**Supporting information for**

# Chemical inhibition of alanylation sensitizes bacteria to phage infection

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**General Remarks**

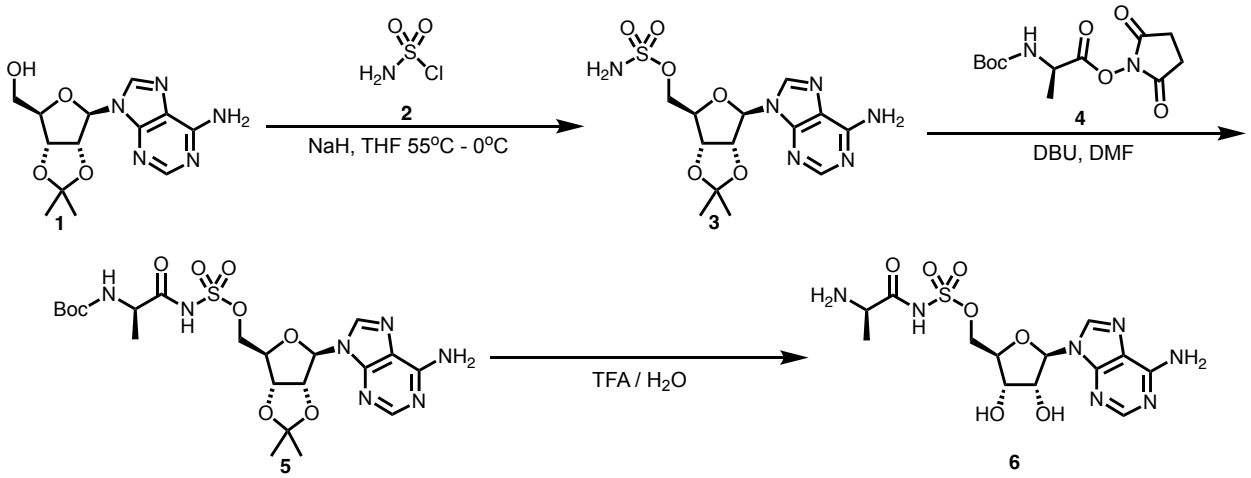
The successive pages will highlight the synthesis and characterization of 2', 3'-*O*-isopropylidene-5´-*O*-sulfamoyl-adenosine (**3**), 2', 3'-*O*-isopropylidene-5'-*O*-[*N*-(*N*-tert-butoxycarbonyl-D-alanyl)-sulfamoyl]-adenosine (**4**), and 5'-*O*-[*N*-(D-alanyl)-sulfamoyl]-adenosine (**5**). All reagents and solvents including but not limited to chloroform, methanol, isopropanol, dimethylformamide (DMF), tetrahydrofuran (THF), sodium hydride (NaH), 1,8-diazabicyclo undec-7-ene, Boc-D-alanine hydroxysuccinimide ester (Boc-D-ala-Osu), and 2', 3'-*O*-isopropylidene-adenosine were obtained commercially (**Supplementary Table 1**). All hydrogen ($^{1}$H, 500 MHz) and carbon-13 ($^{13}$C, 125 MHz) NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d6) using Varian or Bruker NMR. Chemical shifts were reported in parts per million ($\delta$ scale) and coupling constant (J values) are listed in Hertz (Hz). High performance liquid chromatography (HPLC) of final product was performed with Agilent 1260 Infinity II using a Synergi 10 µM HydroRP column. High resolution mass spectrometry data of final product was obtained with Thermo-Scientific Finingan LTQ Orbitrap XL using electrospray ionization (ESI).

***2',3'-O-Isopropylidene-5'-O-sulfamoyl-adenosine* (3):**

To a nitrogen-purged 50 mL round bottom flask with a suspension of 156.8 mg (3.25 mmol) sodium hydride (60% in mineral oil) in 8 mL THF was added four portions of 2´,3´-*O*-isopropyliden-adensosine (**1**) (517.3 mg, 1.63 mmol total). The reaction was stirred for 75 min at 55°C, and the mixture cooled to 0°C. A solution of sulfamoyl chloride (**2**) (297.0 mg, 3.35 mmol) in 4 mL THF was made and added dropwise over 30 min, while the temperature was maintained at 1-3°C. The reaction mixture was stirred for an additional 3 h at 0°C. The reaction was quenched with 2 mL methanol. The solvents were removed in vacuo and crude residue was used for the subsequent reaction with no purification. The presence of desired product was validated by LC-MS and $^{1}$H NMR of the crude mixture.[1]

