The saclayvirus Aci01-1 very long and complex fiber and its receptor at the Acinetobacter baumannii surface

Christine Pourcel (christine.pourcel@u-psud.fr)
Universite Paris-Saclay
https://orcid.org/0000-0002-8951-466X

Malika Ouldali
Universite Paris-Saclay

Paulo Tavares
Universite Paris-Saclay

Christiane Essoh
Universite Peleforo Gon Coulibaly

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The saclayvirus Aci01-1 very long and complex fiber and its receptor at the Acinetobacter baumannii surface

Christine Pourcel#, Malika Ouldali², Paulo Tavares³, Christiane Essoh⁴

¹ Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France

⁵ Department of Biochemistry-Genetic, School of Biological Sciences, Université Peleforo Gon Coulibaly, Korhogo, Côte d'Ivoire

# Address correspondence to: Christine Pourcel

Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France

0000-0002-8951-466X
Abstract

The *Acinetobacter baumannii* bacteriophage Aci01-1 that belongs to the genus *Saclayvirus* of the Caudoviricetes order has an icosahedral head and a contractile rigid tail. We report that Aci01-1 has, attached to the tail conical tip, a remarkable 146 nm-long flexible fiber with seven beads and a terminal knot. Its putative gene coding for a 241.36 kDa tail fiber protein is homologous to genes in Aci01-1-related and unrelated phages. We also identified a putative receptor of the phage on the bacteria capsule that is hypothesized to interact with the Aci01-1 long fiber.

Introduction

*Acinetobacter baumannii* is an opportunistic pathogen that is becoming a major human health threat, namely in hospital settings (nosocomial) infections, due to the development of antibiotic resistant strains [1]. This led the World Health Organization to assign critical priority for development of anti-bacterial strategies to control those infections [2]. Bacterial viruses that kill bacterial cells with high specificity may become central actors to diversify the panel of strategies for effective anti-bacterial treatments [3,4]. Our research aims at characterizing bacteriophages infecting *A. baumannii* and the interactions between these phages as part of the effort to deliver agents for controlling nosocomial infections by this human pathogen.

We described previously three virulent phages of the genus *Saclayvirus*, Aci01-1, Aci02-2 and Aci05 that infect *A. baumannii* strain Ab09 [5]. These phages have closely related genomes of 103kbp, 104kbp, and 103kbp respectively. Here we purified and imaged by electron microscopy (EM) virions of Aci01-1 and Aci01-5. Their most notable feature is the very long and complex tail fiber. We have also isolated bacteria resistant to Aci01-1 infection revealing that the bacterial capsule plays a role in *A. baumannii* sensitivity to Aci01-1.

Bacteriophages Aci01-1 and Aci05 were isolated in Abidjan, Côte d’Ivoire and their genome sequence was previously reported [5]. *A. baumannii* strain Ab09 was isolated from a clinical sample in France [6]. Phage amplification was performed on solid agar LB medium using $10^6$ plaque forming units (PFU) mixed to $10^9$ *A. baumannii* Ab09. After a 6-8 h incubation period at 37°C, phage particles were recovered in 4 mL phosphate buffer saline (PBS), reaching titers of $10^{10}$-10^{11} PFU/mL. The suspension was treated with DNase I (50 µg/mL) and RNase A (10 µg/mL) for 1 h at 37°C and phage particles were precipitated with 6% PEG 8000 overnight at 4°C. After
centrifugation, the pellet was resuspended in phage TBT buffer (Tris-Cl 10 mM, pH 7.5, NaCl 100 mM, MgCl₂ 10 mM). Three chloroform extractions were performed, the supernatant was filtrated through a 0.20 µm filter, and phage particles were pelleted by ultracentrifugation at 154,000 g for 2 h at 4°C. Phage particles were then loaded onto a preformed caesium chloride (CsCl) step gradient centrifuged at 287,000 g for 3 h at 20 °C [7]. A band containing the phages was recovered by puncturing the tube with a needle and the suspension was dialyzed against TBT buffer. To perform electron microscopy (EM) observations, ten microliters of CsCl-purified phage particles were adsorbed onto a grid coated with carbon film (EMS, Hatfield, PA, US), then washed with distilled water followed by negative staining with 1 % uranyl acetate (Sigma, St Louis, MO, US) before imaging [8].

