

# Bacteriophage Sf6 host range mutant that infects *Shigella flexneri* serotype 2a<sub>2</sub> strains

Min Yan Teh<sup>1</sup>, Elizabeth Ngoc Hoa Tran<sup>1</sup> and Renato Morona<sup>1\*</sup>

School of Biological Sciences, Department of Molecular and Biomedical Science, Research Centre for Infectious Diseases, University of Adelaide, Adelaide 5005, Australia

\*Corresponding author: Molecular Life Science Building, Room 4.43, University of Adelaide, Gate 8 off Victoria Drive, Adelaide, South Australia 5005, Australia. Tel: +61 8 8313 4151; Fax: +61 8 8313 7532; E-mail: [renato.morona@adelaide.edu.au](mailto:renato.morona@adelaide.edu.au)

**One sentence summary:** Isolation of bacteriophage Sf6c mutant that infects *Shigella flexneri* serotype 2a<sub>2</sub> strain, the most prevalent serotype that causes shigellosis.

**Editor:** Klaus Hantke

## Abstract

*Shigella flexneri* serotype 2a<sub>2</sub> (II:9;10) is the most prevalent strain in causing bacillary dysentery in developing countries. Chemical modifications such as glucosylation, O-acetylation, and phosphoethanolamine modifications of lipopolysaccharide (LPS) O antigen (Oag) contribute to the emergence of various serotypes. Sf6 is a *Shigella*-specific bacteriophage that infects only a limited range of *S. flexneri* serotypes [X, Y]. LPS Oag is the primary receptor for bacteriophage Sf6 where it uses its tailspike protein (TSP) in binding and hydrolysing LPS Oags. Sf6TSP has recently been shown to be capable of hydrolysing the LPS Oag of Type II strains, albeit modestly. Phage therapy has regained attention in recent years as an alternative therapeutic approach. Therefore, this study aimed to expand the host range of Sf6 to the prevalent *S. flexneri* serotype 2a<sub>2</sub> strain. We discovered a new lytic Sf6 host range mutant that is capable of infecting *S. flexneri* serotype 2a<sub>2</sub> and identified residues in Sf6TSP that may potentially be involved in binding and hydrolysing serotype 2a<sub>2</sub> LPS Oag. This work increased the limited *Shigella*-specific bacteriophage collection and may be useful in the future for phage therapy and/or biocontrolling of *S. flexneri* in contaminated food and water.

**Keywords:** bacteriophage, Sf6, *Shigella flexneri*, serotype 2a, host range mutant, tailspike protein

## Introduction

*Shigella flexneri* is one of the most dominant strains in causing shigellosis in developing countries (Livio et al. 2014). Serotype 2a (29%), 3a (14%), and 1a (9%) are the three most prevalent serotypes in Asian countries (von Seidlein et al. 2006, Ye et al. 2010). Shigellosis is a global burden that has an annual morbidity rate of ~269 million cases and ~210 000 of mortality cases (Khalil et al. 2018). World Health Organisation has listed *Shigella* as a priority organism as it possesses great threat to human health (Tacconelli et al. 2018).

All *S. flexneri* serotypes except serotype 6 share the same O antigen (Oag) polysaccharide backbone that is composed of one N-acetylglucosamine (GlcNAc) residue and three L-rhamnose (Rha<sup>I</sup>-Rha<sup>II</sup>-Rha<sup>III</sup>) residues (Kenne et al. 1977). This forms the Oag polysaccharide of serotype Y. Various chemical modifications such as glucosylation, O-acetylation and phosphoethanolamine modification on different sugars increase Oag diversity and serotypes. *Shigella flexneri* serotypes are defined by a series of type and group O-factors (Boyd 1938, Carlin et al. 1989). Type O-factors are defined by Roman numerals (I, II, III, IV, V, VI, and VII) while group O-factors are defined by Arabic numerals (3,4; 6; 7,8; 9, and 10). Type O-factors are found within one serotype (e.g. serotype 2a and 2b share the type O-factor II) while group O-factors are shared among different serotypes (e.g. group factor 7,8 is found in serotype 2b, 3a, 5b, and X) (Carlin et al. 1989). The group O-factor 3,4 is defined as a backbone epitope and is shared by serotype 2a,

3b, 4a, 5a, and Y (Carlin and Lindberg 1987, Perepelov et al. 2012). The serotype Y strain has the antigenic formula of (-:3,4) because it does not have type O-factor.