***2', 3'-O-isopropylidene-5'-O-[N-(N-tert-butoxycarbonyl-D-alanyl)-sulfamoyl-adenosine* (5):**

With 2',3'-*O*-isopropylidene-5'-*O*-sulfamoyl-adensosine (**3**) in hand, 8 mL of DMF was added to form a solution, followed by subsequent addition of DBU (193 µL, 1.04 mmol). A solution of 372.3 mg (1.04 mmol) Boc-D-ala-Osu (**4**) in 1 mL DMF was added over 30 min to the reaction. The mixture was stirred for 3 h at room temperature. Then organic solvents were removed in vacuo, and the crude residue was used for subsequent reaction with no purification. The presence of desired product was validated by LC-MS and $^{1}$H NMR of the crude mixture.[2]




**Scheme 1. Synthesis of 5'-O-[*N*-(D-alanyl)-sulfamoyl]-adenosine**

***5'-O-[N-(D-alanyl)-sulfamoyl-adenosine*** **(6):**

The 2',3'-*O*-isopropylidene-5'-*O*-[*N*-(*N*-tert-butoxycarbonyl-D-alanyl)-sulfamoyl-adenosine (**5**) residue was taken up in 10 mL of water and 10 mL of TFA was added. The mixture was stirred at room temperature for 3h. After the solvents were removed in vacuo, the residue was dissolved in water and purified by HPLC. The following gradient was used at a flow rate of 10 mL/min. Buffer A was 0.01% (v/v) TFA in water, and buffer B was 0.01% (v/v) TFA in acetonitrile. The sample was injected at 100% (v/v) buffer A, and a linear gradient performed at 0–5% buffer B was performed for 30 min. A second linear gradient was performed over the next 10 min from 5%–100% (v/v) buffer B, followed by holding 100% buffer B for 8 min. The column was then re-equilibrated with 100% (v/v) buffer A. After pooling purified product from multiple runs, 77.8 mg (0.19 mmol, 16%) of pure deprotected adenosine sulfonamide (**6**) was isolated. Analytical data for (**6**): $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 8.49 (s, 1H), 8.26 (s, 1H), 7.83 (s, 3H), 5.94 (d, J = 5.7 Hz, 1H), 4.59 (t, J = 5.3 Hz, 1H), 4.23 – 4.17 (m, 2H), 4.14 – 4.11 (m, 2H), 4.10 – 4.08 (m, 1H), 1.30 (d, J = 7.1 Hz, 3H).$^{13}$C NMR ( 125 MHz, DMSO-$d_6$) δ 17.47, 50.93, 68.80, 71.02, 74.31, 83.03, 88.01, 119.20, 141.49, 148.76, 149.35, 153.17, 172.97. HRMS: 418.1139 [M + $H^+$].

