Supplementary Data

**Revealing Bacteriophage Capabilities: pH and NaCl Concentration Effects on RSJ2 Phage Infectivity and Stiffness**

Udom Sae-Uenga\*, Chooseel Bunsuwansakula, Kittiya Showpanisha, Namthip Phironrita, Chaweewan Sapcharoenkunb, Alongkot Treetongb, Jidapa Thadajarassiric

a National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani 12120, Thailand

b National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani, 12120, Thailand

c Department of Mathematics, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand

\* Corresponding author

**Email address**

udom.sae@biotec.or.th

**Postal address**

National Science and Technology Development Agency (NSTDA)

111 Thailand Science Park, Phahonyothin Road, Khlong Nueng, Khlong Luang

Pathum Thani 12120, Thailand

**Single-step growth curve**

The protocol for the single-step growth curve was previously described (Bhunchoth et al., 2015). The host strain was cultured in CPG medium at 28 ℃ with 230 rpm shaking overnight. The bacterial cells were harvested by centrifugation at 10,000 rpm at 4 ℃ for 10 min. The pellet was resuspended in 10 mL of fresh CPG medium and adjusted to an OD600 of 1.0. The phage sample was added at a multiplicity of infection of 0.1. The mixture was incubated for phage adsorption and centrifuged at 3,800 rpm at 4 ℃ for 15 min. After that, the pellet was resuspended in 10 mL of CPG medium and incubated at 28 ℃ with shaking at 200 rpm. Samples were aliquoted at intervals every hour and up to 8 hours and were proceeded to determine phage titer by the spot-on-lawn method (Ceballos et al., 2020). The colony-forming unit determination was adapted from the previously described protocols (Chen et al., 2003).

**Adsorption test**

*R. solanacearum* strain RS10/3 cultured overnight at 28 ℃ and 230 rpm shaking was diluted to OD600 of 0.25 with CPG medium. A 0.01 mL phage sample in the storage buffers (Table S1) with a known titer was added to 0.25 mL of diluted bacterial culture. After incubating for 30 minutes, the samples were centrifuged at 18,000×*g* for 10 min at 4 °C. 0.01 mL of the supernatant containing the unattached RSJ2 phages to the host cells was added to 0.25 mL of diluted bacterial culture. After incubating for 30 minutes, the sample was mixed with molten 0.45% agar in CPG medium and overlaid on a CPG plate containing 1.5% agar. The number of plaques was recorded after 16–18 hours of incubation at 28 ℃ and converted to PFU per mL. The titers of the attached RSJ2 phages were calculated from the subtraction of the unattached RSJ2 phages and the initial titer. The titers of the attached phage were normalized with the initial titer, yielding the adsorption rate of the RSJ2 phages in each buffer (Sae-Ueng et al., 2020).

**Table S1** Compositions of nine buffers in this study.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| pH | NaCl concentration (M) | MES (M) | Tris-HCl (M) | Bicine (M) | MgSO4 (M) |
| 6 | 0 | 0.05 | 0 | 0 | 0.01 |
|  | 0.1 | 0.05 | 0 | 0 | 0.01 |
|  | 0.5 | 0.05 | 0 | 0 | 0.01 |
| 7.5 | 0 | 0 | 0.05 | 0 | 0.01 |
|   | 0.1 | 0 | 0.05 | 0 | 0.01 |
|   | 0.5 | 0 | 0.05 | 0 | 0.01 |
| 8.3 | 0 | 0 | 0 | 0.05 | 0.01 |
|  | 0.1 | 0 | 0 | 0.05 | 0.01 |
|  | 0.5 | 0 | 0 | 0.05 | 0.01 |