*S. flexneri* 2457T (2a<sub>2</sub>) has two known Oag modifications, glucosylation on Rha<sup>I</sup> (confers type O-factor II), and O-acetylations on Rha<sup>III</sup> at position 3 or 4 (confers group O-factor 9) and on GlcNAc at position 6 (confers group O-factor 10), which are mediated by the *gtrII*BA operon, *oacB*, and *oacD* genes, respectively (Kubler-Kielb et al. 2007, Perepelov et al. 2009, Sun et al. 2014, Wang et al. 2014). Therefore, the antigenic formula of *S. flexneri* 2457T is designated as (II:9;10). Both *gtrII*BA operon and *oacD* gene are carried by the serotype-converting bacteriophage SfII prophage and are inserted between *proA* and *adrA* (Knirel et al. 2015). The *oacB* gene is carried by a transposon-like mobile element which is flanked by integrase and insertion sequences and is located upstream of *adrA* (Wang et al. 2014). Gene deletion of *oacD* only, *oacB* only or both *oacD oacB* in *S. flexneri* 2457T (2a<sub>2</sub>) gave rise to serotype 2a<sub>3</sub> (II:9), 2a<sub>1</sub> (II:10), and 2a (II:3,4), respectively, where serotype 2a<sub>3</sub> and 2a<sub>2</sub> are resistant to bacteriophage Sf6 (Teh et al. 2020).

Bacteriophage Sf6 is a short-tailed dsDNA virus that belongs to the family of *Podoviridae*. Sf6 is a temperate bacteriophage and is part of the subgroup ‘P22-like’ phages because the morphology of Sf6 is very similar to P22 (Casjens and Thuman-Commike 2011). Sf6 is known to infect serotype X and Y of *S. flexneri* strains, converting them into serotype 3a and 3b, respectively (Clark et al. 1991). Our recent study showed that Sf6 is also capable of infect-

Received: November 25, 2021. Revised: January 24, 2022. Accepted: February 24, 2022

© The Author(s) 2022. Published by Oxford University Press on behalf of FEMS. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

ing *S. flexneri* serotype 2a (II:3,4) and 2a<sub>1</sub> (II:10) strains lacking the *oacB* gene, converting them into serotype 3b (III:6) and 3b<sub>1</sub> (III:6;10), respectively (Teh et al. 2020). Sf6 recognises specific LPS Oag via its tailspike protein (Sf6TSP). Sf6TSP is an endorhamnosidase enzyme that binds reversibly to LPS Oag (primary receptor) (Kang et al. 2016) which facilitates the cleavage of Oag repeat units between the 1,3- $\alpha$ -linkage of two rhamnose residues, releasing an octasaccharide product (Lindberg et al. 1978, Chua et al. 1999). Hydrolysis of Oag repeat units brings Sf6 closer to the bacterial surface. Sf6 then binds irreversibly to OmpA or OmpC (secondary receptor) on the outer membrane (Parent et al. 2014).

Sf6TSP exists as a trimer (Muller et al. 2008) and consists of two domains, the highly conserved N-terminal domain (114 residues) that forms the capsid binding domain and the variable C-terminal domain (509 residues) that is responsible in recognising and cleaving the receptor into fragments (Steinbacher et al. 1997, Chua et al. 1999, Casjens et al. 2004). The enzymatic active sites (residues E366 and D399) of Sf6TSP are located on two different subunits of a trimer (Muller et al. 2008). Hence, a trimeric Sf6TSP contains three independent elongated glycan binding sites for LPS Oag fragments (Kunstmann et al. 2020). Sf6TSP has been shown to interact with LPS Oag of serotype 2a weakly (Kunstmann et al. 2018) and we have recently shown that Sf6TSP is capable of modestly cleaving LPS Oag repeat units of serotype 2a<sub>1</sub>, 2a<sub>2</sub>, 2a<sub>3</sub>, and 2a strains (Teh et al. 2020).

To date, there are no *Shigella* vaccines generally available, and the emergence of multi-antibiotics resistance strains have necessitated a need to seek alternative methods of treatment. Phage therapy has regained attention in recent years to treat diseases caused by multi-antibiotic resistance bacteria. This study aims to isolate a Sf6 host range mutant that is capable of infecting the most prevalent *S. flexneri* strain.

A new Sf6 host range mutant that infects *S. flexneri* serotype 2a<sub>2</sub> was isolated in this study. Amino acid residues that are potentially involved in binding and hydrolysing LPS Oag of serotype 2a strains were also identified in this study. Our study contributes to understanding of the interaction between bacteriophage Sf6 and *S. flexneri* serotype 2a.

## Materials and methods

### Bacterial strains and bacteriophage

The strains used in this study are listed in Table 1. Sf6c is a clear plaque derivative of Sf6 and was propagated on *S. flexneri* serotype Y PE577 (Morona et al. 1994).

### Growth media and growth conditions

All strains used in this study were routinely grown in Lysogeny Broth (LB) medium. Bacterial cultures were cultured for 18 h, diluted 1:20 and grown for 4 h with aeration at 37°C.

### Bacteriophage plaque assay and spot assay

The bacteriophage plaque assay was performed by mixing 100  $\mu$ L of bacteriophage ( $10^{-8}$  of an approximately  $1 \times 10^{11}$  pfu mL<sup>-1</sup> stock) with equal amount of bacterial culture and incubated for 4 min at RT followed by 1 min incubation at 45°C. Then, 3 mL of soft agar (0.75% [w/v] LB agar, prewarmed at 45°C) was added into the bacterial/phage mixture and immediately overlaid onto a 25 mL LB agar plate. The plate was incubated at 37°C for 18 h.

