
Modelling Competition for Nutrients between Microbial Populations Growing on Solid Agar Surfaces

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ABSTRACT

Motivation: Growth rate is a major component of the evolutionary fitness of microbial organisms. When nutrients are plentiful, fast-growing strains come to dominate populations whereas slower-growing strains are wiped out. This makes growth rate an excellent (a useful) surrogate for the health of cells. Measuring the health of cells grown in different genetic backgrounds or environments can inform about genetic interaction and drug sensitivity. In high-throughput procedures such as QFA and SGA, arrays of microbial cultures are grown on solid agar plates and quantitative fitness estimates are determined from growth measurements. Diffusion of nutrients along gradients in nutrient density arising between fast- and slow-growing neighbours is likely to affect growth rate and fitness estimates. However, current analyses assume that cultures grow independently. We study data from QFA experiments growing *Saccharomyces cerevisiae* to test a mass action kinetic model of nutrient dependent growth and diffusion. We try to correct for competition to provide more accurate and precise fitness estimates.

Results: Don't know what to say yet.

Availability and Implementation: CANS, a Python package developed for the analysis in this paper, is freely available from [github](https://github.com/danielboocock/CANS).

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1 INTRODUCTION

The bacteria *Escherichia Coli* and yeast *Saccharomyces cerevisiae* are unicellular organisms studied as a model prokaryote and eukaryote respectively. They grow in colonies, where cells may (be clones originating from a single cell or) belong to different genetic strains originating from different individual cells. In favourable conditions, growth is exponential and this makes growth rate a major component of fitness; faster growing strains quickly come to dominate the population. At a certain point growth becomes limited and a stationary phase is reached. For unicellular organisms, growth rate is equal to cell cycle progression rate. Evolutionary pres-

sure has led to rapidly dividing organisms (such as *E. Coli* and *S. cerevisiae*) with compact genomes of essential genes. These genes have been conserved in other species over billions of years of evolution. The eukaryote *S. cerevisiae*, is particularly useful for the study of other eukaryotes such as humans.

The growth rate of microbial organisms is measurable and is often used to determine fitness. In experiments, cell cultures are commonly grown in two types of medium. In spot tests (phenotypic array), cultures are pinned or inoculated on the surface of a solid agar containing nutrients. In liquid culture assays, cultures are mixed in a liquid medium containing nutrients. In both cases cultures are incubated and growth is observed. Identical strains can grow differently between the two mediums and disagreement in fitness estimates is currently an issue Baryshnikova *et al.* (2010a) (I couldn't find a paper specifically talking about this issue but they have a correlation plot Fig2a where correlations are worse with a liquid culture study by Jasnos and Korona; in fact the Baryshnikova paper Fig3c seems to say that they had strong correlation in their "high-resolution liquid growth profiling study"). I do not focus on this issue and exclusively study fitness screens using solid agar.

Fitness estimates can be used to infer genetic interaction or drug response and high-throughput methods allow this to be conducted on a genome-wide scale (see e.g. Costanzo *et al.* (2010); Andrew *et al.* (2013)). In a typical genetic interaction screen, a strain is made with a mutation in a query gene. Double mutants are created by introducing a second deletion in this strain. By comparing the growth of double mutants with a control containing a neutral deletion, genetic interactions can be inferred. If a strain is fitter than the control then the deletion is said to suppress the defect of the query gene. If a strain is less fit than the control then the deletion is said to enhance the defect of the query gene. Either scenario suggests that the two genes interact and have a related function. Due to redundancy, single deletions are often non-lethal. (Remove: Knock downs and conditional mutations can also be used.) This allowed

Costanzo *et al.* (2010) to explore $\sim 75\%$ of the *S. cerevisiae* genome.

Synthetic Genetic Array (SGA) and Quantitative Fitness Analysis (QFA) are high-throughput methods for obtaining quantitative fitness estimates of microbial cultures grown on solid agar plates (Baryshnikova *et al.*, 2010b; Banks *et al.*, 2012). Typically one query gene and many replicates of each deletion are contained in a rectangular array on each plate. Many plates with different query genes and deletions can be grown in high-throughput to explore whole genomes. I study data from QFA which refers to quantitative estimation of fitness by measurement and fitting of growth curves.

In a typical QFA procedure liquid cultures are inoculated onto solid agar in a 384 in a 16x24 array. Initial inoculum density is allowed to vary and can be as low as ~ 100 cells per culture in order to capture as much of the growth curve as possible.

QFA typically uses 384 liquid cultures inoculated onto solid agar and cell density is measured throughout the growth curve. Fitness estimates are made by fitting the measured growth curves.

