Influence of growth efficiency, bacterial density, and metabolism on antibiotic resistance across different drug classes

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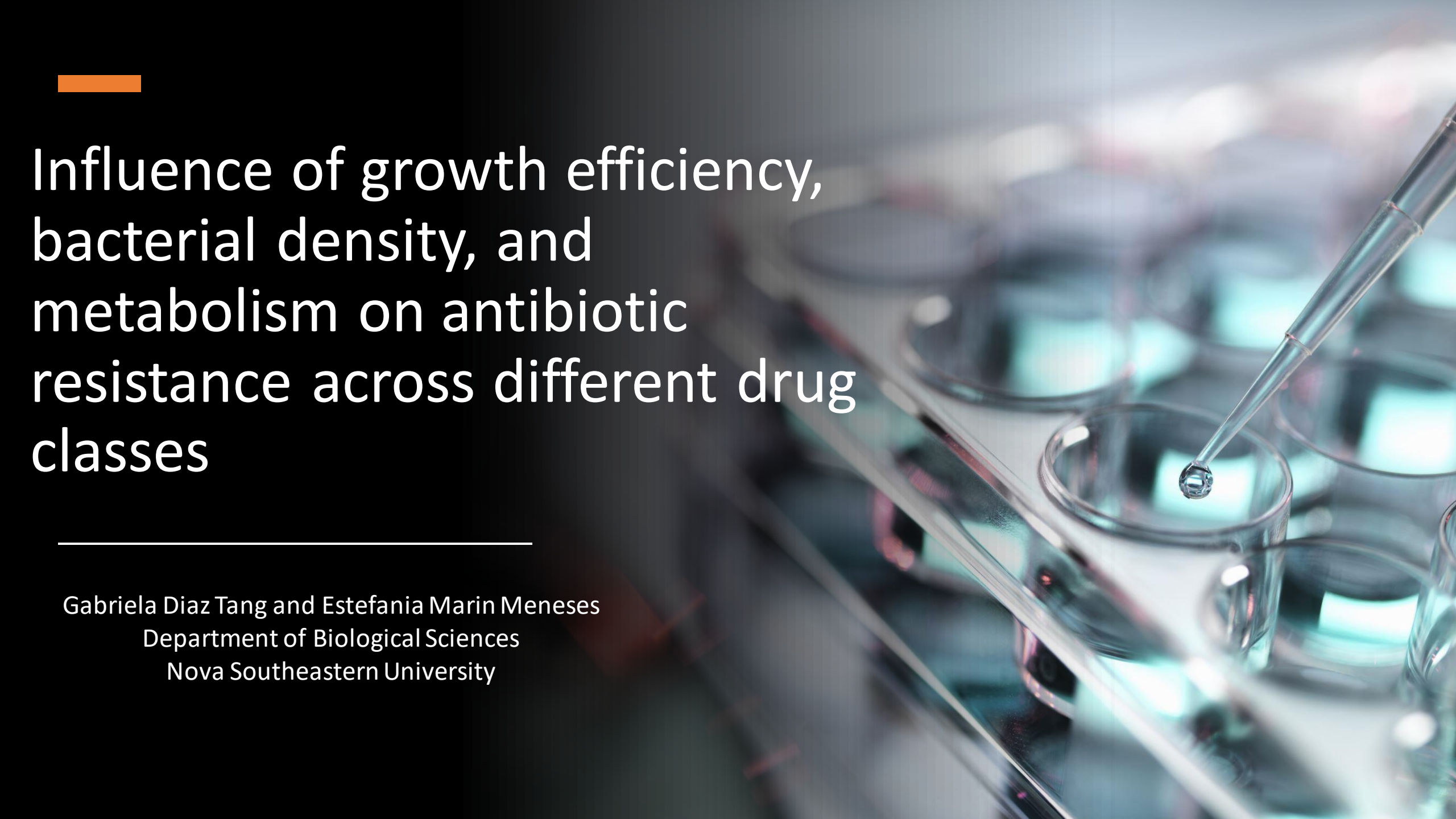
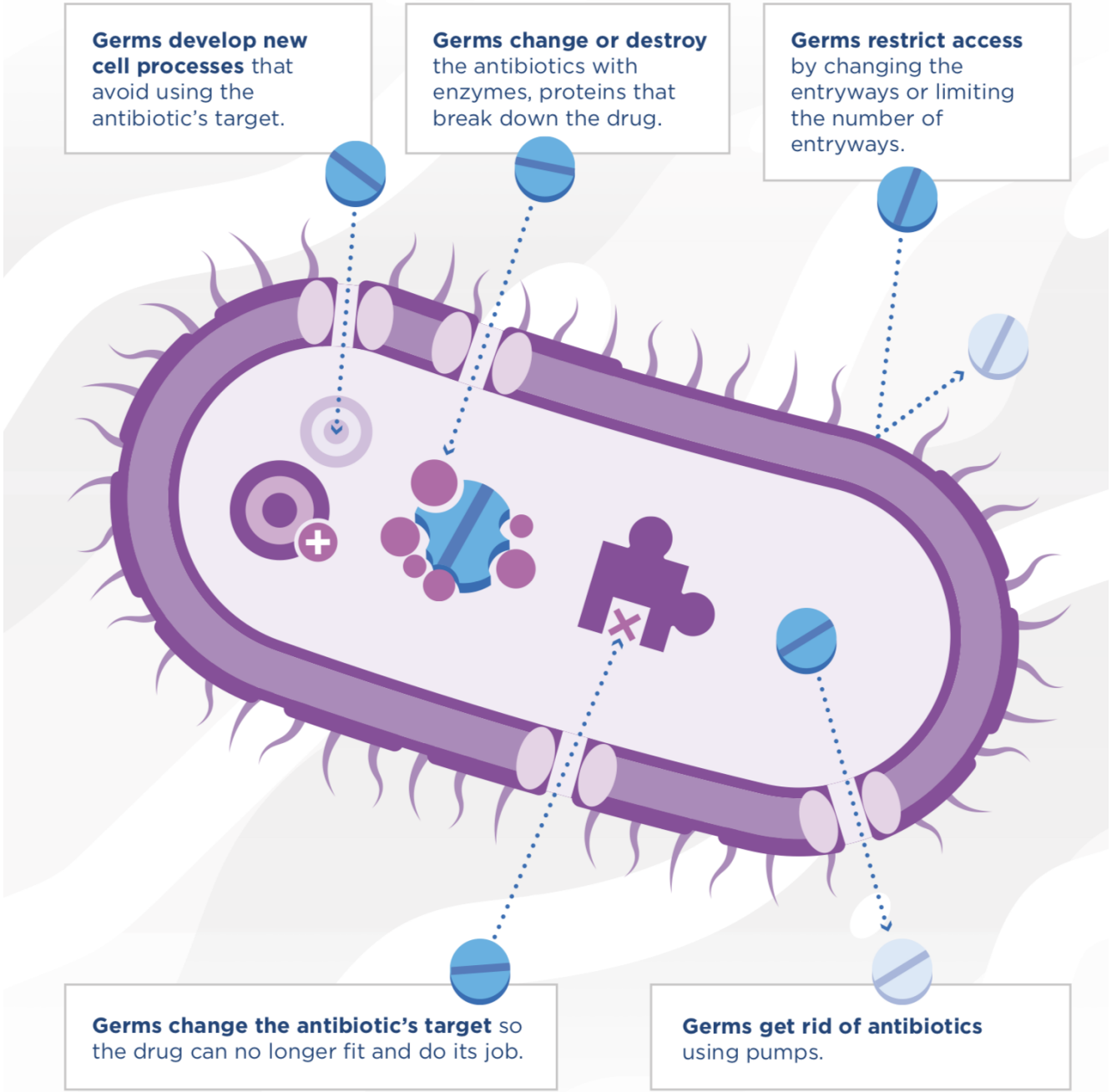


