

# **Biodiversity of new lytic bacteriophages infecting *Shigella* spp. in fresh water environment**

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**Table S1.**

The bacterial strains used in this study, and the host range and efficiency of plating (EOP) for the three isolated bacteriophages

Bacterial species	Source	vB_SfIM_004		vB_SdyM_006		vB_SsoS_008	
		Spot test	EOP	Spot test	EOP	Spot test	EOP
<i>Shigella dysenteriae</i>	PTCC 1188	–		++	0.93 ± 0.06	–	
<i>Shigella dysenteriae</i> (S.d.f1)	Vegetables	–		++	1.00	–	
<i>Shigella dysenteriae</i> (S.d.f2)	Vegetables	–		++	0.77 ± 0.05	–	
<i>Shigella sonnei</i>	ATCC 9290	+	0.14 ± 0.08	–		++	0.88 ± 0.06
<i>Shigella sonnei</i> (w8)	Ready to eat food	–		–		++	1.00
<i>Shigella sonnei</i> (w9)	Vegetables	+	0.25 ± 0.03	–		++	0.68 ± 0.05
<i>Shigella sonnei</i> (w44)	Ready to eat food	–		–		+	0.35 ± 0.04
<i>Shigella sonnei</i> (Sh.s-3 )	Vegetables	+	0.29 ± 0.05	–		+	0.23 ± 0.05
<i>Shigella sonnei</i> (Sh.s-26)	Meat	+	0.33 ± 0.06	–		++	0.70 ± 0.05
<i>Shigella sonnei</i> (Sh.s-77)	Vegetables	–		–		+	0.40 ± 0.01
<i>Shigella flexneri</i>	ATCC 12022	++	0.97 ± 0.01	–		++	0.65 ± 0.05
<i>Shigella flexneri</i>	PTCC 1234	++	0.90 ± 0.05	–		+	0.12 ± 0.07
<i>Shigella flexneri</i>	PTCC 1865	++	0.92 ± 0.03	–		+	0.22 ± 0.03
<i>Shigella flexneri</i> (w7)	Ready to eat food	++	1.00	–		++	0.56 ± 0.02
<i>Shigella flexneri</i> (w16)	Vegetables	++	0.70 ± 0.09	–		++	0.43 ± 0.08
<i>Shigella flexneri</i> (w19)	Vegetables	++	0.75 ± 0.05	–		+	0.30 ± 0.04
<i>Shigella flexneri</i> (w27)	Vegetables	++	0.47 ± 0.06	–		++	0.54 ± 0.01
<i>Shigella flexneri</i> (W2)	Vegetables	++	0.39 ± 0.02	–		++	0.50 ± 0.08
<i>Shigella flexneri</i> (Sh.f-8)	Vegetables	++	0.41 ± 0.02	–		+	0.17 ± 0.02
<i>Escherichia coli</i>	ATCC 25922	–		–		–	
<i>Escherichia coli</i>	Urinary Tract Infection	–		–		–	
<i>Escherichia coli</i>	Bedsore	–		–		–	
<i>Klebsiella pneumoniae</i>	ATCC 13883	–		–		–	
<i>Streptococcus pneumoniae</i>	ATCC 49619	–		–		–	

<i>Pseudomonas aeruginosa</i>	ATCC 27853	—	—	—
<i>Pseudomonas aeruginosa</i>	Burn Wound Infection	—	—	—
<i>Enterobacter cloacae</i>	Bedsore	—	—	—
<i>Enterococcus faecalis</i>	ATCC 29212	—	—	—
<i>Salmonella typhimurium</i>	ATCC 14028	—	—	—
<i>Proteus mirabilis</i>	ATCC 43071	—	—	—
<i>Staphylococcus saprophyticus</i>	ATCC 15305	—	—	—
<i>Staphylococcus epidermidis</i>	ATCC 12228	—	—	—
<i>Staphylococcus aureus</i>	ATCC 35933	—	—	—
<i>Staphylococcus aureus MRSA</i>	ATCC 33591	—	—	—
<i>Bacillus cereus</i>	ATCC 11778	—	—	—
<i>Bacillus subtilis</i>	ATCC 12711	—	—	—
<i>Enterobacter aerogene</i>	ATCC 13048	—	—	—
<i>Streptococcus pyogenes</i>	ATCC 19615	—	—	—
<i>Proteus mirabilis</i>	ATCC 43071	—	—	—

