

Evaluation of the efficacy of bacteriophages against *Staphylococcus aureus*

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Introduction

Staphylococcus aureus (*S. aureus*) is an opportunistic human pathogen that has the ability to cause both health care-associated and community-acquired infections (Chambers, 2001). The infections were once, easily treated with antibiotics before resistance against beta-lactams (eg. Methicillin) and glycopeptides (eg. Vancomycin) began to emerge over the years and caused an increase in mortality and morbidity rates in patients infected with *S. aureus* (Boucher & Corey, 2008; Howe et al., 1998). This has led to an increased interest in the exploration of the use of bacteriophages as a way to combat multi-drug resistant organisms because bacteriophages has bacteriolytic mechanism independent from those of any known antibiotics (Matsuzaki et al., 2005).

Objective

To evaluate the host range of bacteriophages against clinical *S. aureus* isolates.

Methods

The *S. aureus* specific bacteriophages were isolated from wastewater samples collected from Kuching Centralized Sewage Treatment Plant in Sarawak, Malaysia using the double agar layer method as described by Li & Zhang (2014) with modifications. Either *S. aureus* ATCC 25923 or *S. aureus* 995 clinical strain were used as host bacteria.

The bacteriophage isolates were evaluated for their host range activity using the spot test method as described by Lu and Breidt, 2015. A total of 40 clinical strains of *S. aureus* including 4 Methicillin resistant *S. aureus* (MRSA) were obtained from the Sarawak General Hospital. A total of 9 representative non-*S. aureus* strains were also used in this study (Kathleen et al., 2014).

The morphology of the bacteriophage with the most extensive host range was visualized under the transmission electron microscope (TEM) according to Ackermann (2009) with modifications.

The stability of the selected bacteriophage was carried out as described by Li and Zhang (2014).

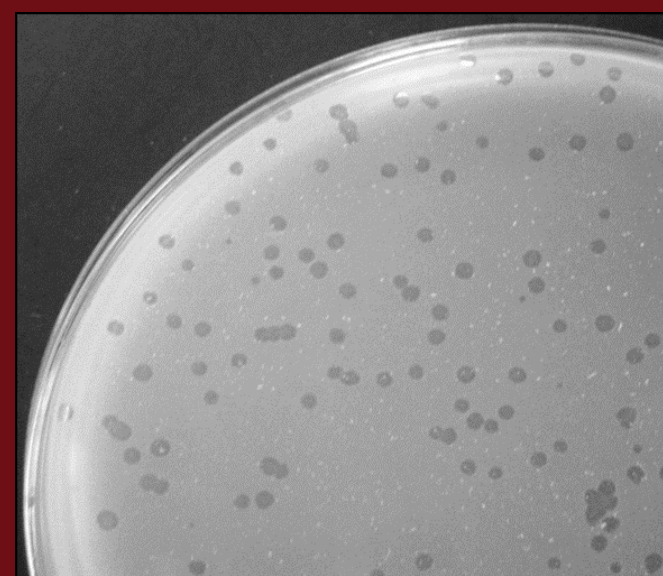


Fig. 1 Plaques formation by double layer agar method with the observed plaque size of 1-2mm of purified Φ NUSA-1