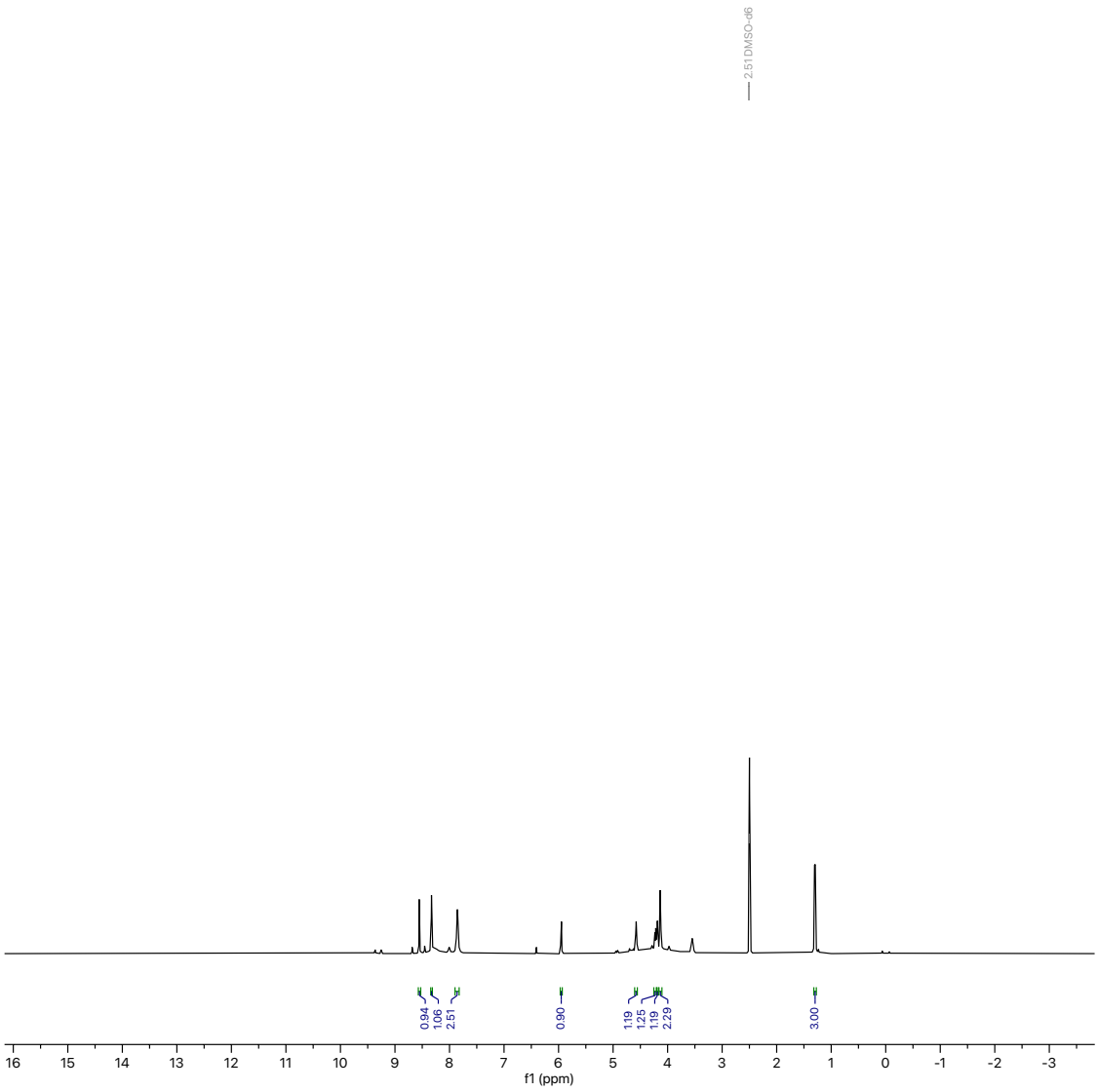



**Figure S1. $^{1}$H NMR of 5'-*O*-[*N*-(D-alanyl)-sulfamoyl]-adenosine (6) taken in DMSO-$D_6$.**

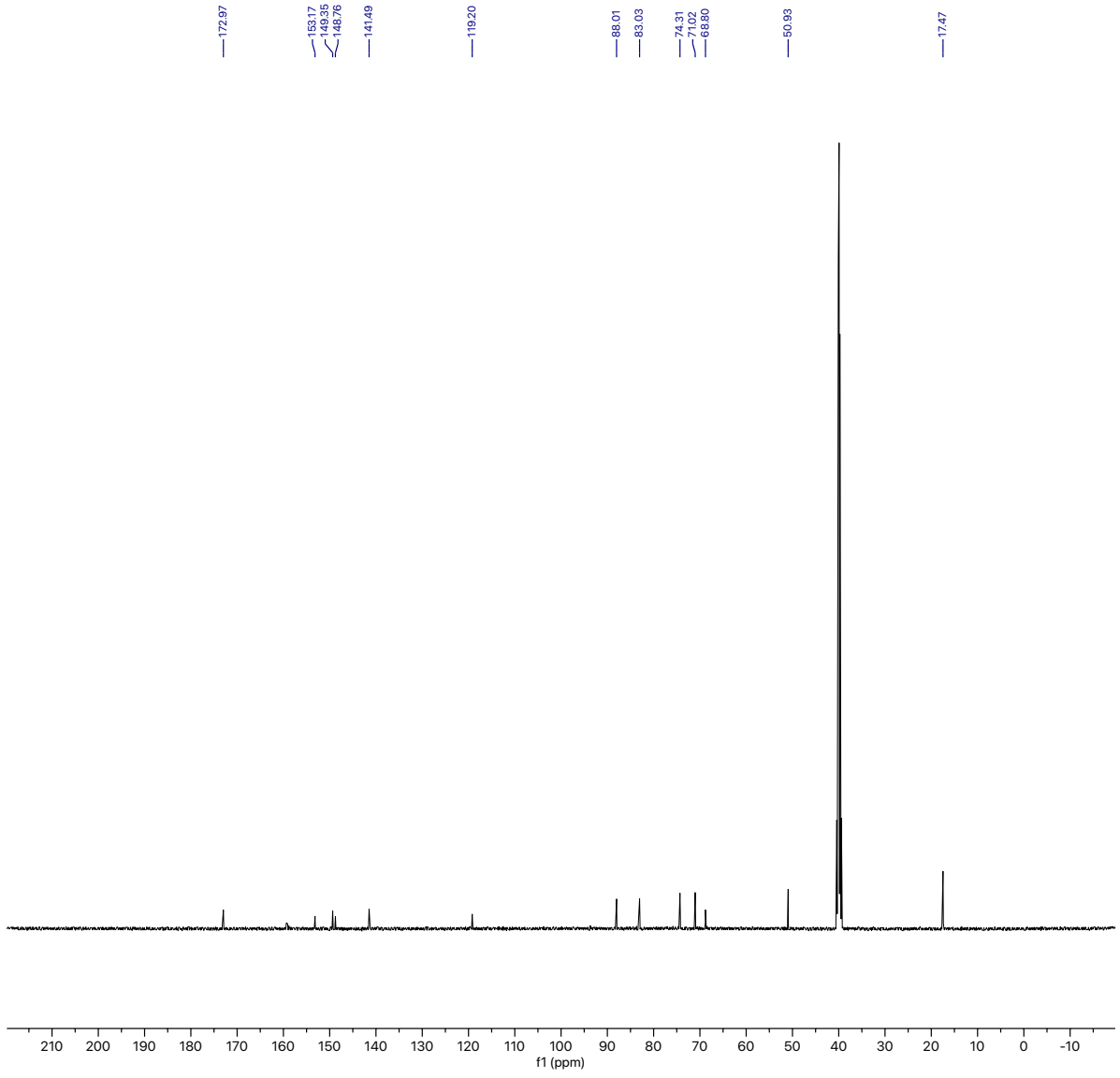



**Figure S2.$^{13}$C NMR of 5'-*O*-[*N*-(D-alanyl)-sulfamoyl]-adenosine (6) taken in DMSO-$D_6$.**

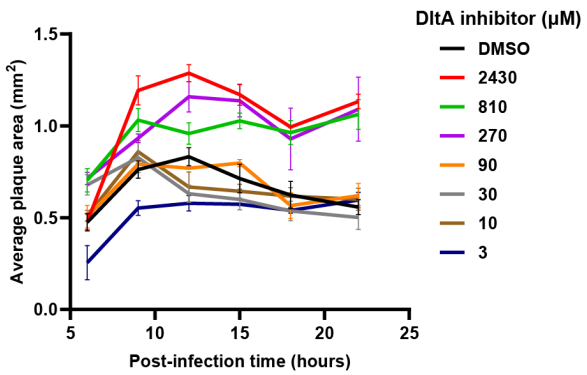



**Figure S3**. **Phi29 response to DltA inhibitor at different concentrations.** Plot of Phi29 plaque area monitored over time in the presence DMSO and different concentrations of DltA inhibitor. Plaque assays were performed in three biological replicates with ~ 20-40 plaques measured per replicate. Error bars represent SEM (n=3).