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++, clear plaque; +, cloudy plaque; —, no plaque. The EOP (efficiency of plating) values were shown as the mean of three repeats  $\pm$  standard deviation (SD)

**Table S2.**

Categorization of the efficiency of plating (EOP) of the *Shigella* phages against different isolates of *Shigella* spp..

	vB_SflM_004	vB_SdyM_006	vB_SsoS_008
<b><i>S. dysenteries</i> (n=3)</b>			
EOP $\geq$ 0.5	–	3	–
$0.1 \leq$ EOP < 0.5	–	0	–
$0.001 <$ EOP < 0.1	–	0	–
<b><i>S. flexneri</i> (n=9)</b>			
EOP $\geq$ 0.5	6	–	4
$0.1 \leq$ EOP < 0.5	3	–	5
$0.001 <$ EOP < 0.1	0	–	0
<b><i>S. sonnei</i> (n=7)</b>			
EOP $\geq$ 0.5	0	–	4
$0.1 \leq$ EOP < 0.5	4	–	3
$0.001 <$ EOP < 0.1	0	–	0

**Table S3.**

The predicted tRNA coding sequences of vB\_SdyM\_006 genome

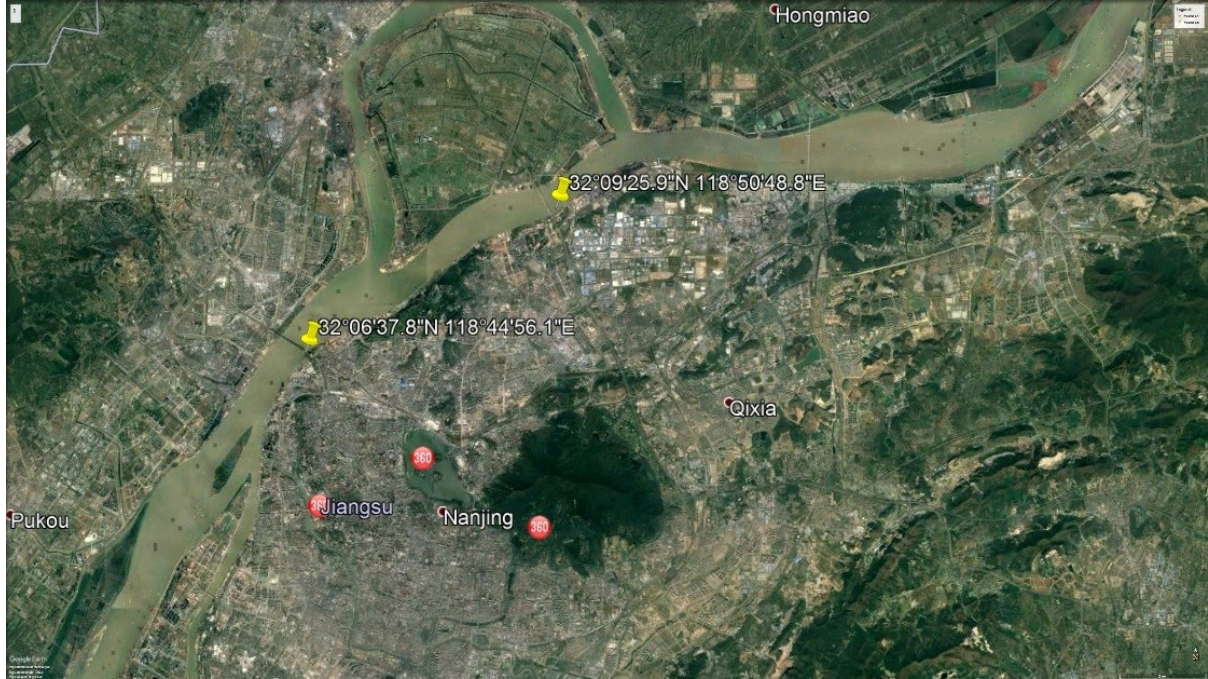
<b>tRNA</b>	<b>Strand</b>	<b>Position</b>	<b>Anticodon</b>	<b>Amino acid</b>
<b>1</b>	Complement	65128..65203	TTC	Glu
<b>2</b>	Complement	65211..65302	TGA	Ser
<b>3</b>	Complement	65651..65726	GTC	Asp
<b>4</b>	Complement	65920..65994	TCC	Gly
<b>5</b>	Complement	66799..66874	CAT	Ile2
<b>6</b>	Complement	66882..66966	GTA	Tyr
<b>7</b>	Complement	67012..67088	TCT	Arg
<b>8</b>	Complement	67403..67477	CCA	Trp
<b>9</b>	complement	67479..67562	GTT	Asn

