Supplementary Materials for

A novel phage indirectly regulates diatom growth by infecting diatom-associated biofilm-forming bacterium

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Materials and methods:

Antibiotic susceptibility test of the host bacteria

Antibiotic susceptibility of *Stappia indica* SNL01 was determined against a set of 32 commercially available antibiotics (Supplementary table.S1) via the Kirby-Bauer disc diffusion method (1). Depending upon the size of growth inhibition zones (efficiency of antibiotic in suppressing strain SNL01 growth) the inhibition effects were categorized as low (smaller inhibition zone), med (medium-sized inhibition zone), and high (large inhibition zone). From the results of the Kirby-Bauer disc diffusion method and depending on the mode of action of antibiotics (Supplementary table.S2) two sets of antibiotic cocktails were prepared (Supplementary table.S3) and tested on the *stappia indica* SNL01- Diatom CCMP 1085 coculture.

Biofilm disruption Assay

Stappia indica SNL01's biofilm-forming capacity and its disruption by phage SI01 was determined as described previously (2). SNL01 was grown overnight till the log phase (10^8 CFU Ml^{-1}). 100 µL of this was inoculated in 600 µL of sterile 2216E medium in six replicates (totally 3 sets) in a 48 well plate. The plate was incubated in dark at 30 ^{0}C for 4 days. Later, 20 µL of concentrated phage lysate (10^8 PFU ML^{-1}) in SM buffer was inoculated into set-I (Treatment). 20 µL of autoclaved phage was added to Set-II (control-II) and 20 µL of sterile SM buffer was added to Set-III (control-I). The wells were gently mixed with a pipette without disturbing the formed biofilm. The plate was incubated overnight at 30 ^{0}C for phage activity. The plate was rinsed twice by slowly submerging it in a fresh sterile NaCl solution (33 ppt) and stained with 200 µL of 0.1 % crystal violet for 15 min. The excess stain was washed with sterile NaCl

solution as described above. Stain adhered cells (biofilm) were solubilized with 70 % fresh ethanol and O.D. 595 was taken via a Biotek Synergy HT microplate reader. Following the acquisition of data, a parametric t-test was conducted for statistical significance calculations via Graphpad Prism v. 9.0.0 (GraphPad Software, San Diego, California USA).

Results:

Antibiotic susceptibility of *S. indica* SNL01

Strain S.indica SNL01 was tested for clinical antibiotic susceptibility via the Kirby-Bauer disc diffusion method (Supplementary table.S1). Though the strain was susceptible to several of the tested antibiotics, the efficiency of these antibiotics in suppressing strain SNL01's growth was not high. Furthermore, the addition of two antibiotic cocktails at different concentrations could not inhibit bacterial growth at optimal range (without affecting the diatom growth, Supplementary table.S3). At higher concentrations, 500 µl- 2.5 ml / 50 ml (Supplementary table.S3) the diatom *Thalassiosira pseudonana* growth was negatively affected.

***S. indica* SNL01 biofilm formation and disruption by phage SI01**

Phage SI01's ability to disrupt *S. indica* biofilm was evaluated by inoculating the phage lysate in a four-day-old biofilm mat of *S. indica* in a 48 well plate. After 12 hours of infection, the difference could be seen visually as clear walls and the clear base of the phage-treated wells, while the base and walls of the control wells were semi-opaque with a creamy white layer. The biofilm formation of the host *S. indica* and disruption by the phage was then confirmed by O.D.600 measurement, after washing the plate and staining with 0.1% crystal violet stain. The O.D.600 of the control sets were 1.2 and 1.4 respectively, while that of the phage treated set

(Treatment-set) was significantly as low as 0.03 (P-value = 0.0004 (Control-I vs treatment) and < 0.0001 (Control-II vs treatment), Fig.S1).

Reference:

1. Gerbig DG, Engohang-Ndong J, Aubihl H. 2013. A New Twist to the Kirby-Bauer Antibiotic Susceptibility Test Activity—Increasing Antibiotic Sensitivity of *Pseudomonas fluorescens* through Thermal Stress. J Microbiol Biol Educ 14:269–270.

