The ISME Journal (2014) 8, 1949–1952

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SHORT COMMUNICATION

Intragenus generalized transduction in *Staphylococcus* spp. by a novel giant phage

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Bacteriophage (phage)-mediated generalized transduction is expected to contribute to the emergence of drug-resistant staphylococcal clones in various environments. In this study, novel phage S6 was isolated from sewage and used to test generalized transduction in human- and animal-derived staphylococci. Phage S6 was a novel type of giant myophage, which possessed a DNA genome that contained uracil instead of thymine, and it could infect all of the tested staphylococcal species. The phage S6 appeared to be similar to the transducing phage PBS1, which infects *Bacillus* spp. Moreover, phage S6 facilitated the transduction of a plasmid in *Staphylococcus aureus* and from *S. aureus* to non-*aureus* staphylococcal species, as well as vice versa. Transduction of methicillin resistance also occurred in *S. aureus*. This is the first report of successful intragenus generalized transduction among staphylococci.

The ISME Journal (2014) **8,** 1949–1952; doi:10.1038/ismej.2014.29; published online 6 March 2014
Subject Category: Microbe-microbe and microbe-host interactions
Keywords: staphylococci; drug-resistance; generalized transduction; bacteriophage; RNA world; sewage.

Antibiotic-resistance genes can be exchanged via horizontal gene transfer among bacteria found in humans and animals (Finley *et al.*, 2013). Generalized transduction, where the transfer of DNA is mediated by a bacteriophage (phage), is an important mechanism that facilitates the horizontal gene transfer of antibiotic-resistant genes. Antibiotic-resistant genes and phages originate from various environments (Weinbauer, 2004; Finley *et al.*, 2013). In particular, sewage is the most concentrated source of both, and phage-mediated gene transfer is likely to occur among staphylococci in sewage (Colomer-Lluch *et al.*, 2011; Finley *et al.*, 2013).

Staphylococcus spp. are Gram-positive bacteria, which are found frequently in humans and animals, and sporadically in various environments (Vos *et al.*, 2009). Some *Staphylococcus* spp. are resistant to methicillin, such as methicillin-resistant *S. aureus* (MRSA) and *S. pseudintermedius*, which often cause serious infections in humans and animals (Doyle *et al.*, 2012). Staphylococci are also likely to exchange genetic elements, possibly via generalized transduction, so they may acquire drug-resistant genes such as methicillin resistance. To the best of our knowledge, however, no direct evidence is available on intragenus generalized transduction in staphylococci (Novick *et al.*, 2010).

In this study, we isolated a novel staphylococcal phage from sewage and used it to test generalized transduction in animal-derived and human-derived *Staphylococcus* spp. in a laboratory setting.

The bacteria used in this study are listed in Supplementary Table S1 and all were cultured in tryptic soy broth, unless stated otherwise. The phage amplification conditions are described in Supplementary Table S2. All of the experiments were replicated three or six times.

Staphylococcal phage S6 was isolated from local sewage samples from Kochi, Japan, using *S. aureus* strain SA27 as the host strain. Electron microscopy, genome size estimation and nucleoside analysis

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Received 18 August 2013; revised 10 December 2013; accepted 24 January 2014; published online 6 March 2014

were conducted to characterize the phage, according to published methods (Uchiyama *et al.*, 2008, 2012; Takemura-Uchiyama *et al.*, 2013). Phage S6 was shown to belong to the family *Myoviridae* with a genome size of ca. 270 kbp (Andrew *et al.*, 2011; Deghorain and Van Melderen, 2012; Figures 1a and b and Supplementary Table S2). Uracil was used as a nucleic acid base instead of thymine in the DNA of phage S6 (Supplementary Figure S1). Tests of the host range using a published procedure (Takemura-Uchiyama *et al.*, 2013) demonstrated that phage S6 exhibited lysis-from-without and/or plaque-forming activities in all of the animal-derived and human-derived *Staphylococcus* spp. we tested, including *S. aureus*, *S. epidermidis*, *S. felis*, *S. arlettae*, *S. kloosii*, *S. pettenkoferi*, *S. schleiferi*, *S. sciuri* and *S. pseudintermedius* (Supplementary Table S1 and Supplementary Figure S2).

Phage S6 was found to be the largest of the known staphylococcal phages, and it cannot be classified into any known staphylococcal phage taxonomy. A search for phages similar to phage S6 showed that *Bacillus* phage PBS1 shared similar morphology and DNA chemistry (Hemphill and Whiteley, 1975). Phage PBS1 has the capacity to transfer large partial genomic fragments among *Bacillus* spp. without bias (Hemphill and Whiteley, 1975; Vettori *et al.*, 1999). Thus, we also examined transduction in staphylococci using phage S6.

