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Characterization and complete genome sequence analysis of *Staphylococcus aureus* bacteriophage SA12

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Abstract *Staphylococcus aureus* is a well-known pathogen that causes several serious diseases in humans and animals. As part of the efforts to control this pathogen, a newly isolated bacteriophage, SA12, which specifically targets *S. aureus,* was characterized, and its genome was completely sequenced. Host range and bacteriophage challenge tests demonstrated its specific and efficient host lysis of *S. aureus*. The genome of phage SA12 consists of 42,902 bp length double-stranded DNA with 58 predicted ORFs-encoding phage structure, DNA manipulation, packaging, host lysis, and regulation proteins. The characterization and genome study of phage SA12 in this report is useful for understanding *S. aureus*-targeting bacteriophages and provides basic information for the further development of phage-based biocontrol agents against *S. aureus*.

Keywords *Staphylococcus aureus* · Bacteriophage · *Siphoviridae* · Host lysis · Genome

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Staphylococcus aureus is one of the important medical pathogens causing postsurgical infections and serious diseases such as abscess, endocarditis, scalded skin, and toxic syndromes in humans and animals [1]. In addition, it is often considered as a food-poisoning pathogen due to the production of several toxins such as toxic shock syndrome toxin-1 (TSST-1) and edema factor (EF), as well as alpha, beta, and delta toxins [2]. It is estimated that approximately 500,000 patients are hospitalized in the United States every year due to the pathogenicity of *S. aureus* [3]. To control this pathogen, antibiotics have been widely used for many years, and antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA), have emerged [4]. To overcome this problem, alternative biocontrol approaches using *S. aureus*-targeting bacteriophages have recently been considered because of their specific infection and host lysis [5–8]. In this study, a novel *S. aureus*-targeting bacteriophage, SA12, was isolated and characterized for application in the specific host lysis of *S. aureus*. Furthermore, the genome of phage SA12 was completely sequenced and analyzed to understand the infection and interaction mechanisms between the phage and the *S. aureus* host.

The novel *S. aureus*-targeting bacteriophage SA12 was isolated from a calf fecal sample (see the Supplementary

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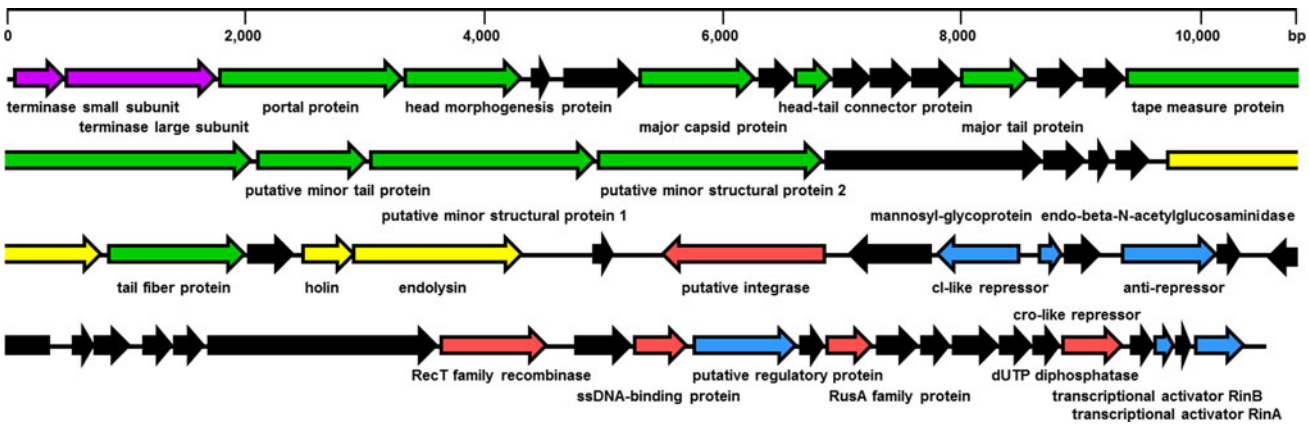

Fig. 1 Genome map of *S. aureus* bacteriophage SA12. The direction of *arrows* indicates the direction of each gene. The color of each gene refers to the functional groups such as structure (*green*), DNA manipulation (*red*), packaging (*purple*), host lysis (*yellow*), and regulation (*blue*)