*A. baumannii* variants resisting phage infection were obtained following infection on solid medium at an input multiplicity of 0.01 Aci01-1 phages/bacterium for 48 h at 37°C. Colonies of different sizes and shapes were picked and purified by two rounds of colony isolation before testing for susceptibility to Aci01-1 infection.

For DNA purification *A. baumannii* bacteria were lysed in lysis buffer (Tris 10 mM, pH 7.8, EDTA 10 mM, NaCl 10 mM, SDS 0.5%), treated with proteinase K at 50 µg/mL for 2 h at 50°C, followed by one phenol and one chloroform extraction. DNA was precipitated with ethanol and resuspended in TE buffer (Tris 10 mM, pH 7.8, EDTA 1 mM). DNA was sequenced in an Illumina MiSeq 300-bp paired-end run with a 900-bp insert library, producing 1.4 to 3.9 million reads. Quality-control was performed with FastQC version 0.11.5, and reads were trimmed under GeneiousR11 (Biomatters Ltd, Auckland, New Zealand) with default trim parameters. To identify mutants in phage resistant variants we compared the genomes of the parental strain to those of the variants using 2-kupl as described [9].

To predict the function of hypothetical proteins, the following programs were used for protein sequence homology and structural elements characterization:

- PsiPred [https://bio.tools/psipred](https://bio.tools/psipred) at ELIXIR
- REPPER [https://toolkit.tuebingen.mpg.de/tools/repper](https://toolkit.tuebingen.mpg.de/tools/repper) at MPI Bioinformatics Toolkit
- RADAR [https://www.ebi.ac.uk/Tools/pfa/radar/](https://www.ebi.ac.uk/Tools/pfa/radar/) at ebi
EM observation of CsCl-purified Aci01-1 phage particles showed that they are myoviruses (morphotype) composed of an icosahedral head of 80 ± 3 nm in diameter and a 128 ± 2 nm contractile tail (Fig. 1a). These are undistinguishable from Aci05 phage particles (not shown). Particles with extended and contracted tails were present in the Aci01-1 preparation (“E” and “C” in Fig. 1a, respectively). In the contracted state, the tail external shaft had a reduced length and the internal tail tube became visible. Phage tails featured a 146±3 nm-long flexible fiber attached to the conical tail tip distal from the head. Seven bead-like structural elements (arrowheads in Fig. 1b) were precisely positioned along the fiber that ended with an elongated complex structure (bracket in Fig. 1b). This fiber was perfectly visible in highly purified phage particles but more difficult to detect in phage particles of crude or partially purified phage preparations. Phages lambda [13], T5 [14] or SPP1 [14] have also an axial tail fiber anchored in the conical tip of the tail tube but the Aci01-1 fiber is much longer and the structure is more complex.

We then searched for putative genes in the Aci01-1 genome (ID NC_048074) that could code for fiber proteins. A very long gene encoding a 2211 amino acids (aa) protein (Aci01-1_031) was identified within the structural proteins genes of Aci01-1. Equally long proteins are encoded by phage Aci05 (genome ID NC_048080) (2223 aa-long; Aci05_030) and other Acinetobacter phages. Using different bioinformatics tools, proteins that are more distant exhibit a modular organization alternating regions with and without homology to the Aci01-1 protein. These are all annotated as putative tail fiber or tail proteins. The Aci01-1 putative tail fiber protein has 34-35 aa repeats as identified using the Rapid Automatic Detection and Alignment of Repeats in protein sequences (RADAR) tool at EBI (Fig. 2a) [15]. These repeats are predicted to form coiled-coils separated by ~500aa as shown with REPeats and PERiodicity (REPPER) (Fig. 2b) [16]. Prediction of the 3D structure and comparison with known protein profiles using Phyre2 showed that the first 1186 amino acids (54% of the sequence) have strong similarity with dynein outer arm proteins. These very large proteins are component of cilia of different organisms playing a major role in the beating [17,18]. In three A. baumannii phages closely related to Aci01-1, vB_AbaM_phiAbaA1 (NC_031280), vB_AbaM_P1 (OL960030) and Abp53 (JF317274) the homologous proteins are 3259aa-, 2902aa- and 1176aa-long, respectively. Lee et al. [19] showed that phage Abp53 protein ORF1176 (AEQ18751) has similarities with the tail protein gp21 (3433aa) of Klebsiella oxytoca siphovirus phiKO2 (NC_005857), a phage that is otherwise very different from Abp53. Interestingly, by inspecting electron micrographs of phiKO2 virions we could find a long fiber, with bead-like elements, attached to the conical tail tip [20]. Similar proteins with size ranging from 1676 to
3702aa can be found in the genome of different lytic or temperate phages. These include the 1300aa-long protein J of phage lambda tail fiber that contacts the receptor LamB on the *Escherichia coli* cell surface [21,22].