For the bacteriophage spot assay, 15  $\mu$ L of bacterial culture was mixed with 0.35 mL of soft agar (0.75% [w/v] LB agar, prewarmed at 45°C) and immediately overlaid onto a 3 mL LB agar in a 12-well

**Table 1.** Strain used in this study.

Strain	Relevant characteristics	Reference
2457T	Virulence plasmid positive <i>S. flexneri</i> serotype 2a <sub>2</sub>	Lab collection
PE577	<i>S. flexneri</i> serotype Y	Lab collection
RMA2159	Virulence plasmid-cured <i>S. flexneri</i> serotype 2a <sub>2</sub>	Lab collection
RMA4266	<i>S. flexneri</i> serotype 5b (PE565)	Lab collection
RMA4267	<i>S. flexneri</i> serotype 4a (PE566)	Lab collection
RMA4269	<i>S. flexneri</i> serotype 3b (PE645)	Lab collection
RMA4270	<i>S. flexneri</i> serotype 2b (PE824)	Lab collection
RMA4271	<i>S. flexneri</i> serotype 3a (PE843)	Lab collection
RMA4272	<i>S. flexneri</i> serotype 4b (PE572)	Lab collection
RMA4274	<i>S. flexneri</i> serotype 1a (PE839)	Lab collection
RMA4275	<i>S. flexneri</i> serotype 1b (PE840)	Lab collection
RMA4278	<i>S. flexneri</i> serotype 5a (PE856)	Lab collection
RMA4335	<i>S. flexneri</i> serotype X (PE576)	Lab collection
MYRM1069	<i>S. flexneri</i> 2457T $\Delta$ <i>oacB</i> serotype 2a <sub>1</sub>	Teh et al. 2020
MYRM1071	<i>S. flexneri</i> 2457T $\Delta$ <i>oacD</i> $\Delta$ <i>gtrIIIBA</i> serotype Y <sub>1</sub>	Teh et al. 2020
MYRM1091	<i>S. flexneri</i> 2457T $\Delta$ <i>oacD</i> serotype 2a <sub>3</sub>	Teh et al. 2020
MYRM1124	<i>S. flexneri</i> 2457T $\Delta$ <i>oacD</i> $\Delta$ <i>oacB</i> serotype 2a	Teh et al. 2020
MYRM1134	<i>S. flexneri</i> 2457T $\Delta$ <i>gtrIIIBA</i> $\Delta$ <i>oacB</i> serotype Y <sub>3</sub>	Teh et al. 2020
MYRM1136	<i>S. flexneri</i> 2457T $\Delta$ <i>gtrIIIBA</i> serotype Y <sub>2</sub>	Teh et al. 2020
MYRM1138	<i>S. flexneri</i> 2457T $\Delta$ All $\Delta$ <i>DIIBA+B</i> serotype Y	Teh et al. 2020

tray. Once the soft agar overlay was dried, 5  $\mu$ L of phage (undiluted phage stock) was spotted onto the overlay and let dry. The plate was incubated at 37°C for 18 h.

### DNA manipulation

The *gp14* gene (encodes TSP) of Sf6(2a)c was PCR amplified and subsequently sequenced with primers MY\_228\_Up\_tsp\_F (5' cttg-caaacaccaggaac 3') and MY\_229\_Down\_tsp\_R (5' gtaagtgcctgactggt 3') that target upstream and downstream of *gp14*, respectively. DNA sequence was analysed by Australian Genome Research Facility (Adelaide, Australia).

### Expansion of Sf6c host range mutant

To isolate a new host range mutant that form clear plaques on *S. flexneri* serotype 2a<sub>2</sub>, Sf6c was first propagated in co-culture of PE577 and *S. flexneri* serotype 2a<sub>2</sub> RMA2159 (ratio 1:1) for multiple rounds. Briefly, 100  $\mu$ L of Sf6c ( $7.8 \times 10^{10}$  pfu mL<sup>-1</sup>) was added into 1:20 of co-culture and grown at 37°C for 24 h with aeration. The co-culture was then pelleted, supernatant that contained phage was transferred to a new glass bottle and treated with chloroform for 30 min at 37°C to remove any live bacteria. One hundred microliters of the supernatant was then mixed with co-culture and the propagation repeated. After 8 rounds of propagation, a plaque assay was performed with RMA2159. Phage from a single clear plaque that formed on RMA2159 was purified, and further propagated on RMA2159 as described above. The newly isolated Sf6c host range mutant was named Sf6(2a)c.