Generalized transduction experiments were conducted as follows. After the electroporation of

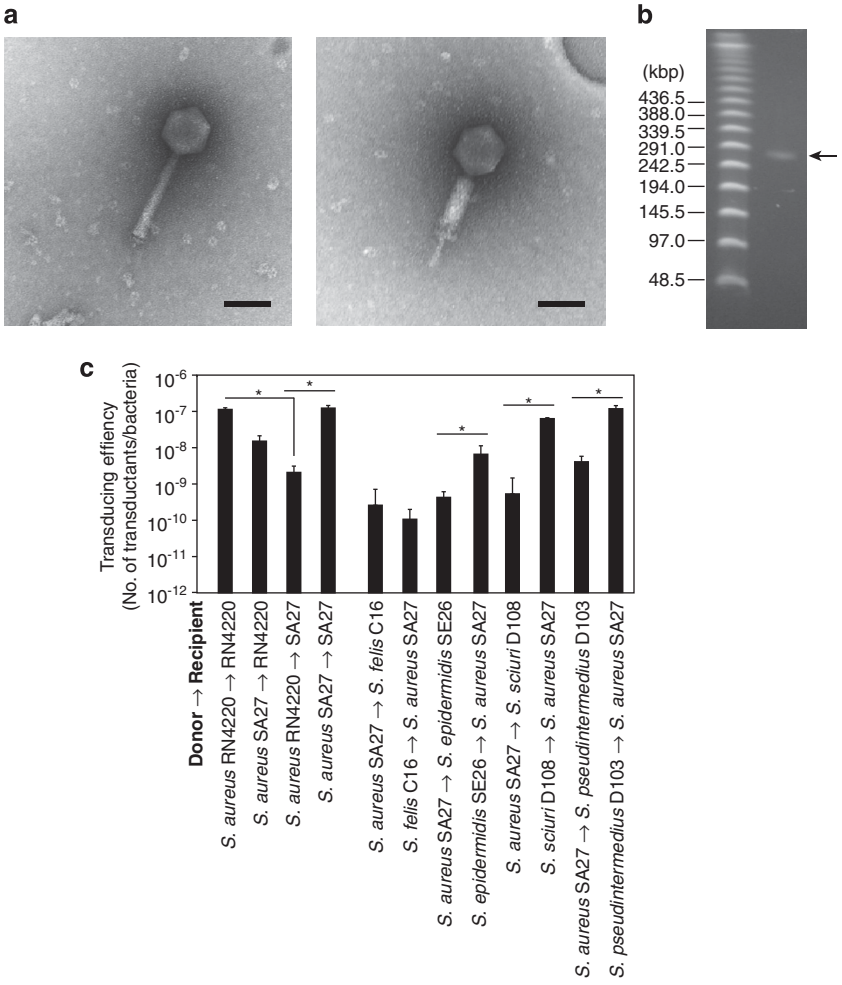

Figure 1 Characteristics of phage S6. (**a**) Morphology. Images of phage S6 with an extended tail and a contractile tail are shown on the left and right, respectively. The bars represent 100 nm. The head diameter and the extended tail length were 118.1 ± 8.5 nm and 236.9 ± 6.6 nm (mean ± s.d., $n = 6$), respectively. (**b**) Genome size estimation by pulsed-field gel electrophoresis. The Lambda Ladder PFG Marker (New England Biolabs, Ipswich, MA, USA) (left) and phage S6 DNA (right) are shown on the electrophoregram. The sizes of the molecular standards are shown at the most left of the electrophoregram. The arrow indicates the location of phage S6 DNA. (**c**) Experimental intragenus and intraspecies generalized transduction in *Staphylococcus* spp. The graph not only shows the efficiencies of transduction mediated by phage S6 from *S. aureus* to *S. aureus,* but also from *S. aureus* to non-*aureus Staphylococcus* spp., and vice versa. The bacteria were animal-derived *Staphylococcus* spp., including *S. felis* strain C16, *S. sciuri* strain D108 and *S. pseudintermedius* strain D103, and human-derived *Staphylococcus* spp., including *S. aureus* strains SA27 and RN4220 and *S. epidermidis* strain SE26. The means and s.d. are shown in the graph (mean ± s.d.; $n = 3$). Statistical differences are indicated by an asterisk (Student's *t*-test, $P < 0.05$).

