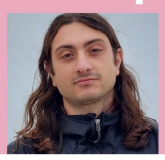
Environmental composition and initial metabolic states shape antifungal resistance evolution in *Candida albicans*



contact:
Burak Demirbas
b.demirbas@uva.nl

Burak Demirbas & Meike Wortel

Molecular Biology and Microbial Food Safety, Swammerdam Institute of Life Science, University of Amsterdam

Evo Sys Bio Lab

Background

- Pathogenic fungi are becoming increasingly **resistant** to antifungal drugs
- *Candida albicans* is a leading cause of infections worldwide
- Clinical isolates show varying **combinations** of **resistance mechanisms**: drug efflux, reduced drug influx, overexpressing drug targets

Why do *Candida* cells combine multiple resistance mechanisms? Adaptation or chance?

Model formulation

Based on an earlier metabolic model for *S. cerevisiae* (Wortel et al., 2016).

Assumptions:
- External conditions (glucose, fluconazole, ethanol) fixed
- Metabolic modes: respiration (**aerobic**) and fermentation (**anaerobic**)
- Fluxes through pathways described by:

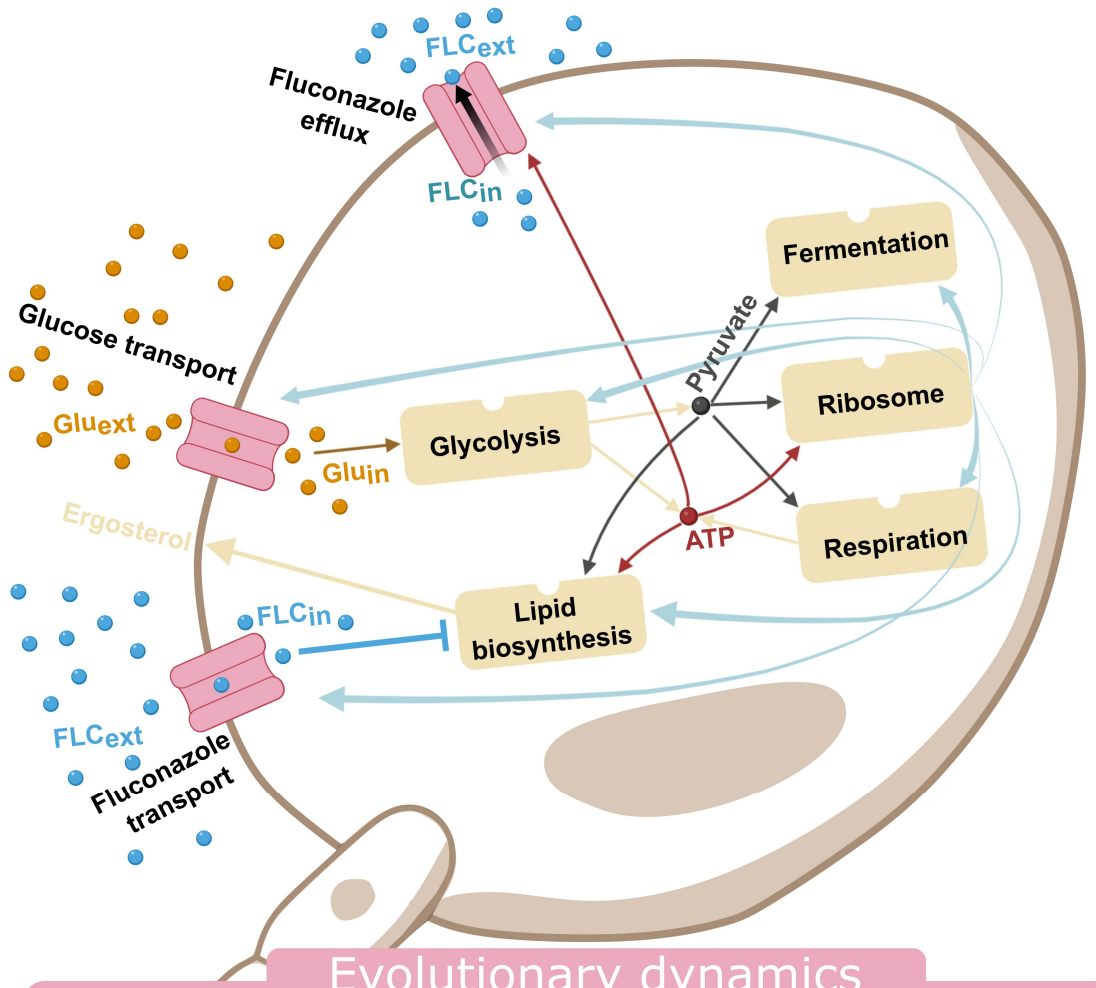
$$flux = k_{cat} * [enzyme\ level] * f(metabolites)$$

- Balanced growth:

$$growth\ rate = \frac{flux\ through\ ribosomes}{total\ enzymes\ needed\ to\ make\ new\ cell}$$