**Table S4.**

Comparison of the basic genomic properties of phages vB\_SflM\_004, vB\_SdyM\_006 and vB\_SsoS\_008 with other similar phages

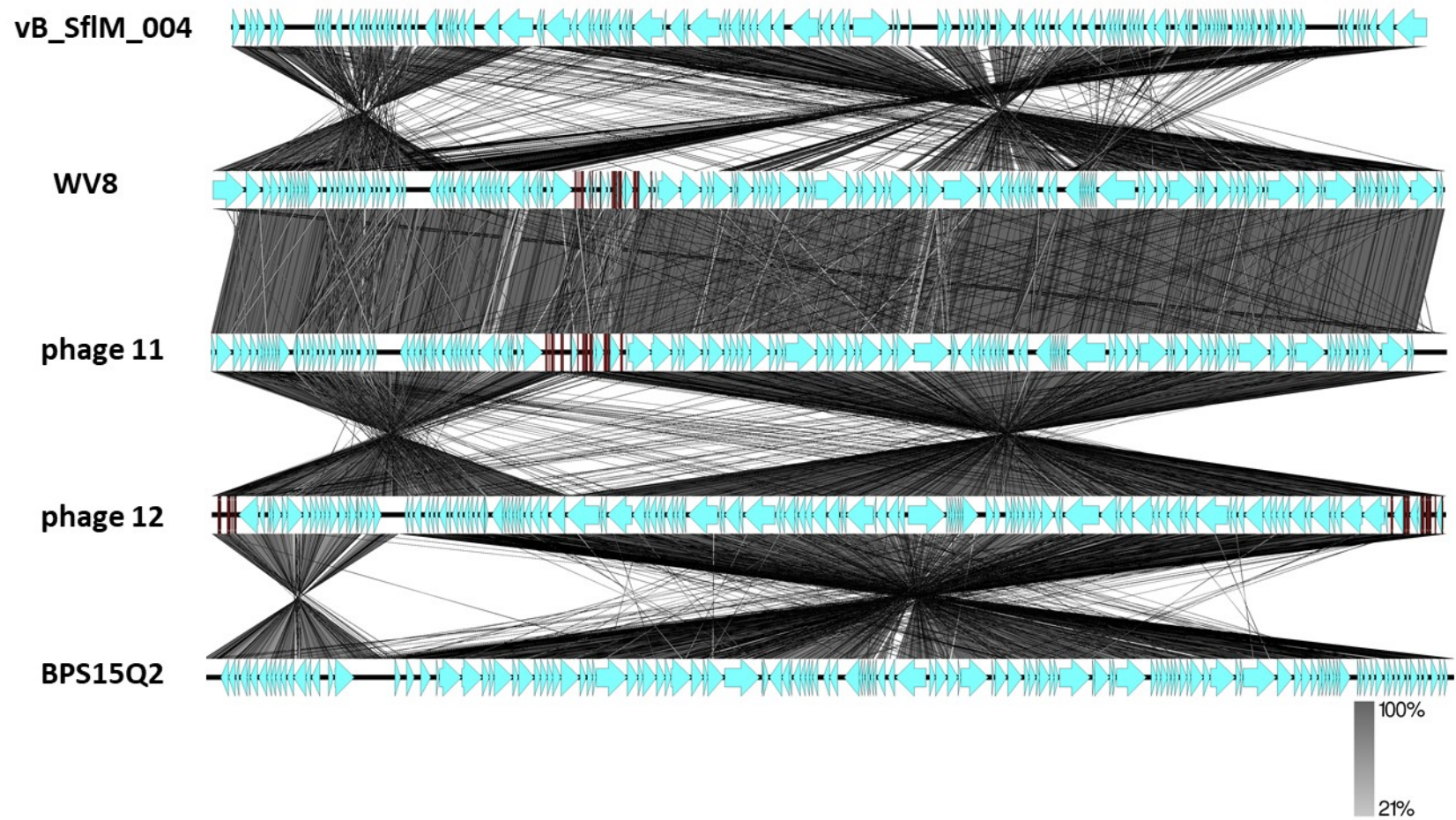
	<b>% identity</b>	<b>Genome length</b>	<b>GC-content</b>	<b>Total/identified ORF</b>	<b>No. of tRNA</b>	<b>Accession no.</b>
<b>vB_SflM_004</b>	100	85,887	38.6	135/48	0	MK295205
<b>WV8</b>	94.2	88487	38.7	137/20	18	EU877232
<b>phage 11</b>	94.2	88771	38.5	148/47	26	KP869109
<b>phage 12</b>	94.2	88632	38.4	132/21	26	KP869110
<b>BPS15Q2</b>	94.1	89817	38.6	130/45		KX405003
<b>vB_SdyM_006</b>	100	166,138	31.5	252/105	9	MK295204
<b>phiP4-3</b>	97	167849	31.4	270/119	8	MG696114
<b>vB_PmiM_Pm5461</b>	86	161989	31.5	252/119	8	KP890823
<b>vB_SsoS_008</b>	100	50,414	46.5	83/34	0	MK335533
<b>T1</b>	91.73	48836	46.5	78/23	0	AY216660
<b>phi2457T</b>	91	50219	46.6	80/21	0	MH917278
<b>SH6</b>	91	50552	46.6	82/26	0	NC_047785