Materials and Methods). Morphological observation of bacteriophage SA12 revealed that it has a 175 ± 8 nm noncontractile tail and a 55 ± 2 nm capsid, indicating that it belongs to the *Siphoviridae* family (Fig. S1). Host range test of phage SA12 indicated that it inhibits the growth of a limited number of *S. aureus* strains, including MRSA, and one *S. epidermidis* strain, suggesting that the host specificity of this phage is relatively high (Table S1). Previously, the host receptor of some *S. aureus*-targeting phages was identified as peptidoglycan-anchored wall teichoic acid (WTA) [9]. To confirm the host receptor of phage SA12, a previously constructed *ΔtagO* mutant, *S. aureus* RN4220*ΔtagO*, and its complemented strain, *S. aureus* c*tagO*, via the expression of *tagO* using pRB474-*tagO* were tested with phage SA12 [10]. As shown in Figure S2 and Table S1, the *ΔtagO* mutant (RN4220*ΔtagO*) presented high resistance to phage SA12. However, its complemented strain, which expressed the *tagO* gene, displayed recovery of sensitivity to phage SA12, suggesting that the host receptor of phage SA12 is peptidoglycan-anchored WTA. To investigate its host lysis activity, a bacterial challenge assay was conducted (Fig. S3). This assay demonstrated that the phage could completely inhibit *S. aureus* ATCC 29213 up to 9 h, indicating that the host lysis activity of phage SA12 is very high. To further understand its host lysis and host interaction mechanisms at the genomic level, the phage SA12 genome was completely sequenced and analyzed.

The complete genome sequence analysis of phage SA12 revealed that the DNA genome was 42,902 bp in length (GC content of 34.54 %) with 58 predicted ORFs and no tRNA (Fig. 1). The average length of the ORFs is 686 bp, and the gene coding percentage is 92.7 %. Annotation and functional analysis of the predicted ORFs revealed five functional groups: structure (portal protein, major capsid protein, head morphogenesis protein, head–tail connector protein, major and minor tail proteins, tape measure protein, minor structure proteins, and tail fiber protein), DNA manipulation (recombinase, ssDNA-binding protein, endodeoxyribonuclease, dUTP diphosphatase, and integrase), regulation (transcriptional activators RinAB, CI-like and Cro-like repressors, and anti-repressor), host lysis (endo-*β*-*N*-acetylglucosaminidase, holin, and endolysin), and packaging (terminase small/large subunits) (Table 1).

The structural genes encode head proteins, tail proteins, and head–tail connection proteins, indicating that this phage genome encodes most proteins required for the phage assembly. While head proteins such as major capsid protein (SA12_029) and head morphogenesis protein (SA12_026) may be functionally similar, they contain different conserved protein domains, phage_capsid (PF05065) and phage_Mu_F (PF04233), respectively. The phage_Mu_F domain was first identified in *Bacillus subtilis* phage SPP1, and may be involved in phage head morphogenesis as a minor capsid protein [11]. Therefore, these two genes most likely work together for phage head construction. The annotation data revealed that the tail gene cluster of this phage (SA12_035-047) encodes major and minor tail proteins (SA12_035 and SA12_039, respectively) as well as tail fiber protein (SA12_047), supported by minor structural proteins (SA12_040-041) and tape measure protein (SA12_038), indicating complete composition of phage tail proteins. While the major tail protein has a typical tail protein domain, phgtail_TP901-1 (PF06199) from *Lactococcus lactis* phage TP901-1, the minor tail protein has a Sipho_tail protein domain (PF05709), partially supporting the idea that this phage belongs to the *Siphoviridae* family. Interestingly, endo-*β*-acetylglucosaminidase or tail-associated cell wall hydrolase (SA12_046) may be a component of the phage tail, which possesses putative lysis activity of host peptidoglycan (PF01832), although, the protein is classified into the host lysis category in Table 1. Tail fiber protein

