Predicting microbial growth in a mixed culture from growth curve data

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**Classification**: Biological Sciences, Population Biology

**Keywords**: population dynamics, microbial growth, competition model, experimental evolution

# Abstract

Estimates of microbial fitness from growth curves are inaccurate. Rather, competition experiments are necessary for accurate estimation of fitness. However, competition experiments require distinct markers, are difficult to perform with isolates derived from a common ancestor or non-model organisms, and do not provide information on the underlying growth differences. Here we describe a new computational approach for predicting growth of microbes in a mixed culture utilizing mono- and mixed culture growth curve data. We validated this approach using growth curve and competition experiments with *E. coli*. Our approachprovides an effective way to predict growth and infer relative fitness in mixed cultures. Furthermore, by integrating several growth phases, it provides an interpretation for microbial fitness.

# Significance statement

We present a new model-based approach for prediction of microbial growth in a mixed culture using data from growth curve experiments, which are considerably easier to perform than competition experiments. Our approach combines growth and competition models and utilizes the total densities of mixed cultures, which are often ignored despite being easy to obtain. We validated our approach using experiments with bacteria and implemented it in an open-source software package. Our new approach shows that mixed growth can be predicted using growth and competition models. It provides a way to infer relative strain or species frequencies even when competition experiments are not feasible, and to determine how differences in growth affect differences in fitness.

# Introduction

Microbial fitness is usually defined as the relative growth of different microbial strains or species in a mixed culture (1). Pairwise competition experiments can provide accurate estimates of relative growth and fitness (2), but are laborious and expensive, especially when used with non-model organisms. Instead, growth curves are commonly used to estimate fitness of individual microbial isolates, despite clear evidence that they provide an insufficient alternative (3, 4).

Growth curves describe the density of cell populations in liquid culture over time and are usually acquired by measuring the optical density (OD) of cell populations. The simplest way to infer fitness from growth curves is to estimate the growth rate during the exponential growth phase by estimating the slope of the log of the growth curve (5), see example in **Figure 1**. Relative growth rates are often used as a proxy for the selection coefficient, *s*, where *1+s* is the standard measure of relative fitness in population genetics (1, 6). However, exponential growth rates do not capture the dynamics of other phases of a typical growth curve, such as the duration of the lag phase and the cell density achieved at stationary phase (7), see **Figure 1A**. Moreover, the maximal specific growth rate is not typical for the entire growth curve (**Figure 1B**). Thus, it is not surprising that growth rates are often poor estimators of relative fitness (3, 4).

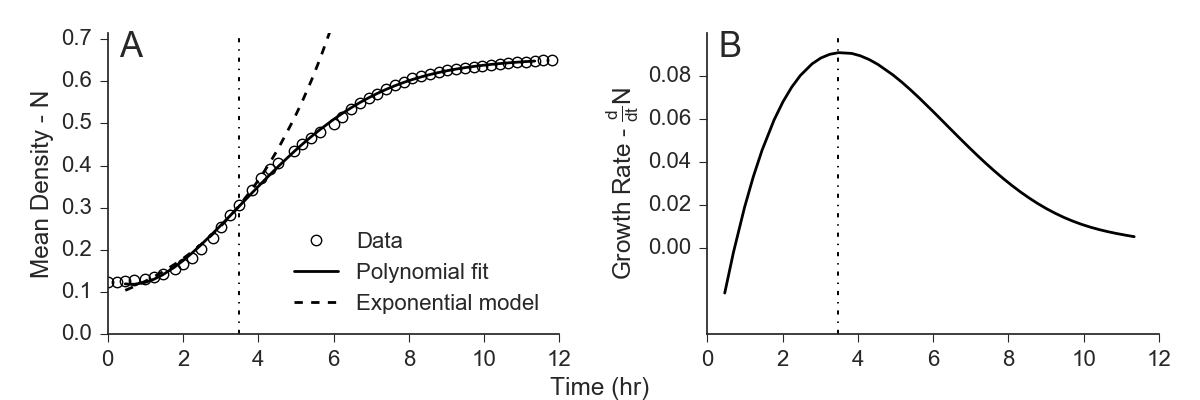
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Figure 1. Common approach for analyzing growth curve data using an exponential model. Growth rates are commonly estimated from growth curves data by taking the log of the growth curve and performing linear regression around the time of maximum growth (see Materials and Methods for specific details). Implicitly, this is equivalent to fitting an exponential growth model *N(t)=N0ert* to the growth curve. (A) The circle markers represent *N(t)* the mean cell density in 22 growth curves. The solid line represents a smooth line through the points (i.e. by fitting a polynomial). The dashed line represents the exponential model *N0ert* fitted to the data, with *r=0.35* and *N0=0.088*. The dotted vertical line denotes *tmax*. (B) The solid curve shows *dN/dt,* the derivative of the mean density (calculated as the derivative of the solid line in panel A). The dotted vertical line denotes *tmax*. Data in this figure corresponds to the growth of strain A1 (red markers in panel A1 of Figure 2).