Mechanism of fluconazole (**FLC**):
- Widely used antifungal drug for treatment of *Candida*
- Inhibits **ergosterol biosynthesis**, weakening cell membrane, included as an inequality constraint:

$$\frac{membrane\ transporter\ proteins}{ergosterol\ in\ membrane} \leq 2$$



Methods

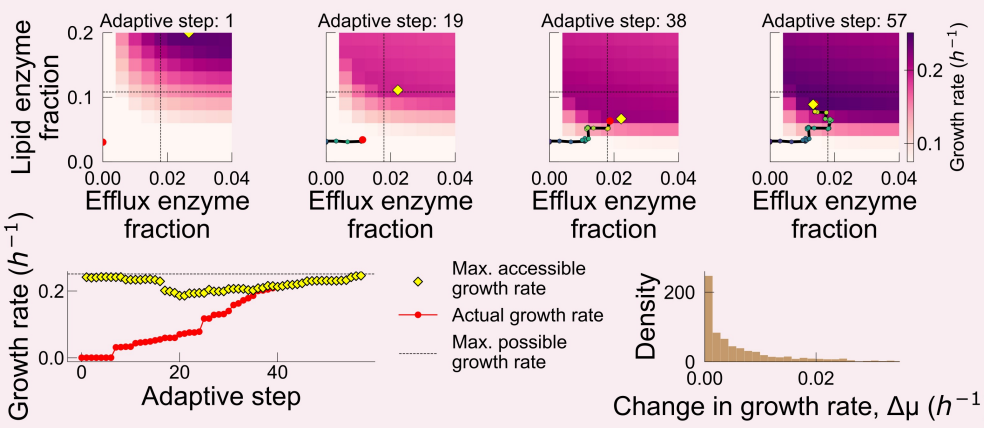
Equations solved either by:
- Non-linear **optimization**:

Allocation of metabolites & enzymes that result in fastest growth
- **Stochastic** evolutionary simulations:

Mutate enzyme levels & select for growth rate

Evolutionary dynamics

Mutation and selection of enzyme allocation (**anaerobic**)

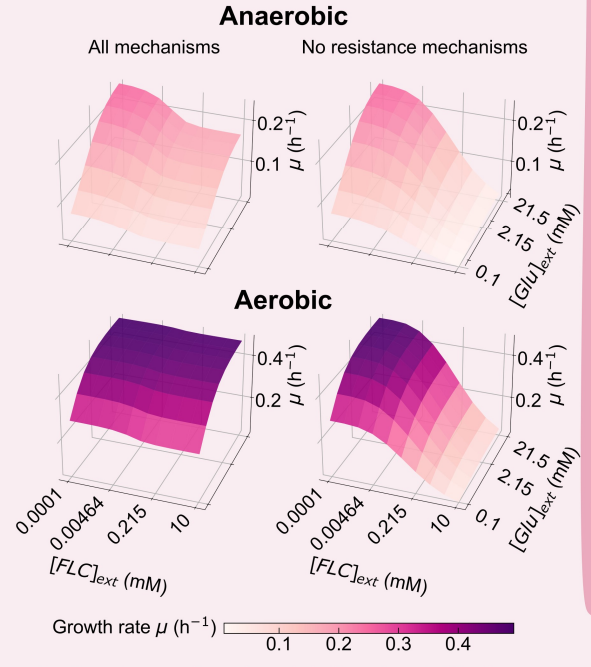


- **Stochastic** walk to global optimum

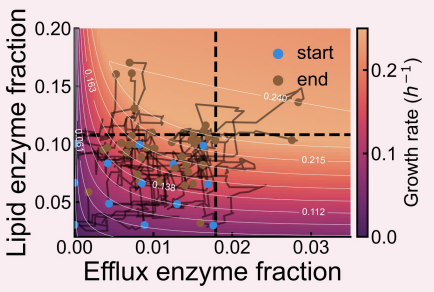
- Metabolic adaptation as well as resistance mechanisms: shift in accessible growth rate
- Effect sizes follow **exponential** distribution

Results

Optimal growth



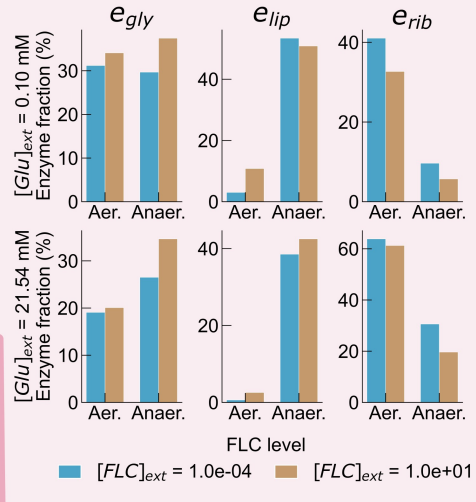
- Optimal growth rates (**μ**): "end point" of evolution, and proxy for resistance
- **Combination** of resistance mechanisms (efflux, target overexpression, reduced influx) very effective at rescuing growth rate
- **Environment** (nutrients, antifungal, oxygen) determines optimal/superior resistance mechanism

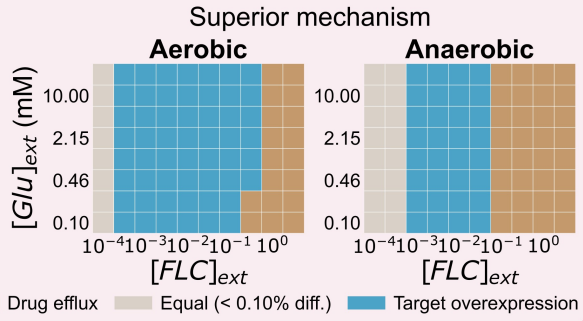


- Initial enzymatic state determines likely evolutionary path
- "Overshooting" of optima (black dashed lines)
- Distinct **resistance phenotypes**, but similar growth rates/resistance levels!

Optimal enzyme allocation

- Metabolic adaptation: increased **glycolysis** and reduced **ribosomes**
- Interestingly, sometimes target (**lipid** enzyme) overexpression does not occur at all
- Large quantitative differences between aerobic and anaerobic conditions





Take-home messages

- There is no universal resistance strategy: environment (nutrients, drugs, oxygen) determines what works best
- Evolution of resistance is accompanied by significant metabolic changes
- Knowing the initial enzymatic states of cells could allow us to predict resistance evolution trajectories

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