Bacteria are one step ahead!

- The CDC reports that each year in the U.S. at least 2 million people get an antibiotic-resistant infection.
- Discovery of new antibiotics has slowed significantly.
- Acquired and Phenotypic resistance render drugs ineffective.

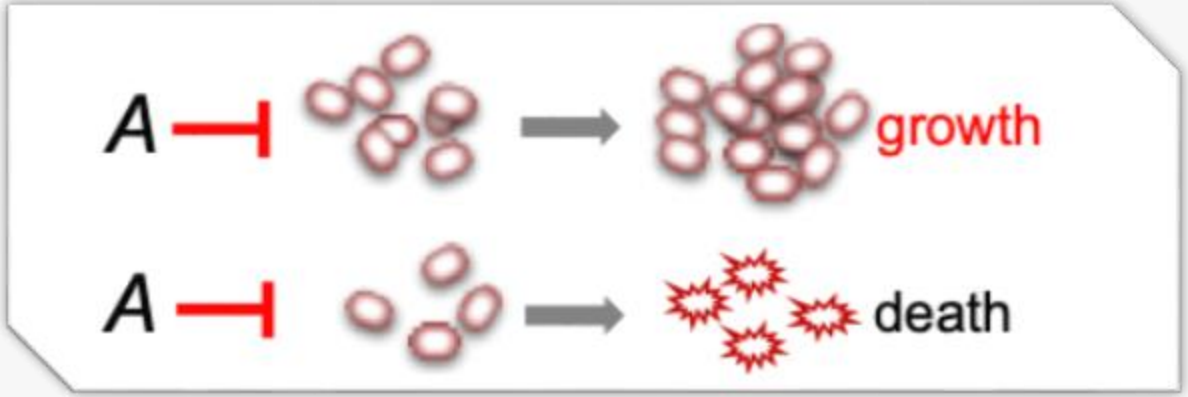


Medlife, 2019

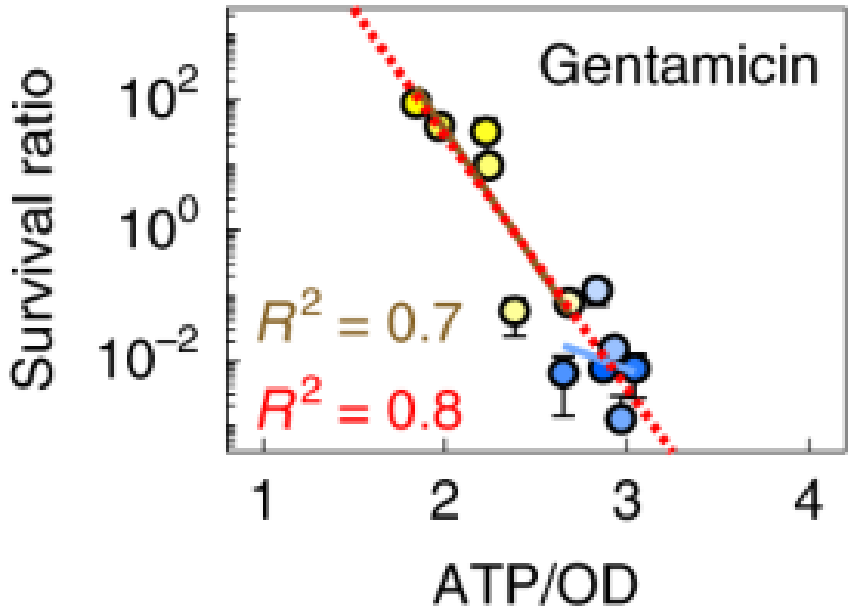
CDC, 2019

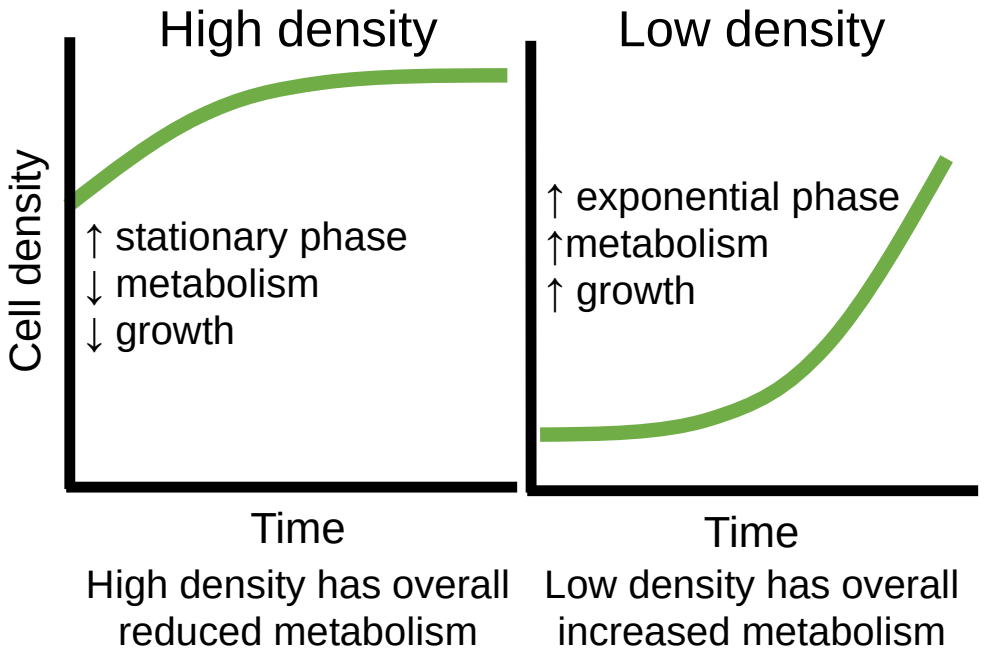
The Inoculum Effect: a mechanism by which bacteria resist drugs

- The initial bacterial density determines MIC of drug required to kill the population
- Observed in nearly all bacteria and antibiotics
- It can spur additional resistance mechanisms
- There's only so much drug you can give a person before it becomes toxic