In contrast, competition experiments can infer relative fitness in a manner that accounts for all growth phases (8). In pairwise competition experiments, two strains, a reference strain and a strain of interest, are grown in a mixed culture. The frequency of each strain in the mixed culture is measured during the course of the experiment using specific markers, either by counting colonies formed by drug resistant or auxotrophic strains (8), by monitoring fluorescent markers with flow cytometry (2), or by counting DNA barcode reads using deep sequencing (9, 10). The selection coefficients of the strains of interest is then estimated from changes in the frequencies of the strains during the competition experiment. Such competition experiments can infer relative fitness with high precision (2), as they directly estimate fitness from changes in strain frequencies over time.

However, competition experiments are challenging because they are more laborious and expensive than simple growth curve experiments. Furthermore, they require that strains of interest be modified to monitor them with genetic or phenotypic assays (see (3) and references therein). Accordingly, competition experiments are often impractical in non-model organisms. Therefore, many investigators prefer to use proxies of fitness such as growth rates from simple growth curve experiments. Even when competition experiments are a plausible approach (e.g. in microbial lineages with established markers (8)), it is important to understand how differences in growth of different strains during different growth phases affect their relative fitness. Such understanding can have a crucial impact on our understanding of microbial fitness and the composition of microbial populations and communities (11).

Here we present a new computational approach thatprovides a predictive and descriptive framework for estimating growth parameters from growth dynamics and predicting relative growth in mixed cultures.

# Results

Our approach consists of three stages: (a) fitting growth models to monoculture growth curve data (**Figure 2A**), (b) fitting competition models to mixed culture growth curve data (**Figure 2B**), and (c) predicting relative growth in a mixed culture using the estimated growth and competition parameters. To test this approach, we measured growth of two *Escherichia coli* strains in mono- and mixed culture over time and used our new approach to predict the relative frequencies of both strains in the mixed culture. We then compared these predictions to empirical measurements of strain relative frequencies.