pCU1 and selection using chloramphenicol ($20\,\mu g\,ml^{-1}$; Augustin *et al.*, 1992), bacteria harboring the plasmid pCU1 were used as donor hosts. The presence of pCU1 in the donor host was confirmed by colony-direct PCR using the primers listed in Supplementary Table S3. The phages were propagated with a suitable donor host bacterium in appropriate culture conditions in the presence of chloramphenicol ($20\,\mu g\,ml^{-1}$; Supplementary Table S2 and Supplementary Figure S3). As a negative control, the phage was propagated on bacteria that did not harbor pCU1 without chloramphenicol. In the transduction experiment using the methicillin-resistant gene, the phage was propagated with MRSA strain COL. After filtration of the phage lysate using a 0.45-μm membrane filter and treatment with DNase I ($10\,\mu g\,ml^{-1}$; 30 min at 37 °C) in a medium containing 5 mM $MgCl_2$, the recipient bacteria (ca. 6.4×10^8 cells ml^{-1}) were cultured with phages at a multiplicity of infection of 1 in 1 ml or 10 ml of tryptic soy broth (30 min, 37 °C). The cultures were plated onto brain–heart infusion plates that contained an appropriate antibiotic (that is, $20\,\mu g\,ml^{-1}$ chloramphenicol or $5\,\mu g\,ml^{-1}$ oxacillin). To validate the transduction experiment, colony-direct PCR was conducted using 10 colonies from each group with the primers listed in Supplementary Table S3. The transduction efficiency was calculated as the ratio of the number of transductants relative to the number of bacteria before inoculation on the antibiotic-containing plate.

Transduction by phage S6 was examined in *S. aureus* and from *S. aureus* to non-*aureus* staphylococci, as well as vice versa, which demonstrated the transfer of the pCU1 plasmid from *S. aureus* to *S. aureus*, *S. epidermidis*, *S. felis*, *S. sciuri* and *S. pseudintermedius,* and vice versa (10^{-7}–10^{-10} transductants per bacteria) (Figure 1c). On the other hand, the phages propagated with bacteria that did not harbor plasmid pCU1 produced no transductants. The efficiencies of transduction from the non-*aureus* staphylococci (*S. epidermidis*, *S. sciuri* and *S. pseudintermedius*) to *S. aureus* were significantly higher than those from *S. aureus* to the non-*aureus* staphylococci.

Finally, phage S6, which was prepared in MRSA strain COL as a donor host, was transduced into strain RN4220. The transduction of methicillin resistance was also successful at $5.2 \times 10^{-11} \pm 9.0 \times 10^{-11}$ transductants per bacteria (mean ± s.d.; $n = 3$).

No previous studies have reported phage infectivity among various staphylococcal species and generalized transduction. The transduction activities of other phages are also of interest in *staphylococci*. Thus, we examined the generalized transduction activity and host range using three types of staphylococcal phages: siphophages ϕMR25 and 80, podophage S13′ and myophage S25-3 (Christie *et al.*, 2010; Hoshiba *et al.*, 2010; Takemura-Uchiyama *et al.*, 2013) (Supplementary Table S2). Phages 80 and ϕMR25 were *S. aureus*-specific phages that exhibited transduction activities in *S. aureus* (Supplementary Table S1 and Supplementary Figure S4). Phages S13′ and S25-3 were *S. aureus*-specific and polyvalent phages, respectively, but they had no transduction activities in *S. aureus* (Supplementary Table S1 and Supplementary Figure S4). Thus, in contrast to phage S6, none of the phages we tested exhibited simultaneous transduction activities and infectivity in staphylococci.

This is the first report of intragenus generalized transduction in *Staphylococcus* spp. that we conducted using a novel type of staphylococcal phage. The methicillin-resistant gene in MRSA is considered to have originated from animal-associated staphylococci (Tsubakishita *et al.*, 2010; Moellering, 2011), so giant myophages such as S6 could contribute to the emergence of new types of MRSA in various environments. Moreover, if RNA is the precursor to life and phages that contain uracil-based DNAs are relics of the RNA world, giant myophages such as phages S6, PBS1 and phiR1-37, may have had important roles in the evolution of bacteria from the last universal common ancestor (Forterre, 2005; Kiljunen *et al.*, 2005). In the future, the experimental evolution of *S. aureus* into MRSA will be investigated using phage S6 to elucidate the origin and emergence of MRSA.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

We thank Dr Glenn W Kaatz, John D Dingell, VA Medical Center, USA, Professor Motoyuki Sugai, Hiroshima University, Hiroshima, Japan and Professor Asako Muraoka, Kochi Junior College, Kochi, Japan, for providing us the plasmid and some bacterial and phage strains. This study was funded by a Grant-in-Aid for Young Scientists (B) (24791025), and the Center for Innovative and Translational Medicine, Kochi System Glycobiology Center, Kochi University, Kochi, Japan.

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