Displacement of Bacterial Plasmids by Engineered Unilateral Incompatibility

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Abstract

Plasmids are self-propagating genetic elements that typically confer selective advantage to their bacterial hosts, including resistance to antibiotics, which pose a significant health concern. Plasmids use copy number control systems to optimize their fitness. Plasmids whose copy number control systems interfere with one another are called incompatible; they cannot be stably maintained in a single host cell. Unilateral incompatibility is achieved by placing the self-suppressing segment of one plasmid’s replication sequence onto an otherwise compatible plasmid. We are exploring the use of unilateral incompatibility for displacement of antibiotic resistance plasmids from environmental populations.

Deterministic Modeling: in-host plasmid dynamics

\[
\frac{d}{dt} y_A(t) = r_A(s_A(t)) y_A(t) - \delta y_A(t) \\
\frac{d}{dt} y_B(t) = r_B(s_B(t)) y_B(t) - \delta y_B(t)
\]

Plasmid concentration

Inhibitor concentration

\[
\frac{d}{dt} s_A(t) = \alpha_A y_A(t) - \beta_A s_A(t) \\
\frac{d}{dt} s_B(t) = \alpha_B y_B(t) - \beta_B s_B(t)
\]

Steady state:

\[
y_B^* = \frac{\beta_B r_B^{-1}(\delta) - \alpha_B r_A^{-1}(\delta)}{\alpha_B}
\]

Displacement successful if either (i) engineered plasmid sufficiently abundant and/or (ii) inhibitor expression sufficiently strong

Stochastic Modeling

Plasmid replication: birth process with per-plasmid replication rate:

\[
\text{Hyperbolic: } k_{tr} \frac{1}{1 + \frac{1}{K_C^*}} \quad \text{or Exponential: } k_{tr} e^{-\frac{1}{K_C^*}}
\]

Cell growth: coordinated cell division cycle

Cell division: binomial distribution of plasmids to daughters

Population maintenance: half of newly formed daughter cells culled

Lab Results

Co-transformations

Chloramphenicol + Kanamycin
Chloramphenicol only
Kanamycin Only

Sequential Transformations

E. coli cells begin with an established population of target plasmids and were subsequently transformed by either the amensal plasmid or a disarmed amensal plasmid that lacked the RNA1 sequence. The two flow cytometry results above were acquired after a two day incubation following sequential transformations. Transformation by the amensal plasmid produced no cells that expressed both green and red fluorescence.

References