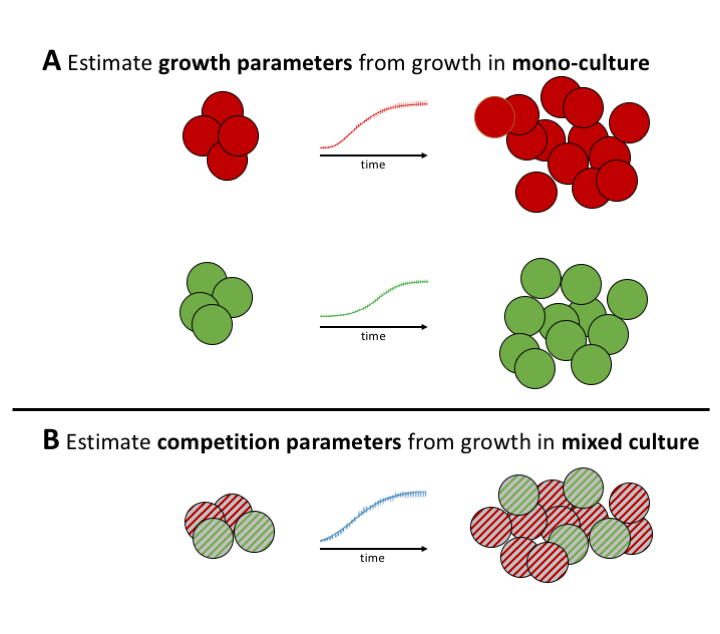


Figure 2. Illustration of the new computational approach. (A) Two strains (here marked as red and green) are grown separately in mono-cultures while measuring the cell culture density (e.g. optical density), and growth models are fitted to the measured growth curves from each experiment, providing estimates for the growth parameters of each strain. (B) The same two strains are grown in a mixed culture, and the *total* cell density of the mixed culture is measured. The resulting growth curve is fitted to a competition model, providing estimates for the competition parameters. Importantly, the cells are *not* identified by their strain, hence the gray-stripes in the figure.

## Experimental design

Three experiments (marked A, B, and C) were performed with different sets of *E. coli* strains marked with green and red fluorescent proteins (GFP and RFP, respectively). In each experiment, 32 replicate monocultures of the GFP strain, 30 replicate monocultures of the RFP strain, and 32 replicate mixed cultures containing the GFP and RFP strains together, were grown in 96-well plate format, under the same experimental conditions.

In experiment A, strain TG1 was labeled with RFP (strain A1) and strain DH5α was labeled with GFP (strain A2). Stationary phase bacteria were diluted into fresh media, yielding a lag phase culture in which lag phase was observably longer for the green strain. In experiment B, strain TG1 was labeled with RFP (strain B1) and strain DH5α labeled with GFP (strain B2). Both strains were pre-grown in fresh media for 4 hours (to exponential growth phase) and then diluted into fresh media, so that there was no observable lag phase. In experiment C, strain K12 MG1655-Δfnr was labeled with RFP (strain C1) and strain JM109 labeled with GFP (strain C2) and was performed as described for experiment A.

The optical density (OD595) of every well (i.e. mono-cultures and mixed culture wells) was measured every 15 minutes using an automatic plate reader for at least 7 hours (markers in **Figure 2**). In addition, samples were collected from a mixed culture well every hour for the first 7-8 hours, and the relative frequencies of the two strains were measured by flow cytometry (markers in **Figure 4**). See Materials and Methods for additional details.

## Estimating growth parameters

**Growth model.** The Baranyi-Roberts model (12) can be used to model growth composed of several phases: lag phase, exponential phase, deceleration phase, and stationary phase (5). The model assumes that cell growth accelerates as cells adjust to new growth conditions, then decelerates as resources become scarce, and finally halts when resources are depleted (13). The model is described by the following ordinary differential equation (see eqs. 1c, 3a, and 5a in (12); for a derivation of eq. 1 and further details, see Appendix 1):

(1)

where is time, is the cell density at time , is the specific growth rate in low density, is the maximum cell density, is a deceleration parameter, and is the adjustment function , which describes the fraction of the population that has adjusted to the new growth conditions by time . In microbial experiments, an overnight liquid culture of microorganisms that has reached stationary phase is typically diluted into fresh media. Following dilution, cells enter lag phase until they adjust to the new growth conditions. We chose the specific adjustment function suggested by Baranyi and Roberts (12), which is both computationally convenient and biologically interpretable: , where characterizes the physiological state of the initial population, and is the rate at which the physiological state adjusts to the new growth conditions.

