Determination of Minimum Inhibitory Concentrations of Antibiotics for *Synechococcus* sp. PCC 7002 in Liquid Growth Media

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- This protocol was designed for determine the minimum inhibitory concentrations (MICs) of various antibiotics of interest for the cyanobacterium *Synechococcus* sp. PCC7002, but can be adapted for determining the MICs of antibiotics for any non-model microorganism.
- The experiment is designed to run in a 96-well plate placed in a shaking incubator.
- The antibiotics and concentrations tested can vary depending on the MICs cited in the literature; this experiment can be run to determine the effectiveness of an antibiotic in new liquid growth media, or to verify literature values.
- We recommend running an analogous parallel assay with *E. coli* DH5α (in LB media at the pH of the media for the non-model organism) to determine if pH or media composition plays a role in the effectiveness of the antibiotic.
- The antibiotic concentrations chosen were relative to standard *E. coli* MICs.
- Testing six antibiotics on one plate allows for the testing of two media controls.

Protocol:

- Antibiotics Tested:
    - Kanamycin (*E. coli* 1x = 30 μg/ml)
    - Carbenicillin (*E. coli* 1x = 50 μg/ml)
    - Tetracycline (*E. coli* 1x = 12 μg/ml)
    - Spectinomycin (*E. coli* 1x = 95 μg/ml)
    - Streptomycin (*E. coli* 1x = 50 μg/ml)
    - Rifampicin (*E. coli* 1x = 50 μg/ml)
- Concentrations Tested (relative to standards for *E. coli*):
    - 0.5x
    - 1.0x
    - 2.0x
    - 4.0x
- Media Tested:
    - ATCC 1047 (pH 8.5)
    - A+ (pH 8.2)
- Antibiotics wells were grown in ATCC 1047 pH 8.5
- Well composition (150 μl total, in a 96-well flat-bottom plate)
    - 145 μl ATCC 1047
    - 5 μl inoculum (saturated culture)

- 96-well plate layout:

- Non-model organism to be tested:

		1	2	3	4	5	6	7	8	9	10	11	12
		0.5x	0.5x	0.5x	1x	1x	1x	2x	2x	2x	4x	4x	4x
A	Kan												
B	Carb												
C	Tet												
D	Rif												
E	Spec												
F	Strep												
G	ATCC 1047 pH 8.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
H	A+	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

- *E. coli* DH5α control:

		1	2	3	4	5	6	7	8	9	10	11	12
		0.5x	0.5x	0.5x	1x	1x	1x	2x	2x	2x	4x	4x	4x
A	Kan												
B	Carb												
C	Tet												
D	Rif												
E	Spec												
F	Strep												
G	LB min pH 8.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
H	Cyano Media	ATCC 1047 pH 8.5	ATCC 1047 pH 8.5	ATCC 1047 pH 8.5	ATCC 1047 pH 8.5	ATCC 1047 pH 8.5	ATCC 1047 pH 8.5	A+	A+	A+	A+	A+	A+

- After inoculating with 5.0 µl of saturated liquid culture(grown up from a single colony), plates were placed in a lighted, shaking incubator (~600 µmol photons/m^2s, 38 ˚C, 250 RPM).
- Sample growth was measured at regular intervals by taking optical density (OD) readings of each well at 600 nm for *E. coli* and 730 nm for PCC7002 using a BioTek plate reader.
    - 600 nm is the standard wavelength for measuring OD for E. coli. 730 nm is the consensus wavelength for measuring OD of PCC7002 cultures (Ruffing et al. 2014). Before experimentation, the literature should be consulted to determine the consensus wavelength for measuring OD for the non-model organism of interest.

- OD measurements were taken over the course of three days, until PCC7002 cultures appeared saturated and no significant changes in the OD730 readings were observed. The length of the data collection period will depend on the doubling time of the organism being assayed.
- A growth curve was created for both PCC7002 and *E. coli* cultures using the averages of the triplicate OD values (standard deviation of each average was calculated to report error).

Reference

Ruffing, A. "Improved Free Fatty Acid Production in Cyanobacteria with Synechococcus sp. PCC 7002 as Host." *Front. Bioeng. Biotechnol.* **2014** 2.