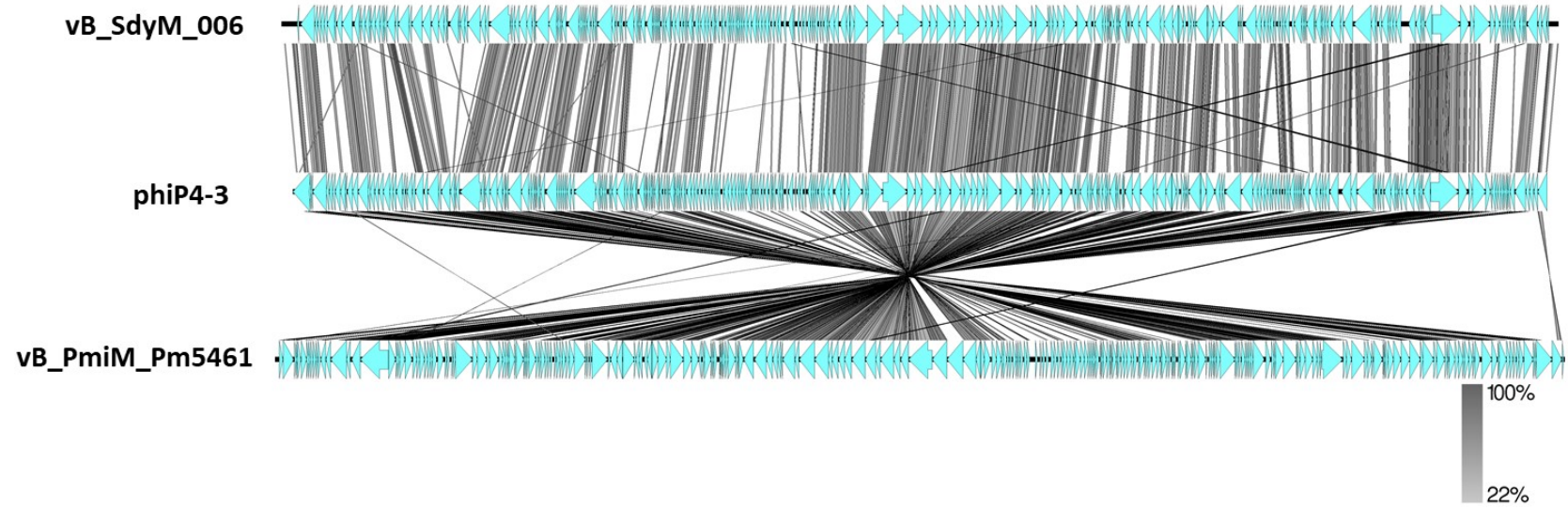


**Fig S1.** Sampling sites along the Yangtze River. The Yellow pins represent latitude: 32°06'37.8"N and longitude: 118°44'56.1"E and latitude: 32°09'25.9"N and longitude: 118°50'48.8"E