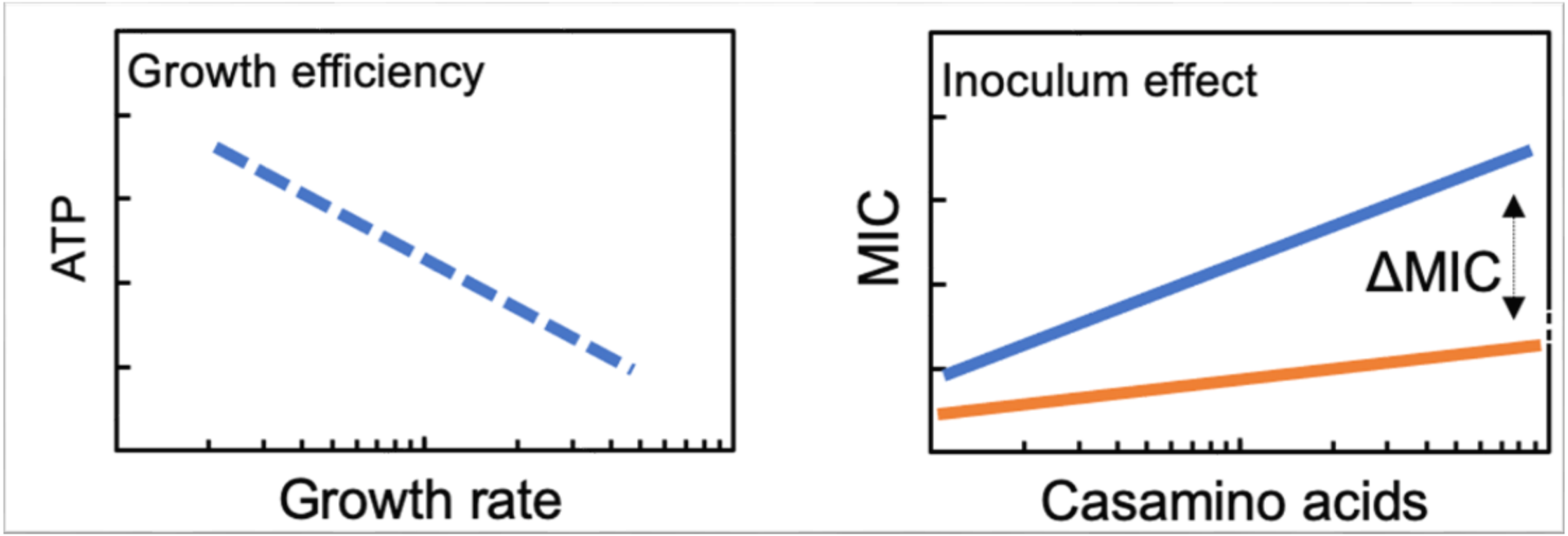


Metabolic rate and bacterial density influence antibiotic efficacy





Lopatkin et al, 2019



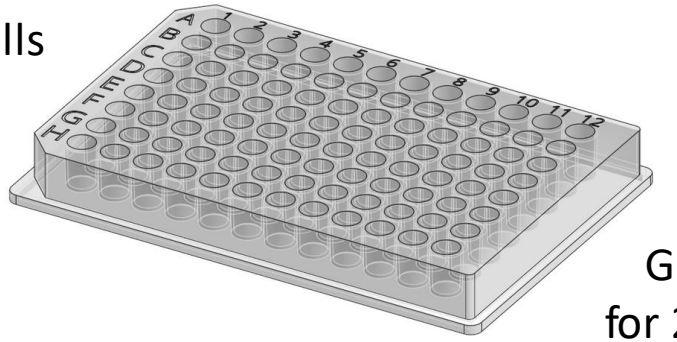
Our Central Hypothesis:

- Interactions between growth, metabolism, and cell density determine IE
- For a given environment where IE occurs, increasing growth efficiency will reduce IE

Experimental approach – Measuring IE

Overnight culture of *E. coli*

Two initial densities
High: 200,000 cells
Low: 20,000 cells



Different - concentrations of antibiotics
- % of glucose (food)
- % of casamino acids

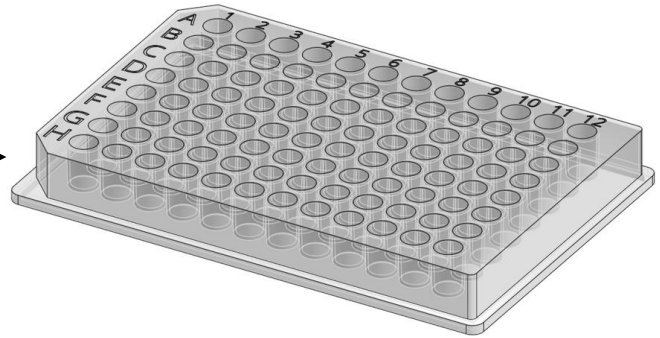
Growth for 24 hours

Measure cell density

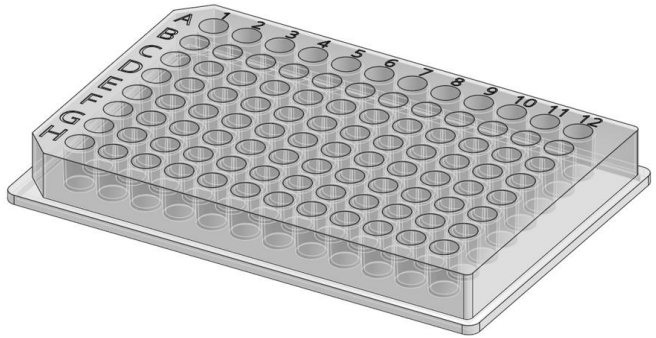
Determine the 1st concentration at which growth is not observed (MIC)

Experimental approach – measuring growth efficiency

Overnight culture of *E. coli*

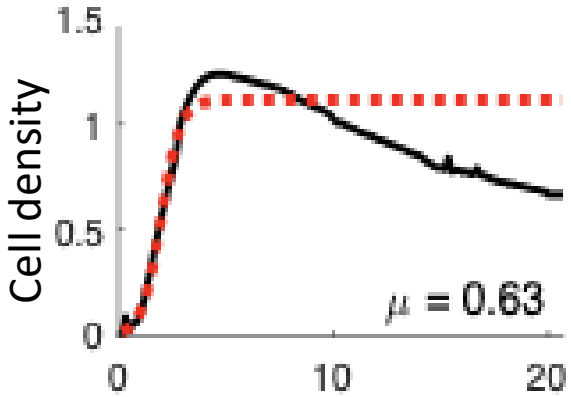


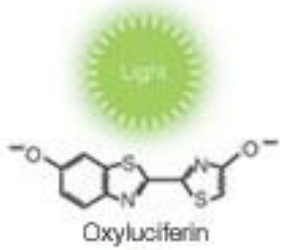
Different - % of glucose
- % of casamino acids



