Apr 15th, 11:00 AM - 12:00 PM

Better than Before and Better Together

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BETTER THAN BEFORE AND BETTER TOGETHER

Fitness as a Function of Diversity in a Simple Microbial Community

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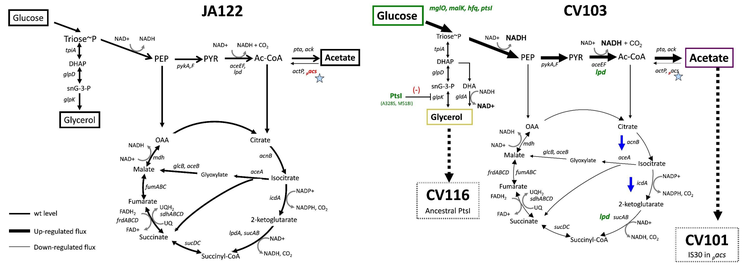
Cooperation as a Function of Complexity

- Fitness is the capacity to survive and produce offspring that themselves survive and reproduce; fitness can be measured as the capacity of a variant type to displace another type in competition for available resources.
- Symbioses, such as mutualism, are pervasive features of the natural world, thus collaboration may be just as important as competition in driving biological innovation.
- Although collaborative interactions are pervasive in Nature, do they actually increase the fitness of collaborating partners?
- Are collaborative systems that are more complex, consisting of multiple variants, more productive than simpler systems consisting of one or few variants?

Previous Work Shows:

Strains	Releant characteristics	Growth rate hr^{-1} ± SEM	Glucose uptake µmol α-MG/min/gm	Steady state [Glucose] µM	Steady state [Acetate] µM	Glycerol uptake pmol/min/10^8cell
JA122=A	Derivative of RH 201, F$^-$ thi1 lacY1 tonA21, supE44 hss1, araD139, lysogenic for λ, contains plasmid pBR322Δ5, Amp^R	0.44 ± 0.01	1.19 ± 0.09	1.84 ± 0.48	194 ± 0.20	99 ± 8
CV103=E3	Derivative of JA122; isolated after 773 generations, forms small colonies on Tryptone Agar (TA), Amp^R	0.40 ± 0.01	2.46 ± 0.16	0.07 ± 0.03	252 ± 70	104 ± 7
CV101=E1	Derivative of JA122; isolated after 773 generations, forms large colonies on TA, Amp^R	0.50 ± 0.02	1.66 ± 0.06	0.88 ± 0.48	0 ± 0	93 ± 7
CV116=E6	Derivative of JA122; isolated after 773 generations, forms large colonies on TA plate, lacks plasmid, Amp^S	0.60 ± 0.01	1.61 ± 0.11	0.19 ± 0.05	40 ± 25	104 ± 14

In 1987 Helling et al. evolved in the laboratory a community of E. coli strains starting with a single common ancestor. The population was cultured for 773 generations with glucose as a limiting resource.



Relative to the common ancestor (**A**), a community of its descendants may better metabolize the limiting nutrient, glucose. One strain, CV103 (**E3**), avidly takes up glucose but metabolizes it wastefully, releasing acetate and glycerol that become differentially accessible to strains, CV101 (**E1**) and CV116 (**E6**). This interaction is known as **Cross-Feeding**.

Questions

Are *E. coli* that evolved into a community more fit than their common ancestor, which was a single clone?

Does collaboration among evolved *E. coli* boost fitness?

If collaborating variants are more fit, are they also, as a group, more productive?

Results

Part 1: The Reconstruction

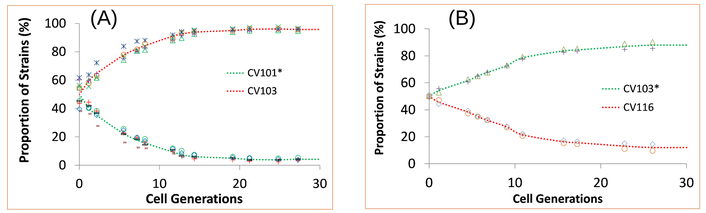


Figure 1 A, B Reconstruction of the consortium with glucose scavenger, strain E3 (CV103) **and either of two waste consuming clones E1 and E6** (CV101 & CV116). E3 is always most abundant; cross-feeding is inferred from the fact frequencies are constant after ~15 generations. When **E** strains are grown, individually or collectively, in the presence of A, their common ancestor, **A** is eliminated after ~20 generations.

Part 2: Fitness Comparison

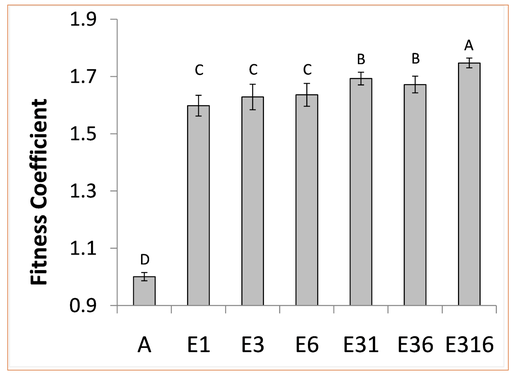


Figure 2. Differences in fitness between individuals and groups, relative to their common ancestor, A. Letters signify groups with statistically equivalent finesses. Fitness (E1+E3+E6)>(E1+E3)=(E3+E6)>E3=E6=E1>A. Fitness coefficients were calculated as the slope of the linear regression ln(experimental/reference), as a function of elapsed generations. Cell generations elapsed equals (time * dilution rate)/ln2.