### Preparation of LPS samples and LPS analysis

The equivalent of  $1 \times 10^9$  bacteria were centrifuged (2200  $\times$  g, 1 min) and resuspended in 50  $\mu$ L lysis buffer, heated at 100°C for 10 min, cooled and treated with 2.5  $\mu$ g mL<sup>-1</sup> Proteinase K

(#AM2542, Thermo Fisher) at 56°C for at least 4 h. The LPS samples were then subjected to SDS-PAGE and silver staining (Teh et al. 2012).

### Preparation of formalin-fixed bacteria

The equivalent of  $1 \times 10^9$  bacteria were centrifuged ( $2200 \times g$ , 1 min), washed once with PBS and fixed with 100  $\mu$ L of 1% (v/v) formalin in PBS at RT for 45 min. The formalin-fixed bacteria were washed once with PBS and resuspended in 100  $\mu$ L PBS.

### LPS hydrolysis assay by bacteriophage

The formalin-fixed bacteria ( $1 \times 10^9$  bacteria  $\text{mL}^{-1}$ ) were centrifuged ( $2200 \times g$ , 1 min), resuspended in 100  $\mu$ L of bacteriophage ( $1 \times 10^{11}$  pfu  $\text{mL}^{-1}$ ) and incubated at 37°C for 45 min. The treated bacteria were washed twice with Milli-Q water and prepared LPS samples as described above.

## Result and discussion

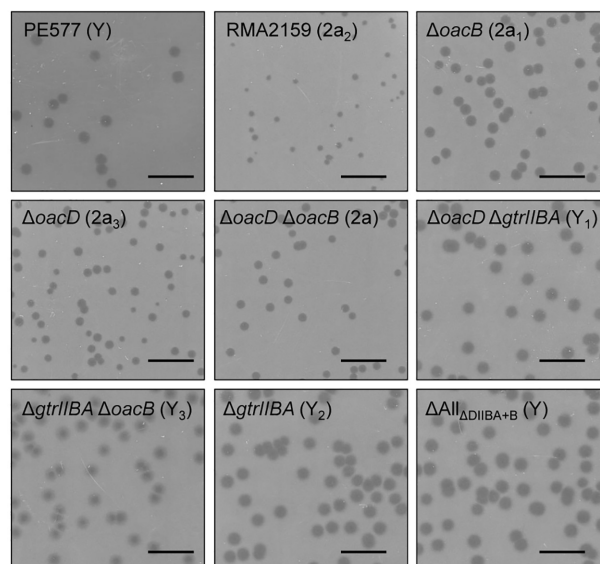
### Sf6 expanded its host range to serotype 2a<sub>2</sub> and 2a<sub>3</sub>

Sf6c is a derivative of Sf6 that forms clear plaques (lytic) on *S. flexneri* serotype Y (Morona et al. 1994). We and others have recently shown that Sf6TSP has some weak interaction with LPS Oag of serotype 2a strains (Kunstmann et al. 2018) and Sf6TSP is capable of hydrolysing LPS Oag of all serotype 2a strains (Teh et al. 2020). Therefore, we hypothesised that propagation of Sf6c in a *S. flexneri* co-culture that contained PE577 (serotype Y; susceptible to Sf6c) and RMA2159 (serotype 2a<sub>2</sub>; resistant to Sf6c) would allow Sf6c to evolve and improve its interaction with serotype 2a strains. RMA2159 was used as it is a virulence plasmid-cured *S. flexneri* 2457T strain and was safer to handle during phage propagation. The process of isolating Sf6c host range mutant was described in the Methods. Sf6c that couldn't plaque on RMA2159 (2a<sub>2</sub>) initially started forming opaque plaques after few rounds of propagation in co-cultures (data not shown) and eventually formed clear plaques on RMA2159 after eight rounds of propagation in co-cultures. We named the newly isolated host range mutant of Sf6c as 'Sf6(2a)c'.