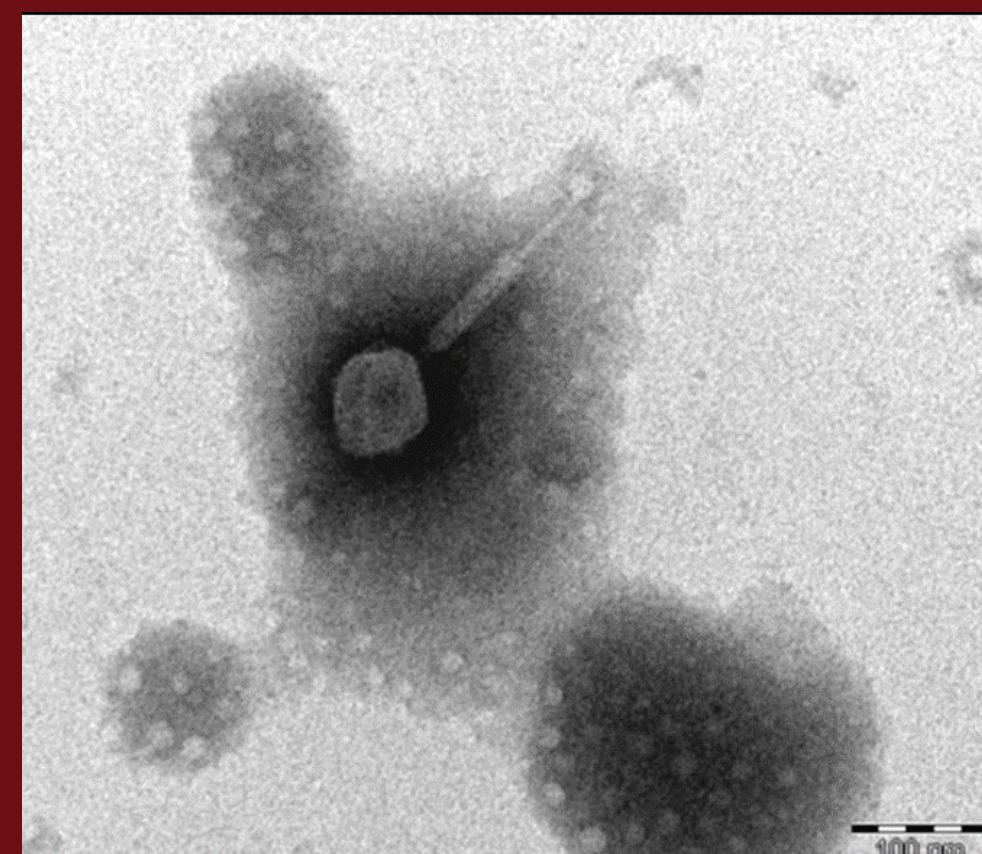


Fig. 2 Transmission electron microscope image of Φ NUSA-1 phage. Micrograph of negatively stained sample with 2% Phosphotungstate with magnification at 80,000x with the bar representing 100nm. Micrograph showing the contractile tail of the phage with clear T=16 hexagonal symmetry capsid head clustered together through the tail fibers.

Results

A total of 5 bacteriophages namely Φ NUSA-1 to Φ NUSA-5 were isolated from the sewage water and visualized as plaques formation on the double agar overlay (Fig. 1).

The host range (Fig. 3) of the bacteriophages on 40 clinical isolates showed that Φ NUSA-1 to be the most virulent bacteriophage killing 85% of all *S. aureus* strains including 3 of 4 MRSA isolates. The descending host range are as follows:

Φ NUSA-1 > Φ NUSA-4 > Φ NUSA-5 > Φ NUSA-2 > Φ NUSA-3.

All bacteriophages isolated were *S. aureus* specific and does not infect the representative non-*S. aureus* strains.

Φ NUSA-1 with the broadest host range was chosen for further characterization.

The morphology of Φ NUSA-1 in Fig. 2 shows that it is non-enveloped, possessing T=16 symmetrical head and contractile tail, consists of a sheath and a central tube. It is stable between pH5-10 (Fig. 4) while remains stable without any measurable drop of viability up to 50°C (Fig. 5).

Strain	<i>Staphylococcus</i> spp.	Φ NUSA-1	Φ NUSA-2	Φ NUSA-3	Φ NUSA-4	Φ NUSA-5
995	<i>aureus</i> ¹	C	C	C	C	C
967209	<i>aureus</i> ¹	C	C	C	C	C
3578	<i>aureus</i> ¹	C	C	C	C	C
914272	<i>aureus</i> ¹	C	C	C	C	C
913842	<i>aureus</i> ¹	C	C	C	C	C
913824	<i>aureus</i> ¹	C	C	C	C	C
932556	<i>aureus</i> ¹	C	C	C	C	C
9176390	<i>aureus</i> ¹	C	C	C	C	C
932373	<i>aureus</i> ¹	C	C	C	C	C
6484	<i>aureus</i> ¹	C	C	C	C	C
6781	<i>aureus</i> ¹	C	C	C	C	C
3854	<i>aureus</i> ¹	C	C	C	C	C
913819	<i>aureus</i> ¹	C	C	C	C	C
635	<i>aureus</i> ¹	C	C	C	C	C
3819	<i>aureus</i> ¹	C	C	C	C	C
1002387	<i>aureus</i> ¹	C	C	C	C	C
966910	<i>aureus</i> ¹	C	C	C	C	C
932365	<i>aureus</i> ¹	C	C	T	C	C
967201	<i>aureus</i> ¹	C	C	N	C	C
966917	<i>aureus</i> ¹	C	T	N	C	T
9774	<i>aureus</i> ¹	C	T	N	C	C
932100	<i>aureus</i> ¹	C	C	N	C	T
932378	<i>aureus</i> ¹	C	C	N	C	C
976391	<i>aureus</i> ¹	C	C	N	C	C
932387	<i>aureus</i> ¹	C	T	N	T	T
6789	<i>aureus</i> ¹	C	C	N	T	T
9772	<i>aureus</i> ¹	C	C	N	C	C
3885	<i>aureus</i> ¹	C	T	N	C	T
966915	<i>aureus</i> ¹	C	T	N	T	T
914349	<i>aureus</i> ¹	C	C	N	T	C
966342	<i>aureus</i> ¹	C	T	N	C	C
ATCC 25923	<i>aureus</i> ²	C	T	N	C	T
999520	<i>aureus</i> ²	C	T	N	T	N
967207	<i>aureus</i> ²	C	T	N	C	N
914266	<i>aureus</i> ²	C	N	N	T	T
914260	<i>aureus</i> ²	T	N	N	C	T
151064*	<i>aureus</i> ²	T	N	N	C	T
9767	<i>aureus</i> ²	T	N	N	N	T
914263	<i>aureus</i> ²	T	N	N	N	T
967304	<i>aureus</i> ²	T	N	N	N	N
9768	<i>aureus</i> ²	N	N	N	N	N
LBG-2P3	<i>saprophyticus</i> ²	N	N	N	N	N
SPD-W1	<i>saprophyticus</i> ²	N	N	N	N	N
SPD-S9	<i>saprophyticus</i> ²	N	N	N	N	N
MY Y-W6	<i>icidurii</i> ²	N	N	N	N	N
MY Y-2F2	<i>xyloosus</i> ²	N	N	N	N	N
SPD-W7	<i>oblongus</i> ²	N	N	N	N	N
SPD-W3	<i>oblongus</i> ²	N	N	N	N	N
SPD-P1	<i>oblongus</i> ²	N	N	N	N	N
LBG-P2	<i>oblongus</i> ²	N	N	N	N	N