Table 1 Functional categories of predicted ORFs in bacteriophage SA12

Group	Subgroup	Locus_tag[a]	Function	BLASTP best match	Identity (%)[b]	Accession No.[c]
Structure	Head	SA12_029	Major capsid protein	Phage major capsid protein [*Staphylococcus aureus* subsp. *aureus* IS-125]	323/324 (99)	ZP_13425340.1
		SA12_026	Head morphogenesis protein	Phage head morphogenesis protein [*Staphylococcus aureus* subsp. *aureus* JH9]	329/331 (99)	YP_001246266.1
	Tail	SA12_035	Major tail protein	Phage major tail protein, TP901-1 family [*Staphylococcus aureus* subsp. *aureus* CIG1150]	192/193 (99)	ZP_13440311.1
		SA12_038	Tape measure protein	Tape measure protein [*Staphylococcus* phage phiMR25]	1133/1154 (99)	YP_001949854.1
		SA12_039	Putative minor tail protein	Putative minor tail protein [*Staphylococcus* phage TEM123]	309/315 (98)	YP_006382275.1
		SA12_040	Putative minor structural protein 1	Phage minor structural protein, N-terminal domain protein [*Staphylococcus aureus* subsp. *aureus* 21201]	614/633 (97)	ZP_12538290.1
		SA12_041	Putative minor structural protein 2	Putative minor structural protein [*Staphylococcus aureus* subsp. *aureus* CIG1605]	635/636 (100)	ZP_13146026.1
		SA12_047	Tail fiber protein	Phage tail fiber [*Staphylococcus aureus* subsp. *aureus* CGS00]	390/390 (100)	ZP_11449784.1
	Head–tail	SA12_025	Portal protein	SPP1 family phage portal protein [*Staphylococcus aureus* M0329]	512/512 (100)	ENJ65982.1
		SA12_031	Head–tail connector protein	Phage gp6-like head–tail connector family protein [*Staphylococcus aureus* subsp. *aureus* CIG1057]	109/110 (99)	ZP_13448453.1
DNA manipulation		SA12_007	RecT family recombinase	Phage RecT family recombinase [*Staphylococcus aureus* M0108]	305/306 (99)	ENI85810.1
		SA12_009	Single-stranded DNA-binding protein	Single-stranded DNA-binding protein [*Staphylococcus aureus* A8796]	156/156 (100)	ZP_06930763.1
		SA12_012	Endodeoxyribonuclease RusA family protein	Crossover junction endodeoxyribonuclease RusA [*Staphylococcus aureus* subsp. *aureus* IS-3]	134/134 (100)	ZP_13201566.1
		SA12_018	dUTP diphosphatase	dUTP diphosphatase [*Staphylococcus aureus* subsp. *aureus* IS-3]	177/178 (99)	ZP_13201575.1
		SA12_052	Putative integrase	No significant similarity found.		
Packaging		SA12_023	Terminase small subunit	Phage terminase small subunit [*Staphylococcus aureus* subsp. *aureus* D139]	146/146 (100)	ZP_06324936.1
		SA12_024	Terminase large subunit	PBSX family phage terminase, large subunit [*Staphylococcus aureus* A9719]	425/425 (100)	ZP_05683726.1
Host lysis		SA12_046	Endo-beta-*N*-acetylglucosaminidase	Mannosyl-glycoprotein endo-beta-*N*-acetylglucosaminidase [*Staphylococcus aureus* subsp. *aureus* DSM 20231]	630/632 (99)	ZP_20913974.1
		SA12_049	Holin	Holin, phage phi LC3 family protein [*Staphylococcus aureus* subsp. *aureus* CGS00]	145/145 (100)	ZP_11449782.1
		SA12_050	Endolysin	Endolysin [*Staphylococcus* phage phiSauS-IPLA88]	472/481 (98)	YP_002332536.1
Regulation		SA12_010	Putative regulatory protein	Phage regulatory protein [*Staphylococcus aureus* A8796]	294/295 (99)	ZP_06930762.1
		SA12_022	Transcriptional activator RinA	Phage transcriptional regulator, RinA family protein [*Staphylococcus aureus* subsp. *aureus* CIG2018]	139/140 (99)	ZP_13479235.1
		SA12_020	Transcriptional activator RinB	Transcriptional activator RinB [*Staphylococcus aureus* A9781]	57/57 (100)	ZP_05642402.1
		SA12_054	CI-like repressor	cI-like repressor [*Staphylococcus* phage 11]	238/239 (99)	NP_803258.1
		SA12_055	Cro-like repressor	Cro-like repressor [*Staphylococcus* phage 11]	72/72 (100)	NP_803259.1
		SA12_057	Anti-repressor	Anti-repressor [*Staphylococcus* phage 11]	261/263 (99)	NP_803260.1