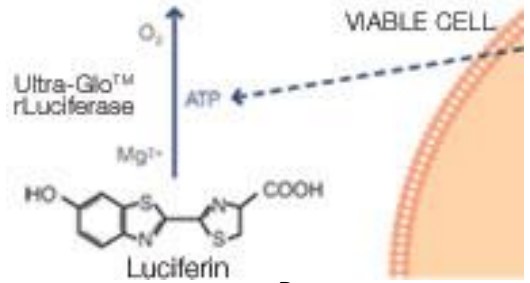
Measure cell density every 10 minutes

Extract growth curve using MATLAB





Bactiter-Glow



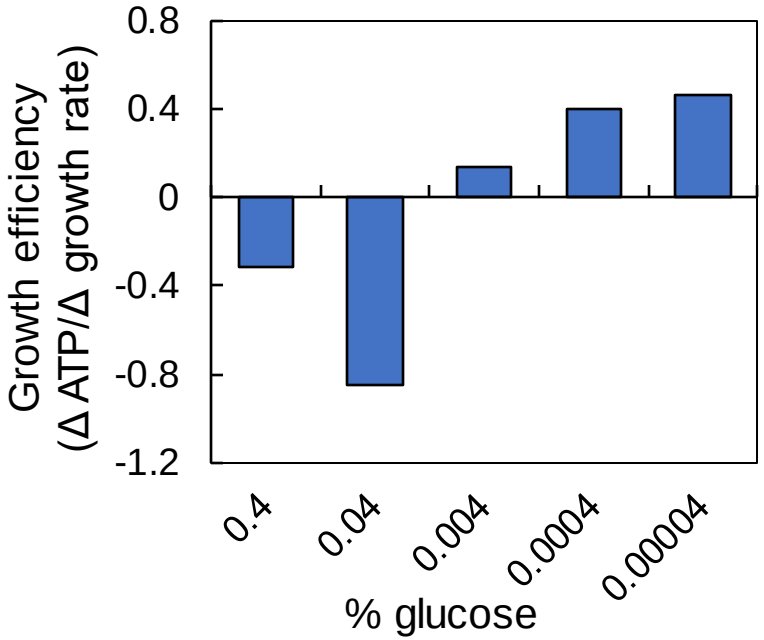
Measure ATP as a proxy for metabolism

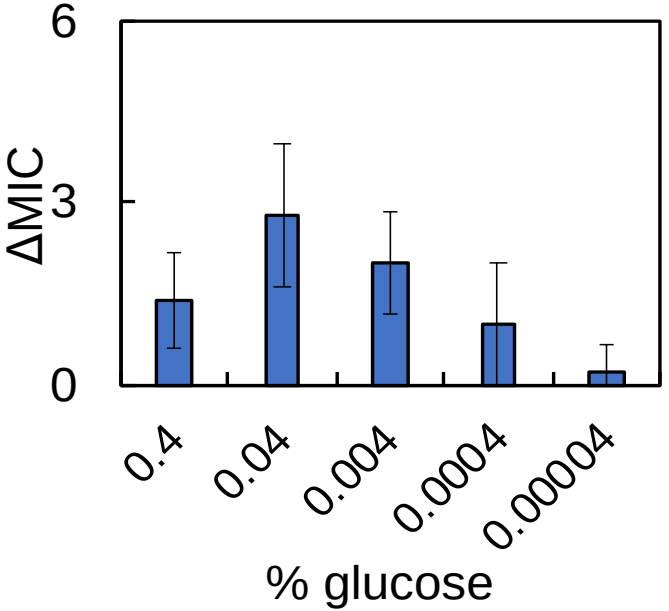
Biorender

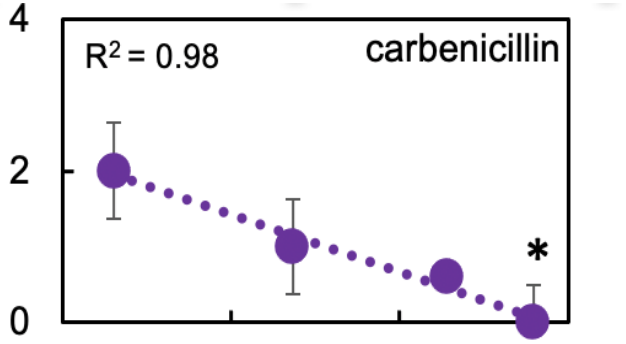
amazon

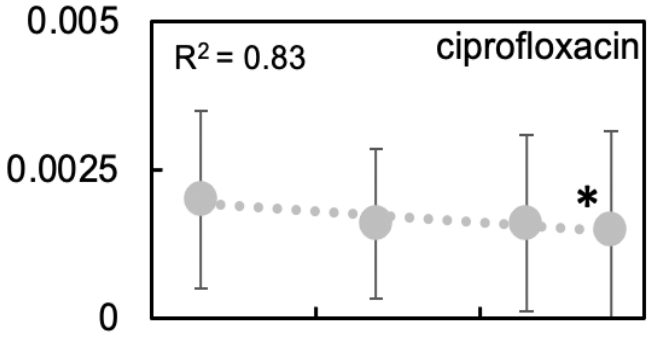
Promega

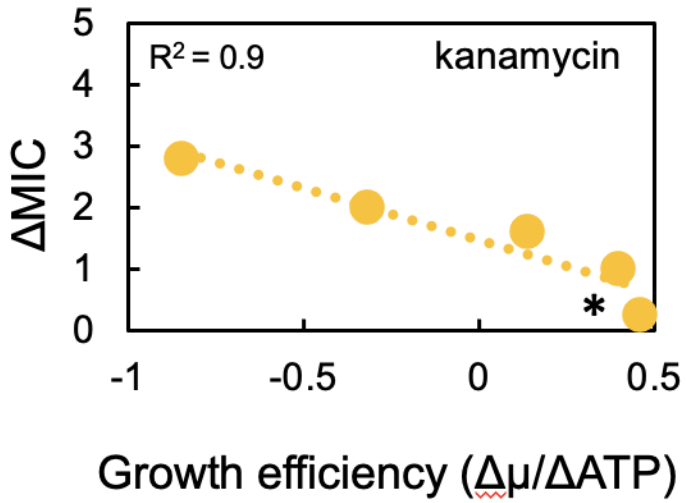
Growth efficiency of glucose determines IE (ΔMIC)