The original features of the Aci01-1 tail fiber prompted us to investigate its bacterial receptor. In order to identify *A. baumannii* genes involved in susceptibility to phage infection, we recovered Ab09 colonies surviving infection by Aci01-1. In three out of eight phage-resistant clones, lysis was observed in dense growth zones on LB agar plates suggesting the presence of the Aci01-1 phage genome in those Aci01-1-resistant bacteria (carrier state). We performed whole genome sequencing of one resistant strain without virus, of one resistant carrier strain and of the Ab09 parental culture used to select for resistant strains. In the non-carrier strain a single point mutation resulting in a threonine to methionine amino acid substitution was found in *gna*, one of the K capsule biosynthesis genes involved in D-GalpNAcA synthesis (equivalent to WpbO in *P. aeruginosa*). The capsule biosynthesis gene cluster of *A. baumannii* Ab09 assembled from reads mapped onto the reference strain ACICU (Genbank CP031380), was shown to be closest to that of strain NIPH KL30 (Genbank MN166189). In the carrier resistant strain no mutation could be detected in the bacterial genome but we found large amounts of non-integrated viral DNA that could be assembled into a complete Aci01-1 103kb genome sequence. This suggested that resistance resulted from episomal maintenance of the Aci01-1 phage DNA in the bacterium, as previously observed with pseudo-lysogens of *P. aeruginosa* [23,24].

We report here that the myovirus Aci01-1 displays a remarkably long and complex fiber fixed to the tail tube conical tip distal from the head, a structure that has not been described so far. Very long fibers were found in flagellotrophic phages to curl around the flagella of the host, such as in *Salmonella* chi [25] but their structure is different from that of the saclaviruses. *A. baumannii* lacks flagella but it can express very long cell extensions such as the fimbrial CsuA/BABCDE dependent pili that play an essential role in adhesion and biofilm formation on abiotic surfaces [26,27]. Sequencing of bacterial strains resisting infection by phage Aci01-1 identified a point mutation on an enzyme involved in biosynthesis of the polysaccharide capsule surrounding the *A. baumannii* surface [28], suggesting that this structure is the receptor for phage Aci01-1. The capsule was previously reported to be the receptor of two other *A. baumannii* phages phiFG02 and phiC001 [29]. It is possible that the long fiber helps the phage to reach its capsular receptor through the thick extracellular material in biofilms and to hydrolyze the capsule polysaccharide, digging the way for the tail tip to reach the bacterial membranes.
Interestingly, we found that several Ab09 phage-resistant variants did not bear any chromosomal mutation but that they were carriers of phage DNA. A similar observation was made for resistant strains to bacteriophages of *P. aeruginosa* [23,24], *Staphylococcus aureus* [30] and *Bacteroides intestinalis* [31]. This extra-chromosomal persistence mechanism of the genome from lytic phages was named pseudo-lysogeny, having been originally described for starved cells carrying lytic phage genomes [32,33]. Our findings suggest that this mechanism may be even more widely represented in phage/bacteria interactions and not limited to stationary phase bacteria. It illustrates the complex relationship between virulent phages and bacterial populations [34,35].

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References


Statements and declarations

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Christine Pourcel, Malika Ouldali, Paulo Tavares and Christiane Essoh. The first draft of the manuscript was written by Christine Pourcel and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The authors have no relevant financial or non-financial interests to disclose.
Figure Legends

**Fig. 1** EM of CsCl-purified Aci01-1 phage particles negatively stained a) Virions with extended (E) or contracted (C) tail shaft. b) Electron micrograph showing the conical tip and the long tail fiber of Aci01-1 with seven bead-like structures (arrowheads) and a complex elongated tip (bracket). The scale bar is 50nm.

**Fig. 2** Analysis of the Aci01-1_031 protein structure a) detection by RADAR of repeats showing first and last residue, alignment scores as described [36] and repeat sequences b) REPPER secondary structure prediction using PSIPRED [37] and PCOILS [38].
Figure 2

(a) Position and score  Sequence

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(b) PSIPRED  PCOILS