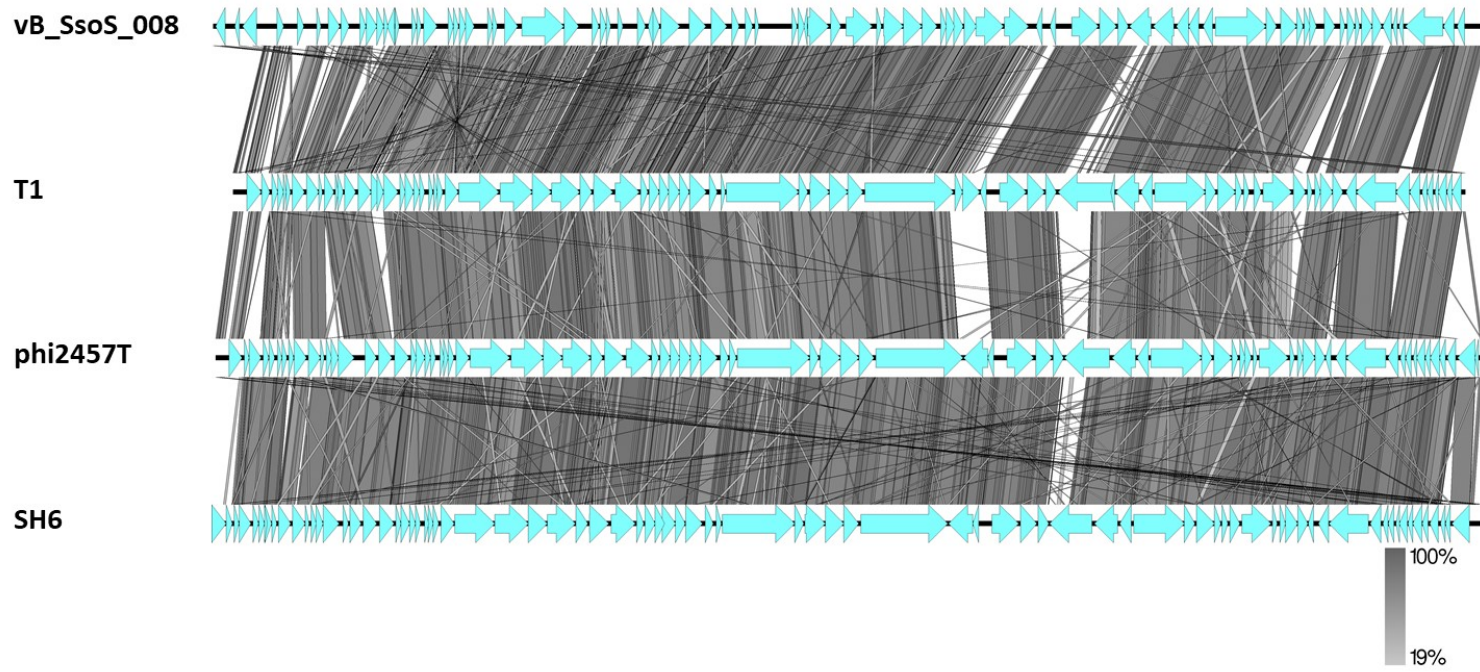




**Fig S2.** BLASTp comparison of *Shigella* vB\_SflM\_004 and the most closet phages. The figure was constructed using EasyFig.



**Fig S3.** BLASTp comparison of *Shigella* vB\_SdyM\_006 and the most closet phages. The figure was constructed using EasyFig



**Fig S4.** BLASTp comparison of Shigella vB\_SsoS\_008 and the most closet phages. The figure was constructed using EasyFig.