**Table S2** The relative titers of the RSJ2 phage in nine buffers at 4 °C for 57 days. The titers were recorded every 7 days and normalized by the titers at 0 days.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| pH | NaCl concentration (M) | 0 days | 1 day | 8 days | 15 days | 22 days | 29 days | 36 days | 43 days | 50 days | 57 days |
| 6 | 0 | 100 | 86 | 66 | 57 | 38 | 23 | 21 | 18 | 7 | 0 |
|  | 0.1 | 100 | 43 | 4 | 3 | 0 | 2 | 0 | 0 | 0 | 0 |
|  | 0.5 | 100 | 53 | 27 | 24 | 12 | 1 | 0 | 0 | 0 | 0 |
| 7.5 | 0 | 100 | 102 | 82 | 47 | 42 | 36 | 29 | 26 | 29 | 15 |
|   | 0.1 | 100 | 99 | 87 | 95 | 80 | 69 | 47 | 33 | 33 | 23 |
|   | 0.5 | 100 | 83 | 79 | 70 | 72 | 63 | 54 | 44 | 41 | 33 |
| 8.3 | 0 | 100 | 97 | 72 | 66 | 54 | 54 | 38 | 40 | 42 | 30 |
|  | 0.1 | 100 | 71 | 48 | 46 | 41 | 31 | 37 | 27 | 32 | 15 |
|  | 0.5 | 100 | 94 | 96 | 100 | 87 | 89 | 84 | 86 | 72 | 63 |

**Table S3** Adsorption rate of the RSJ2 phage in buffers. The adsorption rate was obtained from the triplicate experiments, and the errors were standard deviations.

|  |  |  |
| --- | --- | --- |
| pH | NaCl concentration (M) | Adsorption rate (%) |
| 6 | 0 | 98.6 ± 1.3 |
|  | 0.1 | 98.9 ± 0.6 |
|  | 0.5 | 96.7 ± 1.0 |
| 7.5 | 0 | 98.4 ± 1.1 |
|  | 0.1 | 98.0 ± 0.5 |
|  | 0.5 | 96.4 ± 0.8 |
| 8.3 | 0 | 98.6 ± 0.1 |
|  | 0.1 | 99.0 ± 0.3 |
|  | 0.5 | 98.6 ± 1.2 |

**Table S4** The stiffness of the RSJ2 phage in nine buffers. The stiffness was measured at 25 °C. n was the number of the force-distance curves, and IQR was the interquartile range.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| pH | NaCl concentration (M) | n  | Mean (N/m) | Median (N/m) | Standard deviation (N/m)  | IQR (N/m) |
| 6.0 | 0 | 43 | 0.112 | 0.108 | 0.055 | 0.064 |
|  | 0.1 | 56 | 0.126 | 0.122 | 0.043 | 0.053 |
|  | 0.5 | 38 | 0.099 | 0.087 | 0.052 | 0.080 |
| 7.5 | 0 | 52 | 0.060 | 0.056 | 0.018 | 0.018 |
|   | 0.1 | 41 | 0.053 | 0.052 | 0.015 | 0.020 |
|   | 0.5 | 37 | 0.088 | 0.085 | 0.042 | 0.065 |
| 8.3 | 0 | 47 | 0.052 | 0.045 | 0.027 | 0.030 |
|  | 0.1 | 45 | 0.050 | 0.047 | 0.025 | 0.030 |
|  | 0.5 | 44 | 0.070 | 0.068 | 0.015 | 0.015 |

