. Sontheimer, "CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea," *Nature Reviews Genetics*, vol. 11, no. 3, pp. 181–190, 2010.

[102] R. Gregory, V. A. Saunders, and J. R. Saunders, "Rule-based simulation of temperate bacteriophage infection: restriction-modification as a limiter to infection in bacterial populations," *BioSystems*, vol. 100, no. 3, pp. 166–177, 2010.

[103] V. Casas, J. Miyake, H. Balsley et al., "Widespread occurrence of phage-encoded exotoxin genes in terrestrial and aquatic environments in Southern California," *FEMS Microbiology Letters*, vol. 261, no. 1, pp. 141–149, 2006.

[104] S. F. Altschul, W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, "Basic local alignment search tool," *Journal of Molecular Biology*, vol. 215, no. 3, pp. 403–410, 1990.

[105] *Difco Manual*, Difco Laboratories, Detroit, Mich, USA, 10th edition, 1984.