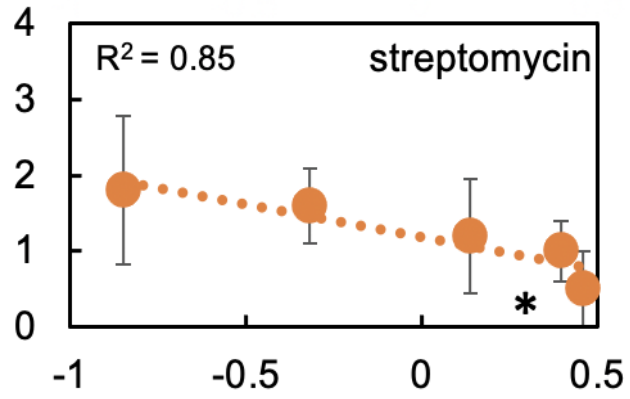


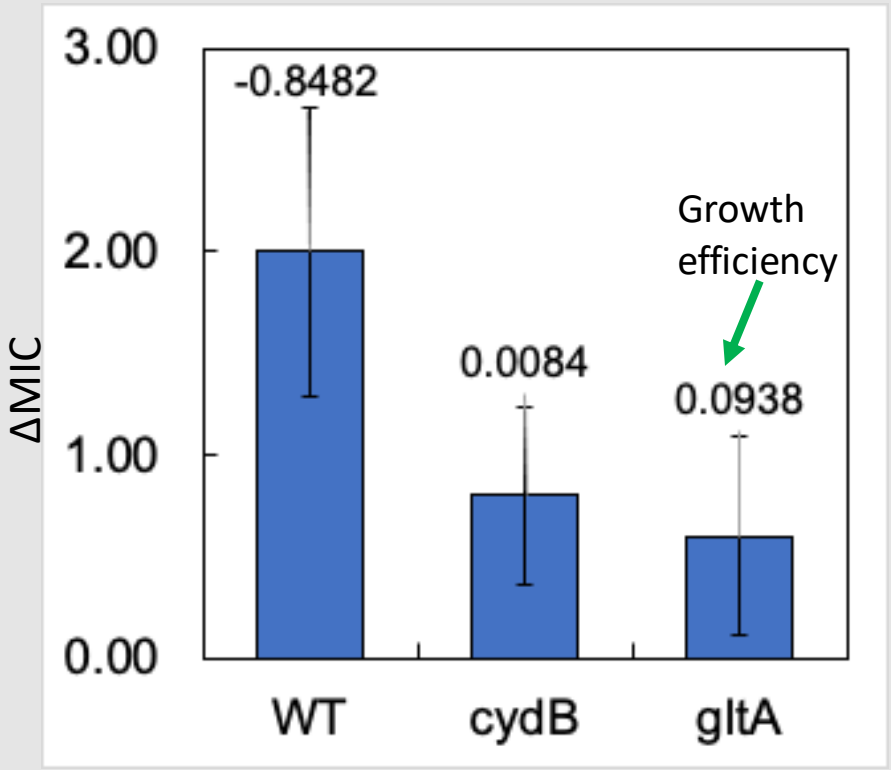






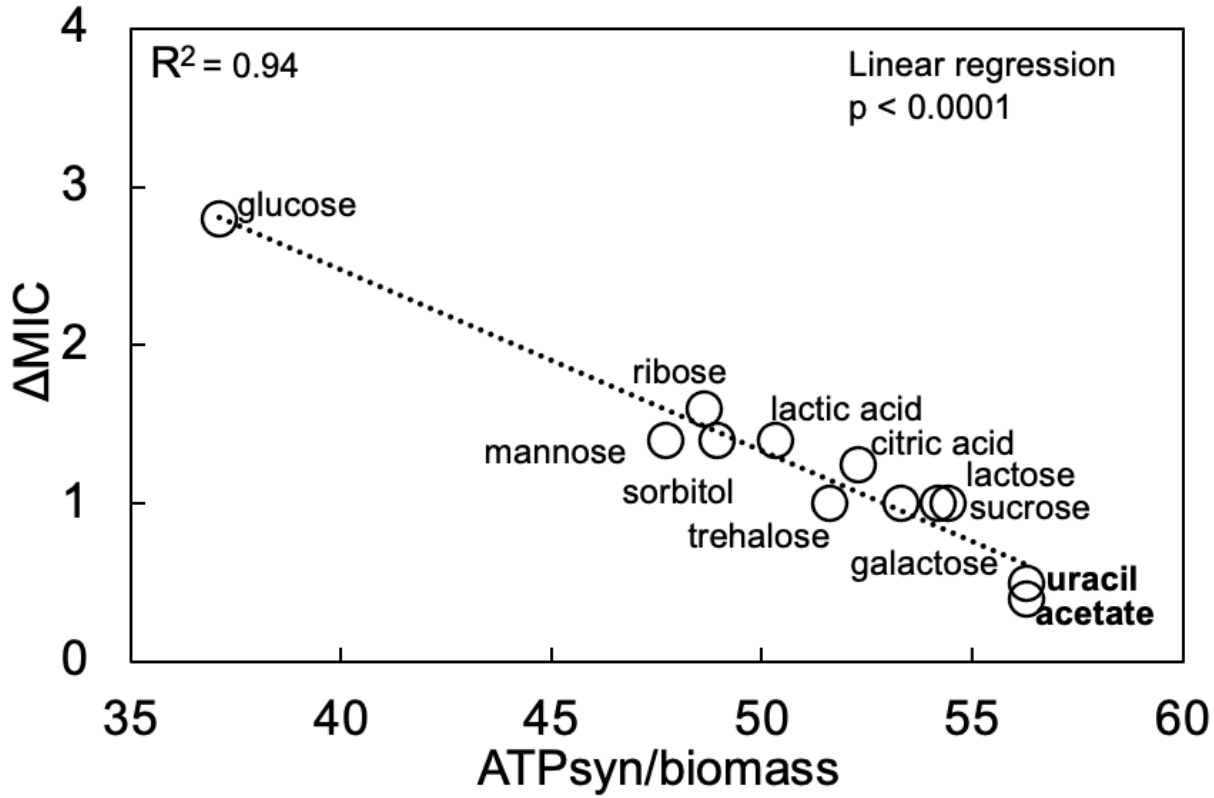


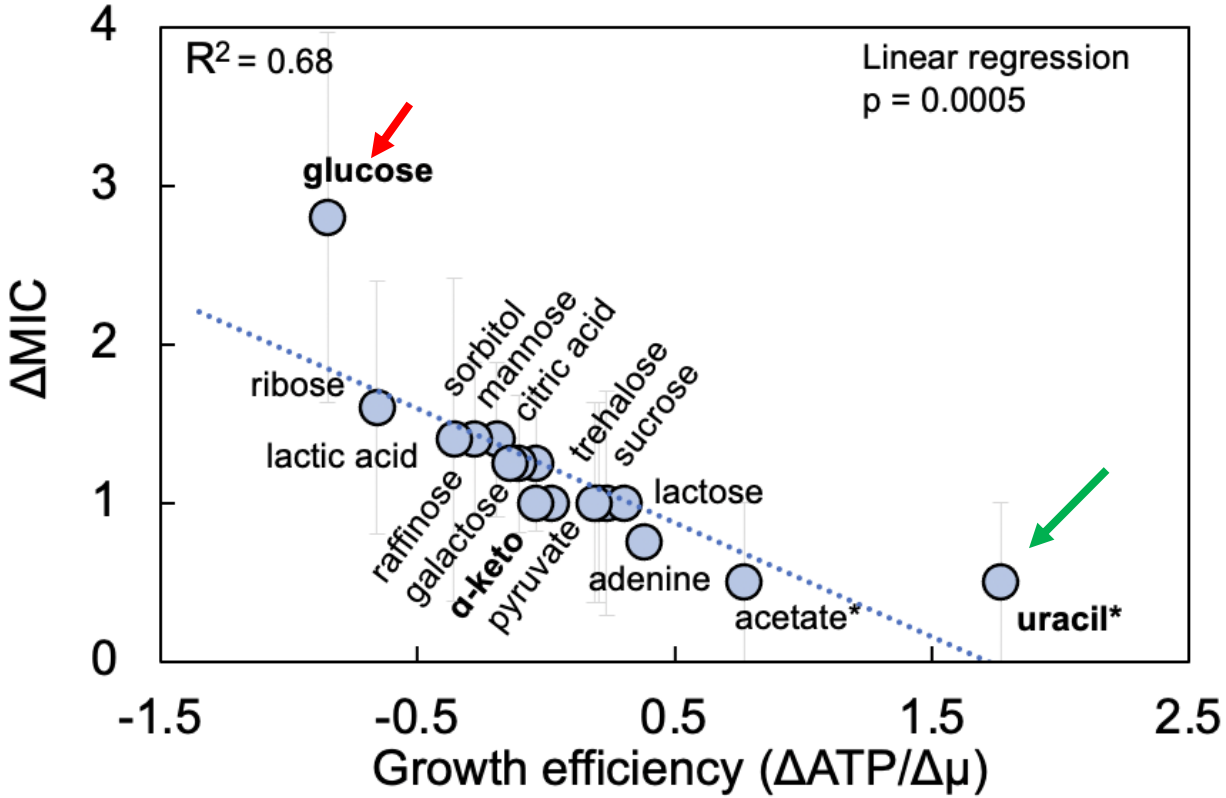




Increasing growth efficiency decreases IE

- Grown in 0.04% glucose, so the only difference is the knockout gene
- Reduced metabolism = increased resistance (higher MIC overall)
- As growth efficiency increases, the strength of IE (ΔMIC) decreases



