

Viability Experiments
Standard Procedure (SOP)

Purpose:

The purpose of the viability experiments I perform is to assess the viability of bacterial strains after treatment with the Segall lab's peptides and other antimicrobial agents.

Protocol:

Overnight cultures in MHB are subcultured 1:100 into 96-well microtiter plates containing 150 μ l MHB. Six separate subcultures are made from each overnight (one for each treatment). At this point a sample is removed for dilution and plating as described below (this is the T -2 timepoint). The plate is incubated for 2 hours at 37 degrees C. Treatments are then added to the cultures. Typically, the treatments match those of the pulse field gels:

- 0.64% DMSO
- 1 μ g/ml MMC
- 32 μ M d-wrwyocr
- 1 μ g/ml MMC with 32 μ M d-wrwyocr
- 64 μ M d-wrwyocr
- 1 μ g/ml MMC with 64 μ M d-wrwyocr

Plate set-up: Each row represents a treatment, each column a strain. I typically run 4 different strains in triplicate (3 replicates x 4 strains = 12 columns)

	1	2	3	4	5	6	7	8	9	10	11	12
A – untreated												
B – DMSO												
C – MMC												
D – 32d8												
E – 32d8 + MMC												
F – 64d8												
G – 64d8 + MMC												
H - untreated												

Here, a sample is taken for dilution and plating as described below (this is the T 0 timepoint). The plate is returned to 37 degrees for three hours. After the three hours, sample is taken for dilution and plating as described below (this is the T 3 timepoint). The plate is replaced to 37 degrees for 19 hours. The final sample is then taken for dilution and plating (this is the T 22 timepoint).

Plating

Plating was performed semi-quantitatively by using a replica-plating tool to transfer inoculum from the dilution plate to LB plates. Plates were incubated at 37°C for 18-24 hours before they were counted. Optionally, optical density readings (OD_{600}) were taken at thirty minute intervals starting at the time of inoculation using a Molecular Devices spectrophotometer SpectraMax Plus 384 microtiter plate reader in order to monitor culture growth.