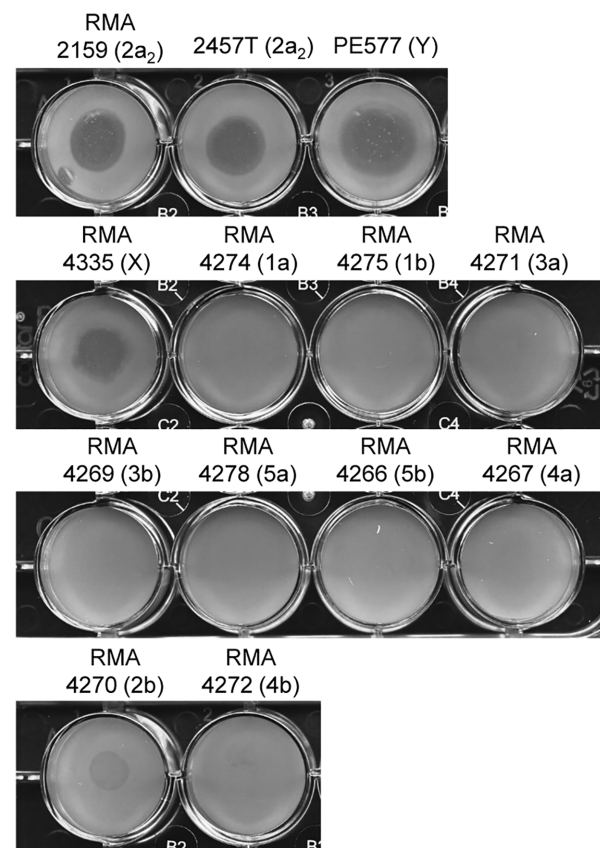
Sf6(2a)c plaque assay results showed that Sf6(2a)c was capable of infecting PE577 (Y), RMA2159 (2a<sub>2</sub>), and all isogenic serotype variant strains of 2457T, such as  $\Delta$ All $_{\Delta$ DIIIB+A+B} (Y),  $\Delta$ oacD  $\Delta$ gtrIIIBA (Y<sub>1</sub>),  $\Delta$ gtrIIIBA (Y<sub>2</sub>),  $\Delta$ gtrIIIBA  $\Delta$ oacB (Y<sub>3</sub>),  $\Delta$ oacB (2a<sub>1</sub>),  $\Delta$ oacD  $\Delta$ oacB (2a) and  $\Delta$ oacD (2a<sub>3</sub>) strains (Fig. 1). Serotype 2a<sub>2</sub> and 2a<sub>3</sub> strains that had previously been shown to be resistant to Sf6c infection were now susceptible to Sf6(2a)c (Teh et al. 2020). This indicated that Sf6(2a)c had evolved and capable of infecting *S. flexneri* serotype 2a strains that were resistant to Sf6c.

### Sf6(2a)c retained its ability to infect serotype Y and X

We found that Sf6(2a)c was still capable of infecting serotype Y strains. We then investigated if Sf6(2a)c was still able to infect serotype X (like Sf6c) and/or had expanded its host range to other serotypes. A spot assay was performed on various serotypes strains (Y, X, 1a, 1b, 2a<sub>2</sub>, 2b, 3a, 3b, 5a, 5b, 4a, and 4b) (Fig. 2). Sf6(2a)c only formed clear spots on the serotype X strain, and hence Sf6(2a)c retained its ability to infect serotype X. Surprisingly, Sf6(2a)c formed an opaque spot on serotype 2b strain (RMA4270), indicating that the growth of serotype 2b strain was affected (Fig. 2). However, Sf6(2a)c could not form plaques on the serotype 2b strain (data not shown), suggesting that Sf6(2a)c has extremely weak interaction with serotype 2b strain and is only able to affect

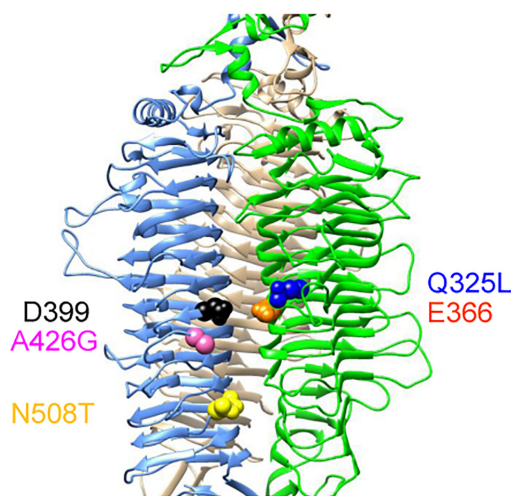


**Figure 1.** Sensitivity of serotype Y and 2a *S. flexneri* strains to the new host range mutant Sf6(2a)c. Bacteriophage Sf6(2a)c plaque assay. About 100  $\mu$ L of overnight cultures of *S. flexneri* were incubated with 100  $\mu$ L Sf6(2a)c ( $10^{-8}$ ) prior to mixed with 3 mL of soft LB agar and overlaid onto 25 mL LB agar plates. The plates were incubated at 37°C, 18 h. Serotypes of each strain are indicated in parentheses. Scale bar indicates 10 mm.



**Figure 2.** Sf6(2a)c spot assay on *S. flexneri* strains with different serotypes. Fifteen microliters of overnight cultures of *S. flexneri* were mixed with 350  $\mu$ L of soft LB agar, overlaid onto 3 mL LB agar in a 12-well tray and let dry. About 5  $\mu$ L of undiluted Sf6(2a)c phage stock was then spotted onto the soft agar. The tray was incubated at 37°C for 18 h. Serotypes of each strain are indicated in parentheses.





**Figure 3.** Location of mutated residues in Sf6(2a)cTSP. Three subunits of native TSP (PBD: 2VVK) are illustrated in light blue, green and grey cartoon. The catalytic residues, D399 and E366 are shown in black and orange spheres, respectively. The mutated residues identified in Sf6(2a)cTSP are depicted in blue (Q325L), pink (A426G), and yellow (N508T) spheres.