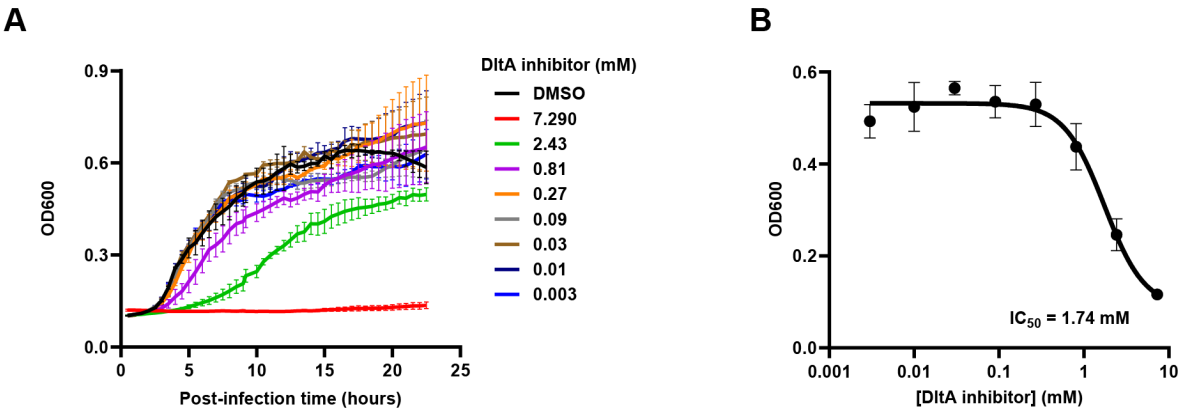



**Figure S4. Dose response of DltA inhibitor with *B. subtlis* PY79 in the absence of phage.** A) Growth curves of PY79 bacteria after different concentration of chemical inhibitor treatment. B) Dose response of inhibitor's impact on OD600 after 9 hours of treatment. Plots are biological replicates. Error bars represent SEM (n=3).