Part 3: Productivity Comparison

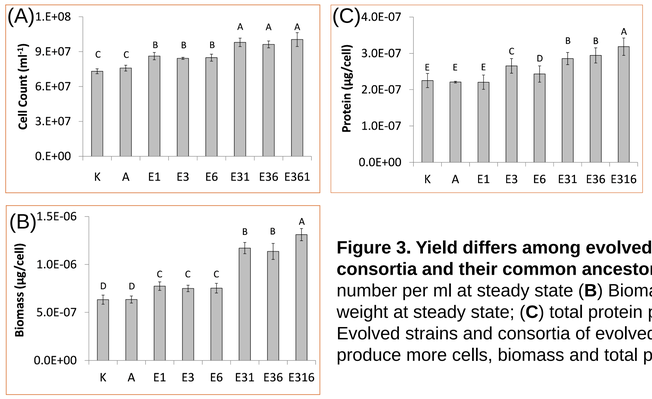


Figure 3. Yield differs among evolved strains, consortia and their common ancestor. (**A**) cell number per ml at steady state (**B**) Biomass dry weight at steady state; (**C**) total protein per cell. Evolved strains and consortia of evolved strains produce more cells, biomass and total protein.

Methods

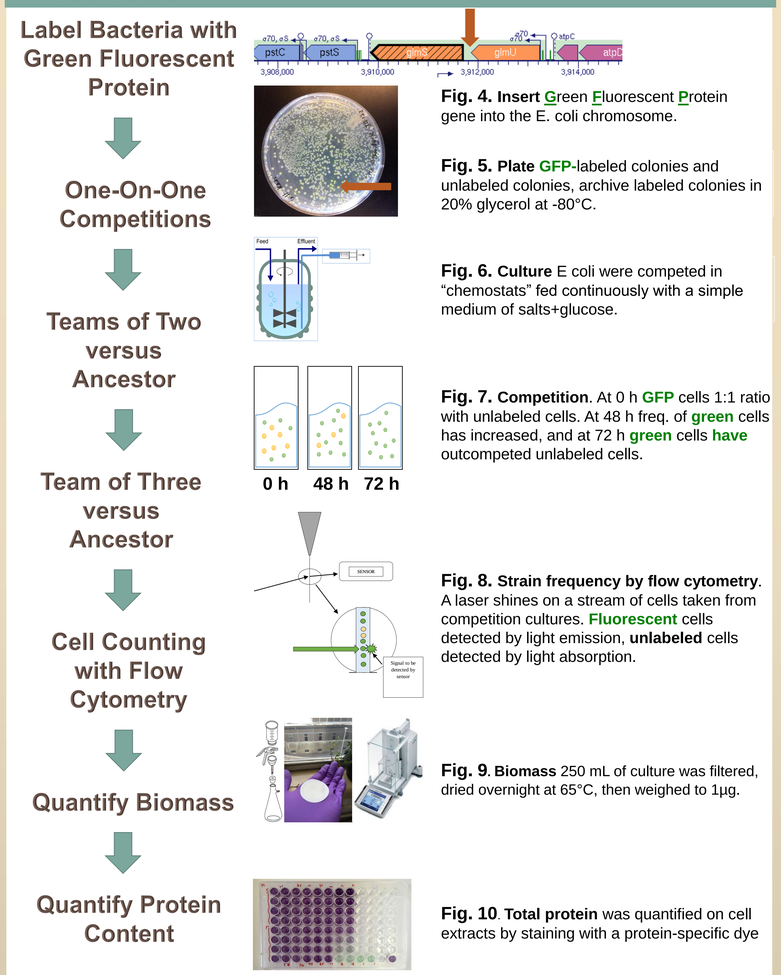


Fig. 4. Insert Green Fluorescent Protein gene into the E. coli chromosome.

Fig. 5. Plate GFP-labeled colonies and unlabeled colonies, archive labeled colonies in 20% glycerol at -80°C.

Fig. 6. Culture E coli were competed in "chemostats" fed continuously with a simple medium of salts+glucose.

Fig. 7. Competition. At 0 h **GFP** cells 1:1 ratio with unlabeled cells. At 48 h freq. of **green** cells has increased, and at 72 h **green** cells **have** outcompeted unlabeled cells.

Fig. 8. Strain frequency by flow cytometry. A laser shines on a stream of cells taken from competition cultures. **Fluorescent** cells detected by light emission, **unlabeled** cells detected by light absorption.

Fig. 9. Biomass 250 mL of culture was filtered, dried overnight at 65°C, then weighed to 1µg.

Fig. 10. Total protein was quantified on cell extracts by staining with a protein-specific dye

Conclusions

- **Co-evolved communities can be reconstructed in lab.**
- **Evolved clones are all more fit than their common ancestor, but not more fit than each other.**
- **Community fitness is greater than individuals' fitness, but fitness is not additive**
- **Community fitness and productivity increases as a function of its genetic complexity.**

Acknowledgements

DDY, AA and FR were funded by NNX12AD87G-EXO from NASA, AC and AR by University of Toronto Cancer Center