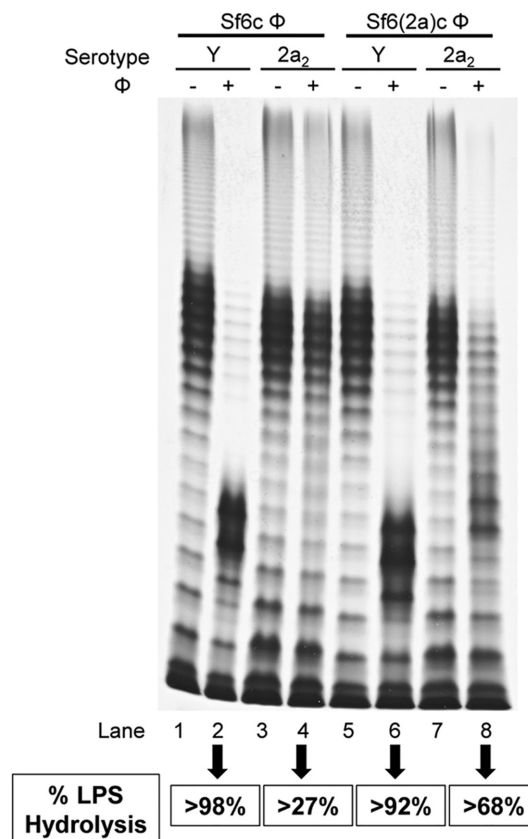
the growth of serotype 2b at high titre. The serotype 2b LPS Oag has glucosylation on both Rha<sup>I</sup> and Rha<sup>III</sup> residues, while serotype 2a<sub>2</sub> and 2a<sub>3</sub> LPS Oags have both glucosylation and O-acetylation on Rha<sup>I</sup> and Rha<sup>III</sup>, respectively (Teh *et al.* 2020). A recent study reported that O-acetylation or glucosylation on Rha<sup>III</sup> had a similar effect on Oag helical conformation (Hlozek *et al.* 2020). Therefore, Sf6(2a)c which has the ability to infect 2a<sub>2</sub> and 2a<sub>3</sub> strains may then be able to interact with the LPS Oag of serotype 2b, albeit weakly.

### Sf6(2a)c tailspike protein has mutations that correlate to infection of serotype 2a<sub>2</sub> and 2a<sub>3</sub> strains

TSP (encoded by *gp14*) is the protein that mediates binding of Sf6 to LPS Oag of *S. flexneri*. Therefore, we hypothesised that Sf6(2a)c has mutated residues within the binding groove of TSP in order to increase its binding affinity to LPS Oag of serotype 2a<sub>2</sub> and 2a<sub>3</sub> strains. Sequencing of the Sf6(2a)c *gp14* gene revealed three amino acids change: Q325L, A426G and N508T. Coincidentally, these amino acids mutations are in close proximity to the enzyme's active site residues (E366 D399), where both Q325 L and A426G are located within the glycan binding groove while N508T is located below the groove (Fig. 3). In a trimeric TSP, Q325 L is located on the same subunit as E366, while A426G and N508T are located on another subunit like D399 (Fig. 3). A recent MD simulations study reported that two Sf6TSP E366A D399A variants (V204C and S246C) that are in close proximity to the Oag binding site, had a slight increase in binding affinity to serotype Y LPS Oag (Kunstmann *et al.* 2020). This indicated that Sf6TSP can be engineered to increase its binding affinity to LPS Oag. In Sf6(2a)c, its TSP has mutated as a result of adaptation to improve its interaction with LPS Oag of serotype 2a<sub>2</sub> and 2a<sub>3</sub>. The adaptive mutational alterations in the *tsp* gene arose spontaneously, and the sequences are not present in the genomes of the host strains.

### Sf6(2a)cTSP has improved serotype 2a LPS Oag hydrolysis

We have previously shown that while Sf6TSP can hydrolyse serotype 2a<sub>2</sub> LPS Oag, it does so modestly, in a dose-dependent



**Figure 4.** LPS hydrolysis assay mediated Sf6c or Sf6(2a)c bacteriophage on *S. flexneri* serotype Y (PE577) and 2a<sub>2</sub> (RMA2159) strains. Overnight cultures of *S. flexneri* ( $1 \times 10^9$  bacteria mL<sup>-1</sup>) were formalin-fixed for 45 min at RT, washed twice with PBS and resuspended in 100 μL of bacteriophage ( $1 \times 10^{11}$  pfu mL<sup>-1</sup>). The mixture was incubated at 37°C for 45 min, washed twice with Milli-Q water and resuspended in 50 μL lysis buffer. LPS samples were then subjected to SDS-PAGE and LPS silver staining as described in the Methods. The untreated and bacteriophage-treated LPS Oag repeat units 11–17 were measured by densitometry using ImageLab 6.1 and the results are presented as percentage of LPS hydrolysis/reduction.