[a] Each predicted ORFs of phage SA12 was labeled with locus_tag

[b] Identities were calculated between amino acid sequences

[c] GenBank accession numbers

(SA12_047) is generally involved in the host specificity by interaction with host receptor [12]. The receptor study of phage SA12 demonstrated that the WTA of *S. aureus* was a specific target of phage SA12, suggesting that the tail fiber of phage SA12 most likely interacts with the WTA of *S. aureus* (Fig. S2 and Table S1). Interestingly, this protein contains a collagen protein domain (PF01391) and is highly conserved, similar to the other tail fiber proteins in *S. aureus* phages [13], suggesting that the structure of the tail fiber protein in *S. aureus* phages may be similar to the collagen helix structure. To complete phage assembly, the phage head and tail should be linked to each other. The portal protein (SA12_025) and head–tail connector protein (SA12_031) may play roles in this linkage between the head and the tail [14]. These linkage proteins are highly similar to those of *B. subtilis* phage SPP1, suggesting that the phage minor head and head–tail connector proteins may be derived from a common origin.

The DNA manipulation and packaging category proteins are functionally related to phage genome replication and its packaging. RecT family recombinase (SA12_007), ssDNA-binding protein (SA12_009), integrase (SA12_052), and endodeoxyribonuclease RusA (SA12_012) may be involved in phage genome replication. After replication of phage genomic DNA, terminase large and small subunit proteins (SA12_023-024) may precisely cut phage genomic DNA for its packaging into the phage head [15].

The host lysis gene cluster in phage SA12 encodes endolysin (SA12_050), holin (SA12_049), and endo-β-*N*-acetylglucosaminidase (SA12_046). An InterProScan analysis of the endolysin revealed three protein domains: the CHAP (PF05257) [16], amidase_2 (PF01510), and bacterial SH3 domains (PF08460) [17]. Though the CHAP and amidase domains are related to peptidase or amidase activity for host lysis, interestingly, the bacterial SH3 domain may be involved in host cell wall binding, most likely the specific localization of endolysin before host lysis. For host lysis just before phage burst-out, holin generally creates holes in the host inner membrane to expose the peptidoglycan layer to endolysin. This holin contains a protein domain, the phage_holin_1 domain (PF04531) containing a transmembrane region. This transmembrane region may be involved in the transfer of this holin to the inner membrane before creating holes [18]. As previously described, whereas endo-β-*N*-acetylglucosaminidase (or tail-associated cell wall hydrolase) may be one of the components for phage tail, the protein belongs to the host lysis category due to its host lysis activity.

In the regulation category, putative regulatory proteins may be involved in local control of gene expression for DNA manipulation. The phage genome has two transcriptional activators, RinA and RinB. These proteins may be related to the control of packaging and structure reconstruction [19]. Interestingly, this phage genome contains CI-like and Cro-like repressors as well as an anti-repressor [20]. These proteins are known to be generally involved in determination of the lytic/lysogenic cycle [21]. However, a few genes encoding CII and other termination factors, such as N and Q, are missing in this lytic/lysogenic cycle determination gene cluster, most likely due to insufficient information on *S. aureus*-targeting phage genomes in the sequence databases. Although, SA12 phage displayed host lysis activity, as described above, additional induction experiments with SA12 phage-resistant host cells (collected at 12 h after SA12 phage infection for challenge assay in Fig. S3) using mitomycin C revealed that a portion of the SA12 phage-resistant host cells contain lysogens (data not shown), suggesting that the SA12 phage is a temperate phage. The Phage Classification Tool Set (PHACTS) analysis of SA12 phage indicated that it is a temperate phage (data not shown) [22].

The isolation and characterization of the novel *S. aureus*-phage SA12 revealed specific infection against only *S. aureus* via WTA as a host receptor and via host lysis activity. Subsequent genome sequence analysis of this phage also provided further characteristic insights into its structure, DNA manipulation, packaging, host lysis, and regulation. The results of this study may be very useful for understanding *S. aureus*-targeting bacteriophages and may provide basic information for the further development of phage-based biocontrol agents effective against *S. aureus*.

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