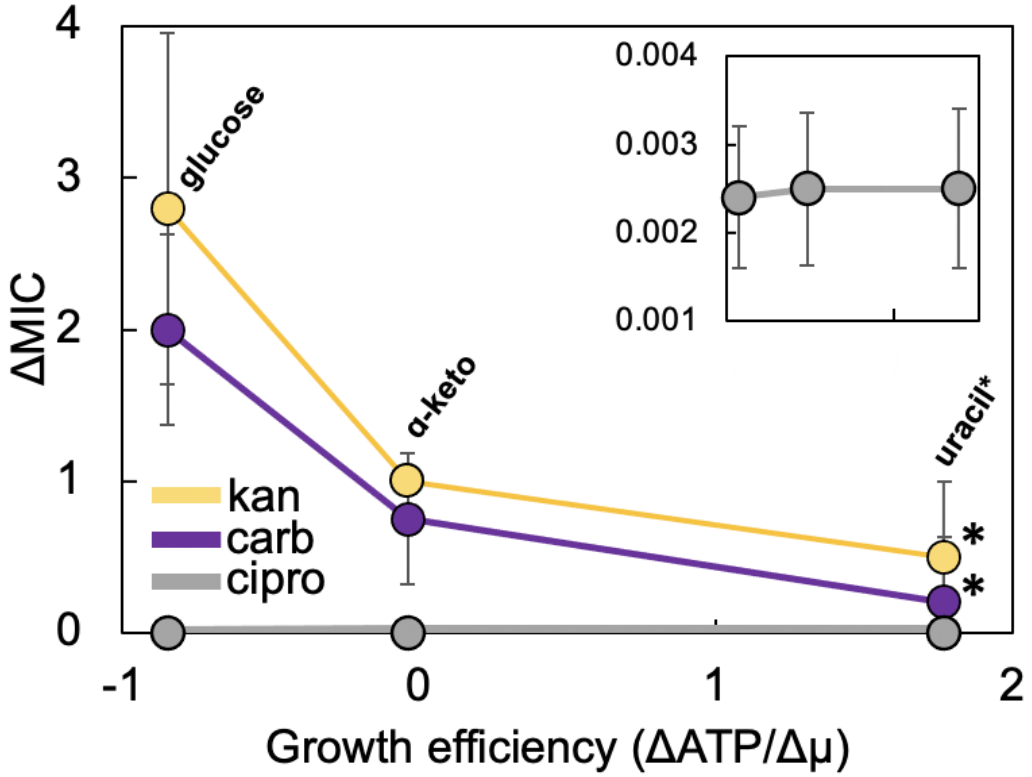


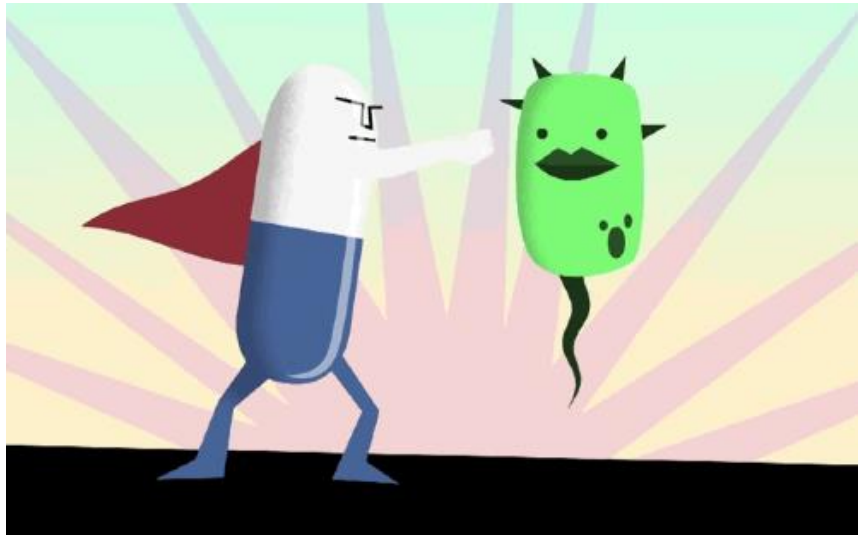
Flux balance analysis can accurately predict growth efficiency

- Changing carbon sources/metabolites alters growth efficiency
- Lowest growth efficiency for glucose = increased IE
- Uracil abolishes IE

Growth efficiency determines IE for many growth environments

- Coupling antibiotic treatment with metabolic adjuvants increased growth efficiency, which reduced ΔMIC for three antibiotic classes. (* indicates ΔMIC is no different than zero).





What do these results mean in the greater context of antibiotic resistance?

- Growth efficiency can determine the strength of the inoculum effect
- Relationship between growth rate and metabolism that depends upon the food source
- Co-administering a metabolite as an adjuvant along with antibiotics could reduce or eliminate inoculum effect
- Extend the usefulness of existing drugs
- Testing in animal models

WhatCulture, 2016

The Smith Lab

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JewishVeg, 2020

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