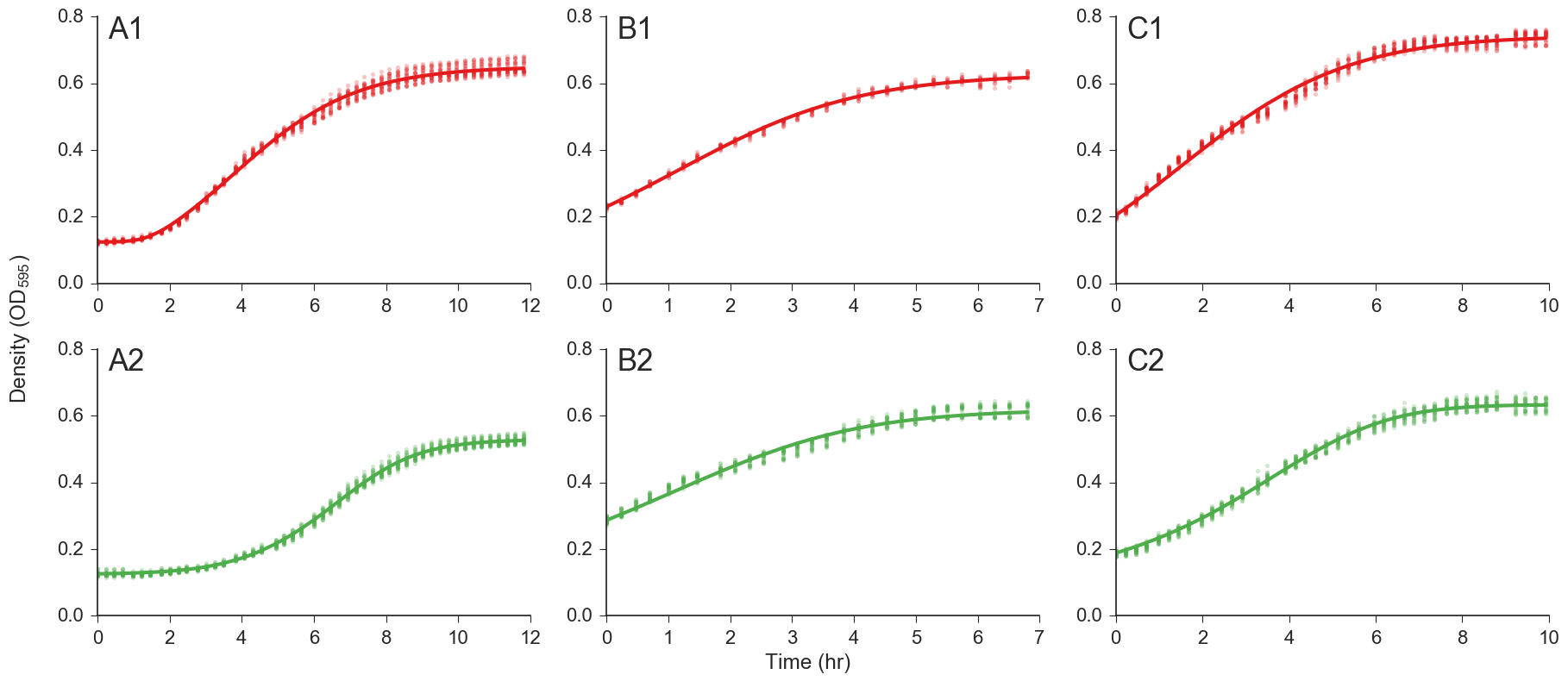
The Baranyi-Roberts differential equation (eq. 1) has a closed form solution:

(2a)

(2b)

where is the initial population density. For a derivation of eq. 2 from eq. 1, see Appendix 1.

**Model fitting.** Growth model parameters were estimated by fitting the model (eq. 2) to the monoculture growth curve data of each strain. The best-fit models (lines) and experimental data (markers) are shown in **Figure 3**; see **Table S1** for the estimated growth parameters. From these best-fit models we also estimated the maximum specific growth rate , the minimal specific doubling time (minimal time required for cell density to double), and the lag duration; see **Table 1**. Different strains differ in their growth parameters: for example, strain A1 (red strain in experiment A) grows 41% faster than the strain A2 (green), has 23% higher maximum density, and a 60% shorter lag phase (**Figure 3**).



**Figure 3. Fitting growth model to growth curve data.** The panels show mono-culture growth curve data (markers) and best-fit growth models (lines; eq. 2). Panel labels correspond to the measured strains, where letters denote the experiment (A, B, and C) and numbers denote the strain (1 and 2); see Experimental design. See Table 1 for estimated growth parameters. 30-32 replicates per experiment per strain.