manner (Teh *et al.* 2020). We then investigated if the Sf6(2a)cTSP was able to hydrolyse serotype Y (PE577) and 2a<sub>2</sub> (RMA2159) LPS Oags. We initially attempted to overexpress and purify Sf6(2a)cTSP for use in LPS hydrolysis assays but were unsuccessful due to low yield and stability. Therefore, LPS hydrolysis assays were performed on formalin-fixed bacteria using Sf6c and Sf6(2a)c phage. The results were analysed with SDS-PAGE and silver staining (Fig. 4). Oag repeat units 11–17 of the untreated and Sf6c- or Sf6(2a)c-treated LPS Oags of each strain were quantified by densitometry and the results were presented as a percentage of LPS Oag reduction or hydrolysis in Fig. 4.

As expected, Sf6c-mediated LPS hydrolysis of PE577 (Y) and RMA2159 (2a<sub>2</sub>) were > 98% and > 27%, respectively (Fig. 4, Lane 2 and 4, respectively). This is consistent with our previously reported data by using Sf6TSP protein (Teh *et al.* 2020). This is also similar for Sf6(2a)c-mediated LPS hydrolysis where serotype Y LPS Oag had > 92% hydrolysis (Fig. 4, Lane 6). Excitingly, there was a significant increase in Sf6(2a)c-mediated LPS hydrolysis of RMA2159 (> 68%), in comparison to Sf6c-mediated LPS hydrolysis (> 27%) of the same strain (Fig. 4, Lane 8 and 4, respectively). This data suggested that the amino acids mutations within the Sf6(2a)cTSP protein enhanced LPS hydrolysis of serotype 2a LPS Oags and subsequently allowing infection of 2a strains by Sf6(2a)c

phage. Notably, Sf6(2a)c-mediated LPS hydrolysis on RMA2159 was incomplete and this is consistent with our previous finding that Sf6TSP was not able to achieve complete hydrolysis on LPS Oag of *S. flexneri* 2457T-derived strains (Teh et al. 2020). The data also suggested that partial hydrolysis of LPS Oags is sufficient for Sf6(2a)c to infect a serotype 2a strain.

## Conclusion

A new host range mutant of Sf6c that infects the most prevalent *S. flexneri* serotype (2a<sub>2</sub>) was successfully isolated in this study. We showed that Sf6(2a)cTSP had improved LPS hydrolysis ability on serotype 2a<sub>2</sub> strain and infection. The isolation of Sf6(2a)c contributes to our understanding of the interaction between Sf6TSP and its Oag receptor. Sf6(2a)c may be useful for phage therapy or biocontrolling of *S. flexneri* in contaminated food or water.

## Funding

This work was supported by the Australian Research Council Discovery Project [DP170104325] to R. Morona.