2. Kabwe M, Brown T, Speirs L, Ku H, Leach M, Chan HT, Petrovski S, Lock P, Tucci J. 2020. Novel Bacteriophages Capable of Disrupting Biofilms From Clinical Strains of *Aeromonas hydrophila*. Front Microbiol 11.

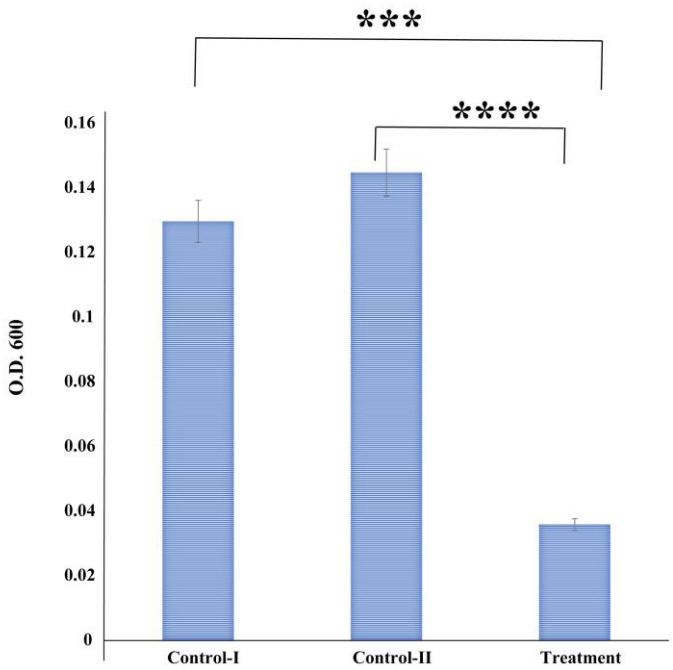

Fig.S1: Bar graph showing O.D. 600 measurement of control vs phage treated S. indica SNL01 biofilm. The data is an average of six replicates. Control-I vs Treatment showed a significant difference with a p-value of 0.0004 (***). Control-II vs Treatment showed a significant difference with a p-value of <0.0001 (****).

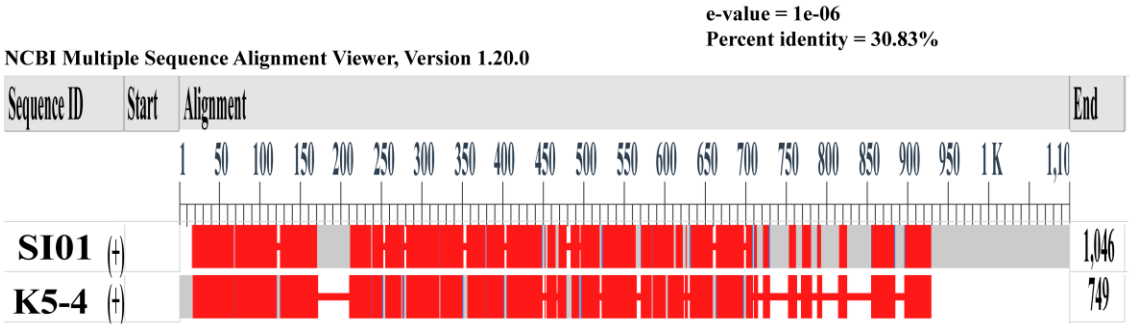

Fig.S2: Graphical alignment of Gene_4 of SI01 phage with tail spike protein-coding depolymerase of Klebsiella phage K5-4. Region 1-150 shows higher similarity.

Table.S1: Kirby-Bauer antibiotic susceptibility test on *S. indica* SNL01 strain.

Table.S2: Antibiotic selection criteria for antibiotic combinations.

Table.S3: Antibiotic combinations used to treat *S. Indica* SNL01 associated with diatom *T. pseudonana*, their concentration, and effect on SNL01 and *T. pseudonana*.

Table.S4: Gene annotation information of the 50 identified ORFs.

Table.S5: Information about the closely related phages of phage SI01