**Table S5** Pairwise independent-samples *t*-test of the phage stiffness

|  |  |  |  |
| --- | --- | --- | --- |
| Pair no. | Buffer 1 | Buffer 2 | *p*-value |
| 1 | pH = 6.00 M NaCl | pH = 6.00.1 M NaCl | 0.14783 |
| 2 | pH = 6.00 M NaCl | pH = 6.00.5 M NaCl | 0.26470 |
| 3 | pH = 6.00 M NaCl | pH = 7.50 M NaCl | 5.24E-05 |
| 4 | pH = 6.00 M NaCl | pH = 7.50.1 M NaCl | 3.41E-05 |
| 5 | pH = 6.00 M NaCl | pH = 7.50.5 M NaCl | 0.03575 |
| 6 | pH = 6.00 M NaCl | pH = 8.30 M NaCl | 1.98E-05 |
| 7 | pH = 6.00 M NaCl | pH = 8.30.1 M NaCl | 1.11E-05 |
| 8 | pH = 6.00 M NaCl | pH = 8.30.5 M NaCl | 4.96E-05 |
| 9 | pH = 6.00.1 M NaCl | pH = 6.00.5 M NaCl | 0.00556 |
| 10 | pH = 6.00.1 M NaCl | pH = 7.50 M NaCl | 5.42E-05 |
| 11 | pH = 6.00.1 M NaCl | pH = 7.50.1 M NaCl | 1.02E-05 |
| 12 | pH = 6.00.1 M NaCl | pH = 7.50.5 M NaCl | 5.26E-05 |
| 13 | pH = 6.00.1 M NaCl | pH = 8.30 M NaCl | 9.84E-05 |
| 14 | pH = 6.00.1 M NaCl | pH = 8.30.1 M NaCl | 4.29E-05 |
| 15 | pH = 6.00.1 M NaCl | pH = 8.30.5 M NaCl | 3.64E-05 |
| 16 | pH = 6.00.5 M NaCl | pH = 7.50 M NaCl | 2.62E-05 |
| 17 | pH = 6.00.5 M NaCl | pH = 7.50.1 M NaCl | 6.87E-05 |
| 18 | pH = 6.00.5 M NaCl | pH = 7.50.5 M NaCl | 0.34678 |
| 19 | pH = 6.00.5 M NaCl | pH = 8.30 M NaCl | 6.37E-05 |
| 20 | pH = 6.00.5 M NaCl | pH = 8.30.1 M NaCl | 3.18E-05 |
| 21 | pH = 6.00.5 M NaCl | pH = 8.30.5 M NaCl | 0.00074 |
| 22 | pH = 7.50 M NaCl | pH = 7.50.1 M NaCl | 0.06137 |
| 23 | pH = 7.50 M NaCl | pH = 7.50.5 M NaCl | 3.32E-05 |
| 24 | pH = 7.50 M NaCl | pH = 8.30 M NaCl | 0.08647 |
| 25 | pH = 7.50 M NaCl | pH = 8.30.1 M NaCl | 0.03260 |
| 26 | pH = 7.50 M NaCl | pH = 8.30.5 M NaCl | 0.00338 |
| 27 | pH = 7.50.1 M NaCl | pH = 7.50.5 M NaCl | 3.01E-05 |
| 28 | pH = 7.50.1 M NaCl | pH = 8.30 M NaCl | 0.80783 |
| 29 | pH = 7.50.1 M NaCl | pH = 8.30.1 M NaCl | 0.53271 |
| 30 | pH = 7.50.1 M NaCl | pH = 8.30.5 M NaCl | 1.17E-05 |
| 31 | pH = 7.50.5 M NaCl | pH = 8.30 M NaCl | 5.57E-05 |
| 32 | pH = 7.50.5 M NaCl | pH = 8.30.1 M NaCl | 2.15E-05 |
| 33 | pH = 7.50.5 M NaCl | pH = 8.30.5 M NaCl | 0.00847 |
| 34 | pH = 8.30 M NaCl | pH = 8.30.1 M NaCl | 0.75604 |
| 35 | pH = 8.30 M NaCl | pH = 8.30.5 M NaCl | 0.00013 |
| 36 | pH = 8.30.1 M NaCl | pH = 8.30.5 M NaCl | 1.64E-05 |

**Fig. S1.** One-step growth curve of the RSJ2 phage and the growth curve of the *Ralstonia solanacearum* strain RS10/3 after incubating with the RSJ2 phage. CFU estimates the number of viable bacterial cells. The error bars represented the standard deviation from the triplicate experiments.



**Fig. S2.** Numerical analysis of the relative titers of the RSJ2 phage in nine buffers at 4 °C for 57 days (Table S2). The buffer conditions were stated on top of each panel. The fitting model was the exponential decay model: *y = y0\*exp(-kx)*. y0 is the initial relative phage titer, and *k* is the decay rate constant.



**Fig. S3.** Force-distance curves from indenting on the RSJ2 phage particle and a substrate.