**Table 1. Estimated growth parameters.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Experiment A | | Experiment B | | Experiment C | |
| *Strain*  *Parameter* | **A1 (red)** | **A2 (green)** | **B1 (red)** | **B2 (green)** | **C1 (red)** | **C2 (green)** |
| Initial density | 0.124 | 0.125 | 0.23 | 0.286 | 0.204 | 0.188 |
| Max density | 0.650 (0.643, 0.658) | 0.528 (0.525, 0.532) | 0.628 (0.624, 0.632) | 0.619 (0.612, 0.625) | 0.741 (0.735, 0.746) | 0.633 (0.627, 0.638) |
| Max specific growth rate | 0.376 (0.371, 0.382) | 0.268 (0.262, 0.275) | 0.369 (0.355, 0.384) | 0.256 (0.251, 0.261) | 0.42 (0.391, 0.426) | 0.228 (0.226, 0.231) |
| Min doubling time | 1.844 (1.809, 1.88) | 2.695 (2.636, 2.77) | 2.451 (2.397, 2.506) | 4.372 (4.269, 4.481) | 2.075 (2.035, 2.124) | 3.117 (3.087, 3.147) |
| Lag duration | 1.578 (1.513, 1.64) | 3.93 (3.82, 4.028) | 0.014 (0.002, 0.029) | 0.004 (0.002, 0.013) | 0.039 (0.033, 0.081) | 0.711 (0.684, 0.749) |

Parentheses provide 95% confidence intervals, calculated using bootstrap (1,000 samples). Min doubling time is the minimal time required to double the population density. Densities are in OD595; growth rate in hours-1; doubling time and lag duration in hours. See Table S2 for additional parameter estimates.

## Estimating competition coefficients

**Competition model.** To model growth in a mixed culture, we assume that interactions between strains in a mixed culture are solely due to resource competition. Therefore, all interactions are described by the deceleration of the growth rate of each strain in response to the depletion of resources due to growth of both strains. We have derived a two-strain Lotka-Volterra competition model (14) based on resource consumption (see Appendix 2):

|  |  |  |
| --- | --- | --- |
|  |  | (3a)  (3b) |

where is the density of strain , and are the values of the corresponding parameters for strain (obtained from fitting the growth model (eq. 2) to monoculture growth curve data), and are competition coefficients, the ratios between inter- and intra-strain competitive effects. Note that each strain can have a different limiting resource and resource efficiency, based on the maximum densities and competition coefficients determined for each strain.

**Model fitting.** The competition model (eq. 3) was fitted to growth curve data from the mixed culture, in which the total OD of both strains in mixed culture was recorded over time (i.e. the bulk density, not the frequency or density of individual strains, see **Figure 2B**). The fitting provides estimates for the competition coefficients and was performed by minimizing the squared differences between (the sum of the integrals of the system in eq. 3; integrals solved numerically using LSODA solver) and the total OD from the mixed culture (**Figure 4**). See Materials and Methods fir additional details. Part of the strength of our approach stems from its use of data measuring the total density of mixed cultures, which is usually ignored when estimating fitness from growth curves (5).

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Figure 4. Fitting competition model to growth curve data from a mixed culture. The panels show mixed-culture growth curves data (blue markers show total density) and best-fit competition models (solid blue lines; eq. 3) and correspond to experiments A, B, and C. 32 replicates per experiment. Dashed black lines show the prediction of the exponential model (see Figure 1).

## Prediction and validation of relative growth

**Model prediction.** With estimates of all the competition model parameters, we solved the competition model (eq. 3) using numerical integration (LSODA solver), thereby providing a prediction for the cell densities and of the two strains growing in a mixed culture. From these predicted densities, the relative frequencies of each strain over time were estimated as .

**Experimental validation.** Wecompared our model predictions to experimental relative frequencies obtained using flow cytometry from mixed culture samples. Experimental results (green and red markers) and model predictions (green and red dashed lines) are shown in **Figure 5**, together with the exponential model predictions (black dashed lines; see **Figure 1** for details on the exponential model). Our model performed well and clearly improved upon the exponential model for predicting competition dynamics in a mixed culture.