In SGA 1536 cultures are pinned onto solid agar and an endpoint assay of culture area is used as a growth estimate.

In QFA typically 384 liquid cultures are inoculated onto a solid agar plate and cell density is measured throughout the growth curve. Fitness estimates are made by fitting the measured growth curves.

QFA collects more information about growth by taking images of plates at points throughout the growth curve. QFA can be performed using either the pinned cultures (used in SGA) or dilute liquid cultures (“spots”) of lower initial cell density. Although pinned QFA allows for more cultures per plate (1536 vs 384 in spotted), spotted QFA allows for more accurate fitting of growth models as growth curves are more complete (see Figure ??) (Lawless *et al.*, 2010). Comparison of spotted and pinned QFA cultures in Figure ??c shows how spotted cultures are composed of many individual colonies which increase in size and thickness, whereas pinned cultures are composed of a single uniform colony which grows radially. The number of individual colonies in a spotted culture is high enough that lag and other stochastic effects should average out.

1.1 Subsection

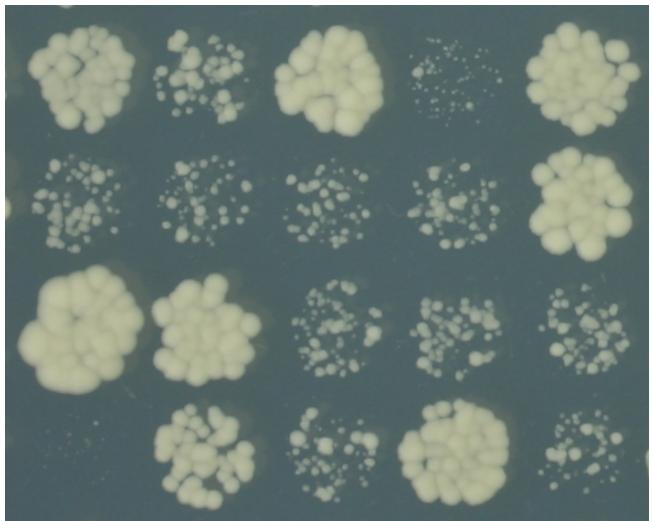


Figure 1: 4x5 section of a QFA plate. Taken from a 16x24 format solid agar plate inoculated with dilute *S. cerevisiae* cultures. Image captured at $\sim 2.5d$ after inoculation and incubation at $27^\circ C$.

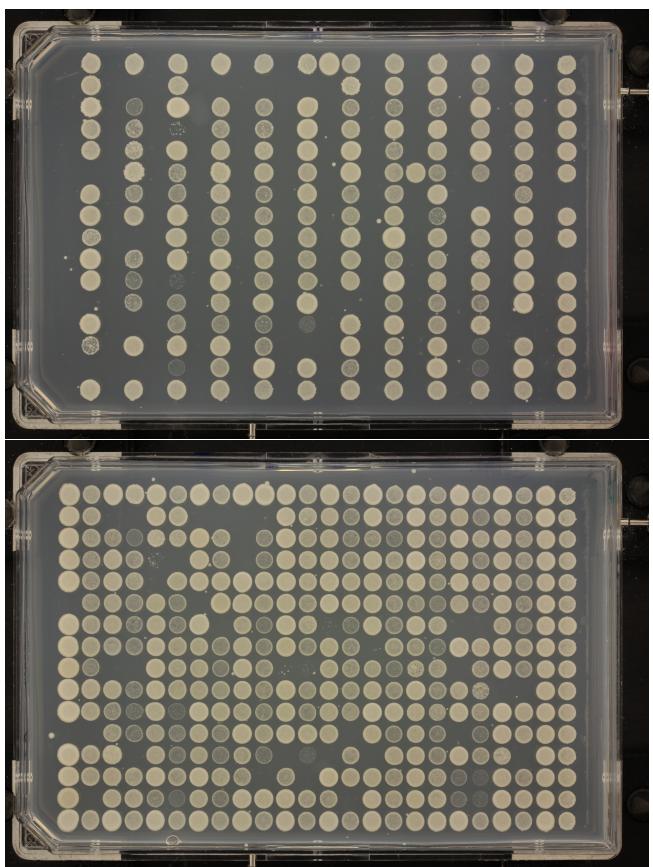


Figure 2: An experiment designed to examine competition. A) QFA plate inoculated with a more concentrated *S. cerevisiae* inoculum (no cells inoculated on alternative columns). B) Same as in A, but with strains of similar growth rate inoculated in the positions missing in A.

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