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

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August 13, 2016

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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 (Baryshnikova *et al.*, 2010b; Banks *et al.*, 2012). Typically one query gene and replicates of deletion are contained in a rectangular array on a solid agar 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 (containing nutrients (already mentioned above)) often with 384 cultures in 16x24 rectangular array. Initial inoculum density can be varied to capture more or less of the growth curve. Dilute cultures are inoculated with  $\sim 100$  starting cells (Addinall *et al.*, 2011). Plates are grown in incubation. Photographs of whole plates are taken at timepoints throughout growth which typically covers several days and captures the exponential and stationary growth phases. Photographs are processed by Colonyzer (Lawless *et al.*, 2010) to produce cell density estimates from the optical density of cultures. Past analysis independently fit the logistic model, which describes self-limiting growth, to the timecourse of each culture and quantitative fitness estimates are defined in terms of the growth constant,  $r$ , and carrying capacity,  $K$ . In contrast, SGA typically uses an array of 1536 pinned cultures and an endpoint assay of culture area (a single measurement) to quantify growth. The differential form and solution of the logistic model (Verhulst, 1845) (probably don't need this reference) are given in Equations 1, where  $C$  represents cell density and  $C_0$  is cell density at time zero.

$$\dot{C} = rC \left(1 - \frac{C}{K}\right) \quad (1a)$$

$$C(t) = \frac{KC_0 e^{rt}}{K + C_0(e^{rt} - 1)} \quad (1b)$$

Fitting the logistic model to QFA data requires plate level or culture level parameters for  $C_0$  and culture level parameters for  $r$  and  $K$  making 769 or 1152 parameters per plate of 384 cultures.

The growth constant  $r$  could be used as a fitness measure. However, Addinall *et al.* (2011) define a more complicated fitness measure as the product of Maximum Doubling Rate (MDR) and Maximum Doubling Potential (MDP) which they derive from logistic model parameters. MDR measures the doubling rate at the beginning

of the exponential growth phase, when growth is fastest, and MDP is the number of divisions which a culture undergoes from inoculation to the stationary phase.

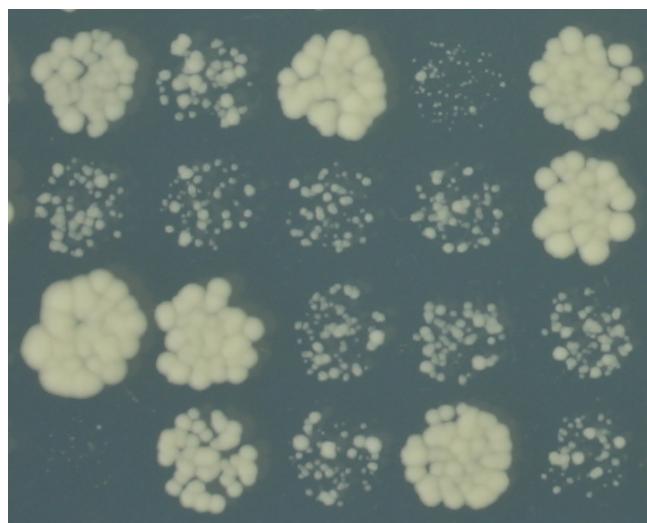
$$MDR = \frac{r}{\log\left(\frac{2(K-C_0)}{K-2C_0}\right)} \quad (2a)$$

$$MDP = \frac{\log\left(\frac{K}{C_0}\right)}{\log(2)} \quad (2b)$$

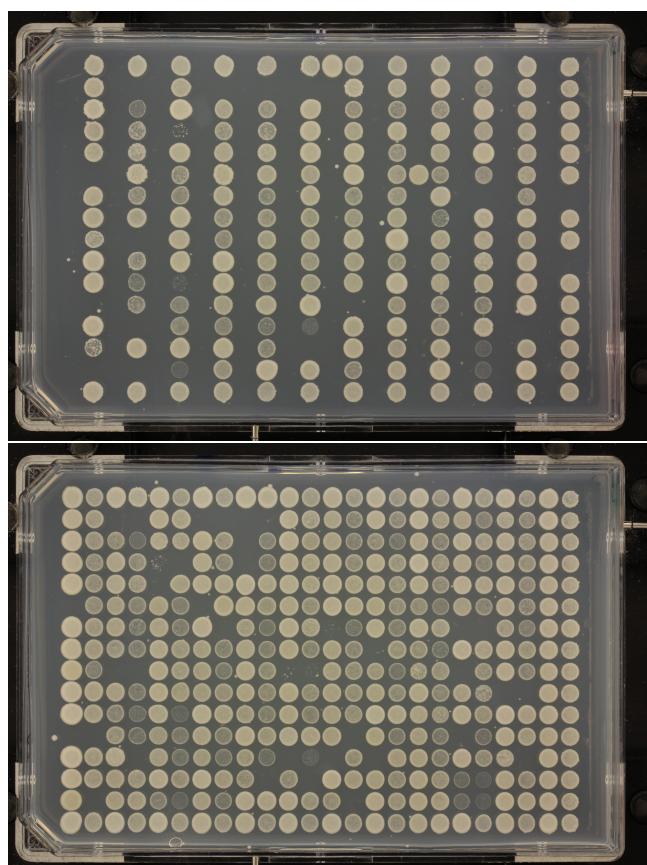
To improve the quality of fits QFA now uses the generalised logistic model which requires an extra parameter for each culture.  $r$  of the standard and generalised logistic model are not equivalent or comparable so comparison relies on MDR as a fitness measure. The analysis of QFA data using both the standard and generalised logistic models is available through the QFA R package (Lawless *et al.*, 2016).

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 ~2.5d after inoculation and incubation at 27°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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