Fig. 3 Bacteriophage host range. A total of 5 bacteriophage isolates were tested against 40 clinical *S. aureus* and 9 non-*S. aureus* strains using the spot test method.

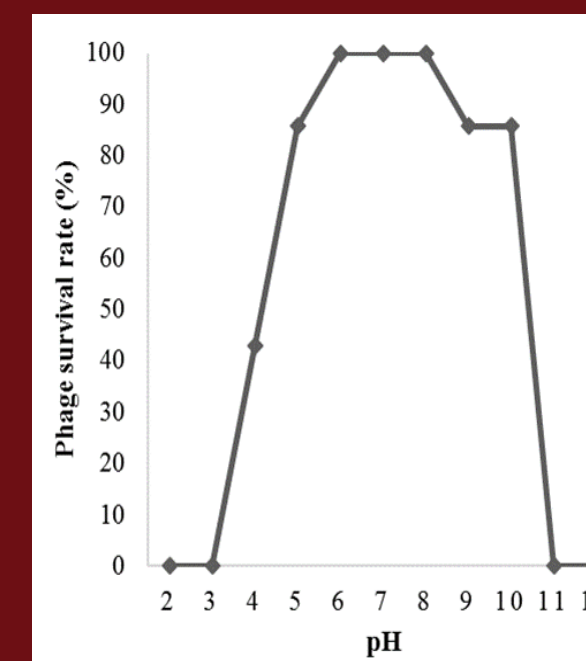


Fig. 4 pH stability test for Φ NUSA-1. Φ NUSA-1 was incubated at different pH ranging from pH2 to pH12 for 3 hours and immediately assayed using the double agar overlay method.

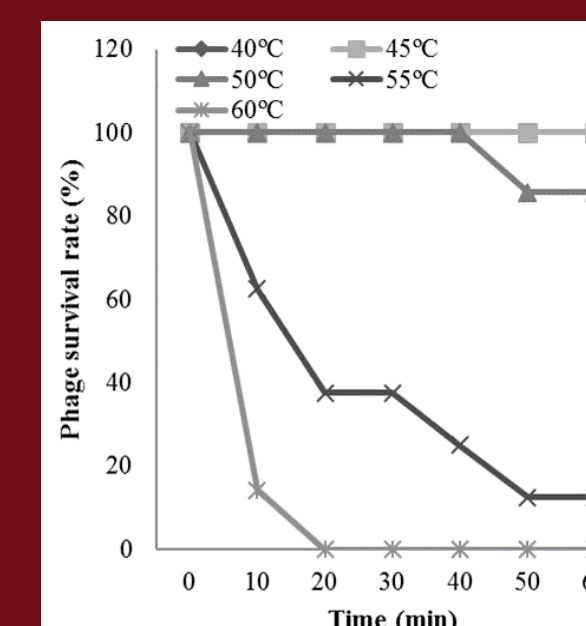


Fig. 5 Thermostability test for Φ NUSA-1 from 40-60°C with the increment of 5°C. Samples were collected at 10 mins intervals for 60 minutes and tested using the double agar overlay method.

Discussion & Conclusions

Bacteriophage Φ NUSA-1 belongs to the family *Myoviridae*, subfamily *Spounavirinae*, genus “Twort-like viruses” (Ackermann 2001, 2007).

It is interesting to note that the ATCC 25923 was only sensitive to 2/5 of the bacteriophages which suggests that ATCC strain of *S. aureus* may not be the optimal host to be used for the isolation of bacteriophages.

Φ NUSA-1 is stable between pH 5-10 and thermostable up to 50°C suggesting that the bacteriophage is stable at physiologic conditions and at room temperature.

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