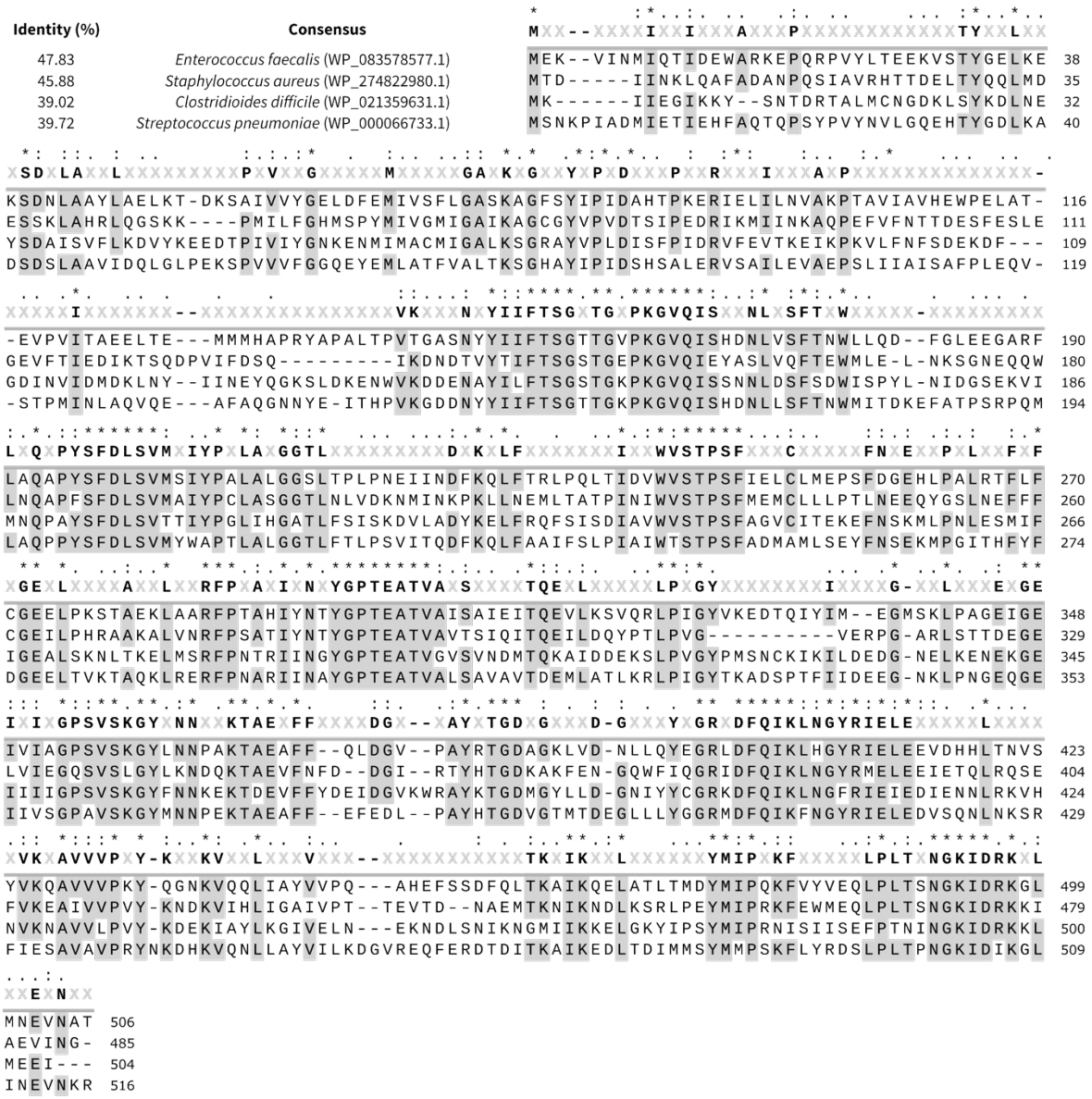



**Figure S5. DltA protein is widespread in firmicutes.** Alignments and percent identity scores of DltA protein found across different pathogens to the *B*. *subtilis* (WP_369340711.1) DltA protein query sequence using Blastp.

**Table S1. Chemical reagents for the synthesis of 5'-*O*-[*N*-(D-alanyl)-sulfamoyl]-adenosine.**

| Regent | Source |
|---|---|
| Reaction solvents | Sigma Aldrich |

| | |
|---|---|
| Sodium hydride | Sigma Aldrich |
| Boc-D-alanine hydroxysuccinimide ester | BACHEM |
| 2', 3'-*O*-isoproylidene-adenosine | Sigma Aldrich |

**Table S2. List of bacteria strains used in this work and the source obtained**

| Strain | Source |
|---|---|
| *B*. subtilis PY79 | Bacillus Genetic Stock Center (BGSC), (1A747) |
| *B*. subtilis W23 | BGSC, (2A9) |
| *B*. subtilis Δ6 | BGSC, (1A1299) |
| *B*. *subtilis* 168 trpC2 Δ*dltA*::*ErmR* | BGSC, (BKE38500) |
| *B*. *subtilis* PY79 Δ*dltA*::*ErmR* | This work |
| *B*. *subtilis* PY79 (*amyE*::$_{Phyper\text{-}spank}$—*sigX-spC*) (ET28) | Prof. Sigal Ben-Yehuda, The Hebrew University of Jerusalem[3] |

*Erm = Erythromycin
**Cam = Chloramphenicol

**Table S3. List of phages used and source obtained.**

| Phage | Source |
|---|---|
| Phi29 | BGSC |
| SPP1 | BGSC |
| SPO1 | Prof. Daniel Kearns, Indiana University |
| SP50 | University of Laval |
| PBS1 | Daniel Schwartz, Indiana University |
| vB_BsuM-Goe2 | Daniel Schwartz, Indiana University |

| | |
|---|---|
| SP10 | University of Laval |

**Table S4. Primers used in this work and source obtained.**

| Primer | Source |
|---|---|
| ErmR815 (Forward)<br>5′ CCTTAAAACATGCAGGAATTGACG 3' | Integrated DNA Technologies (IDT) |
| 3pR GT (Reverse)<br>5' CATCAGAACGGCGTGATAG 3' | IDT |

References


(1) May, J. J.; Finking, R.; Wiegeshoff, F.; Weber, T. T.; Bandur, N.; Koert, U.; Marahiel, M. A. Inhibition of the D-alanine:D-alanyl carrier protein ligase from Bacillus subtilis increases the bacterium's susceptibility to antibiotics that target the cell wall. *FEBS Journal* **2005**, *272* (12), 2993-3003. DOI: 10.1111/j.1742-4658.2005.04700.x.

(2) Castro-Pichel, J.; García-López, M. T.; De las Heras, F. G. A facile synthesis of ascamycin and related analogues. *Tetrahedron* **1987**, *43* (2), 383-389. DOI: 10.1016/s0040-4020(01)89967-1.

(3) Tzipilevich, E.; Pollak-Fiyaksel, O.; Shraiteh, B.; Ben-Yehuda, S. Bacteria elicit a phage tolerance response subsequent to infection of their neighbors. *EMBO J* **2022**, *41* (3), e109247. DOI: 10.15252/embj.2021109247.