**Conflict of interest statement** . None declared.

## References

- Boyd JS. The antigenic structure of the mannitol-fermenting group of dysentery bacilli. *J Hyg (Lond)* 1938;**38**:477–99.
- Carlin NI, Lindberg AA. Monoclonal antibodies specific for *Shigella flexneri* lipopolysaccharides: clones binding to type IV, V, and VI antigens, group 3,4 antigen, and an epitope common to all *Shigella flexneri* and *Shigella dysenteriae* type 1 stains. *Infect Immun* 1987;**55**:1412–20.
- Carlin NI, Rahman M, Sack DA et al. Use of monoclonal antibodies to type *Shigella flexneri* in Bangladesh. *J Clin Microbiol* 1989;**27**:1163–6.
- Casjens S, Winn-Stapley DA, Gilcrease EB et al. The chromosome of *Shigella flexneri* bacteriophage Sf6: complete nucleotide sequence, genetic mosaicism, and DNA packaging. *J Mol Biol* 2004;**339**:379–94.
- Casjens SR, Thuman-Commike PA. Evolution of mosaically related tailed bacteriophage genomes seen through the lens of phage P22 virion assembly. *Virology* 2011;**411**:393–415.
- Chua JE, Manning PA, Morona R. The *Shigella flexneri* bacteriophage Sf6 tailspike protein (TSP)/endorhamnosidase is related to the bacteriophage P22 TSP and has a motif common to exo- and endoglycanases, and C-5 epimerases. *Microbiology* 1999;**145**:1649–59.
- Clark CA, Beltrame J, Manning PA. The *oac* gene encoding a lipopolysaccharide O-antigen acetylase maps adjacent to the integrase-encoding gene on the genome of *Shigella flexneri* bacteriophage Sf6. *Gene* 1991;**107**:43–52.
- Hlozek J, Ravenscroft N, Kuttel MM. Effects of Glucosylation and O-Acetylation on the conformation of *Shigella flexneri* serogroup 2 O-Antigen vaccine targets. *J Phys Chem B* 2020;**124**:2806–14.
- Kang Y, Gohlke U, Engstrom O et al. Bacteriophage Tailspikes and bacterial O-Antigens as a model system to study weak-affinity protein-polysaccharide interactions. *J Am Chem Soc* 2016;**138**:9109–18.
- Kenne L, Lindberg B, Petersson K. Basic structure of the oligosaccharide repeating-unit of the *Shigella flexneri* O-antigens. *Carbohydr Res* 1977;**56**:363–70.
- Khalil IA, Troeger C, Blacker BF et al. Morbidity and mortality due to *Shigella* and enterotoxigenic *Escherichia coli* diarrhoea: the global burden of disease study 1990–2016. *Lancet Infect Dis* 2018;**18**:1229–40.
- Knirel YA, Sun Q, Senchenkova SN et al. O-antigen modifications providing antigenic diversity of *Shigella flexneri* and underlying genetic mechanisms. *Biochemistry (Moscow)* 2015;**80**:901–14.
- Kubler-Kielb J, Vinogradov E, Chu C et al. O-Acetylation in the O-specific polysaccharide isolated from *Shigella flexneri* serotype 2a. *Carbohydr Res* 2007;**342**:643–7.
- Kunstmann S, Engstrom O, Wehle M et al. Increasing the affinity of an O-Antigen polysaccharide binding site in *Shigella flexneri* bacteriophage Sf6 Tailspike protein. *Chem Eur J* 2020;**26**:7263–73.
- Kunstmann S, Scheidt T, Buchwald S et al. Bacteriophage Sf6 Tailspike protein for detection of *Shigella flexneri* pathogens. *Viruses* 2018;**10**:431.
- Lindberg AA, Wollin R, Gemski P et al. Interaction between bacteriophage Sf6 and *Shigella flexneri*. *J Virol* 1978;**27**:38–44.
- Livio S, Strockbine NA, Panchalingam S et al. *Shigella* isolates from the global enteric multicenter study inform vaccine development. *Clin Infect Dis* 2014;**59**:933–41.
- Morona R, Mavris M, Fallarino A et al. Characterization of the *rfc* region of *Shigella flexneri*. *J Bacteriol* 1994;**176**:733–47.
- Muller JJ, Barbirz S, Heinle K et al. An intersubunit active site between supercoiled parallel beta helices in the trimeric tailspike endorhamnosidase of *Shigella flexneri* phage Sf6. *Structure* 2008;**16**:766–75.
- Parent KN, Erb ML, Cardone G et al. OmpA and OmpC are critical host factors for bacteriophage Sf6 entry in *Shigella* a. *Mol Microbiol* 2014;**92**:47–60.
- Perepelov AV, L'Vov VL, Liu B et al. A similarity in the O-acetylation pattern of the O-antigens of *Shigella flexneri* types 1a, 1b, and 2a. *Carbohydr Res* 2009;**344**:687–92.
- Perepelov AV, Shekht ME, Liu B et al. *Shigella flexneri* O-antigens revisited: final elucidation of the O-acetylation profiles and a survey of the O-antigen structure diversity. *FEMS Immunol Med Microbiol* 2012;**66**:201–10.
- Steinbacher S, Miller S, Baxa U et al. Phage P22 tailspike protein: crystal structure of the head-binding domain at 2.3 Å, fully refined structure of the endorhamnosidase at 1.56 Å resolution, and the molecular basis of O-antigen recognition and cleavage. *J Mol Biol* 1997;**267**:865–80.
- Sun Q, Knirel YA, Wang J et al. Serotype-converting bacteriophage SfII encodes an acyltransferase protein that mediates 6-O-acetylation of GlcNAc in *Shigella flexneri* O-antigens, conferring on the host a novel O-antigen epitope. *J Bacteriol* 2014;**196**:3656–66.
- Tacconelli E, Carrara E, Savoldi A et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018;**18**:318–27.
- Teh MY, Furevi A, Widmalm G et al. Influence of *Shigella flexneri* 2a O antigen acetylation on its bacteriophage Sf6 receptor activity and bacterial interaction with human cells. *J Bacteriol* 2020;**202**:e00363–20.
- Teh MY, Tran EN, Morona R. Absence of O antigen suppresses *Shigella flexneri* IcsA autochaperone region mutations. *Microbiology* 2012;**158**:2835–50.
- von Seidlein L, Kim DR, Ali M et al. A multicentre study of *Shigella* diarrhoea in six Asian countries: disease burden, clinical manifestations, and microbiology. *PLoS Med* 2006;**3**:e353.
- Wang J, Knirel YA, Lan R et al. Identification of an O-acyltransferase gene (*oacB*) that mediates 3- and 4-O-acetylation of rhamnose III in *Shigella flexneri* O antigens. *J Bacteriol* 2014;**196**:1525–31.
- Ye C, Lan R, Xia S et al. Emergence of a new multidrug-resistant serotype X variant in an epidemic clone of *Shigella flexneri*. *J Clin Microbiol* 2010;**48**:419–26.