Figure 5. Predicting competition results. Comparison of experimental data (markers) and model prediction (dashed lines; see Figure S3 for confidence intervals) of relative strain frequencies in a mixed culture. Red and green dashed lines show our model predictions for the red and green strains (strains 1 and 2 of each experiment); dashed black lines show exponential model predictions (see Figure 1). Error bars show standard deviation (hardly seen in A and C). Inferred time-averaged selection coefficients of red strains are *s=0. 376* in experiment A, *s=0.182* in experiment B, and *s=0.124* in experiment C (fitness of red strain relative to green strain is *1+s*).

# Discussion

We developed a new computational approach to predicting relative growth in a mixed culture from growth curves of mono- and mixed cultures, without the need to measure the frequencies of single isolates within the mixed culture. We tested and validated this new approach, which performed far better than the approach commonly used in the literature.

Our approach only assumes that the assayed strains grow in accordance with the growth and competition models: namely, that growth depends on resource availability. Therefore, this approach can be applied to data from a variety of organisms, experiments, and conditions.

Growth curve experiments, in which only optical density is measured, require much less effort and resources than pairwise competition experiments, in which the cell frequency or count of each strain must be determined (2, 3, 8, 15). Current approaches to estimating fitness from growth curves mostly use either the growth rate and/or the maximum population density as a proxy for fitness (5). However, these proxies for fitness based on a single growth parameter cannot capture the full scope of effects that contribute to differences in overall fitness (11, 16). Most obviously, they fail to account for the lag and deceleration phases of growth. In contrast, our new approach integrates several growth phases, allowing a more accurate estimation of relative growth and fitness from growth curve data. Different growth phases can also be integrated into a single parameter by measuring or calculating the *area under the cur*ve (AUC) of the growth curve (17). This approach is easy to understand and to implement, and the AUC seems to correlate with both the growth rate and the maximum density (17). But the biological interpretation of the AUC, how it is affected by the different growth parameters, and how it affects relative fitness and competitions results, is unclear.

Even when competition experiments can be performed, they cannot explain how differences in fitness relate to differences in growth: is strain 1 fitter than strain 2 due to faster growth rate or due to shorter lag phase? By inferring relative fitness from growth parameters, our approach sheds light on the source of differences in fitness. Furthermore, one can change specific growth parameters and simulate competitions, thereby predicting the effects of such changes on competitions. Another interesting approach for relating differences in growth during different growth phases to fitness has recently been described by Li et al. (11). By changing the duration of experiments with yeast strains, they changed the relative importance of the different growth phases. “Fitness profiles” – measurements of fitness vs. experiment duration – were then used to find the underlying cause of fitness gain in strains that previously evolved to a glucose-limited environment. This is a very promising approach, but also very labor intensive and expensive compared to our approach.

We have released *Curveball*, an open-source software package which implements our new approach (<http://curveball.yoavram.com>). This software is written in Python (18), an open-source and free programming language, and includes a user interface that does not require prior knowledge in programming. It is *free* and *open* (i.e. *libre* and *gratis*), so that additional data formats, growth and competition models, and other analyses can be added by the community to extend its utility.

## Conclusions

We developed and tested a new approach for analyzing growth curve data, and applied it to predict the relative growth of individual strains within a mixed culture. This approach can improve fitness estimation from growth curve data, has a clear biological interpretation, and can be used to predict and interpret growth in a mixed culture and results of competition experiments.

# Materials and Methods

**Strains and plasmids.** *Escherichia coli* strains used were DH5α (Berman lab, Tel-Aviv University), TG1 (Ron lab, Tel-Aviv University), JM109 (Nir lab, Tel-Aviv University), and K12 MG1655-Δfnr (Ron lab, Tel-Aviv University). Plasmids contain a GFP or RFP gene, and genes conferring resistance to kanamycin (KanR) and chloramphenicol (CapR) (Milo lab, Weizmann Institute of Science (19)).

**Media.** All experiments were performed in LB media (5 g/L Bacto yeast extract (BD, 212750), 10 g/L Bacto Tryptone (BD, 211705), 10 g/L NaCl (Bio-Lab, 190305), DDW 1 L) with 30 μg/mL kanamycin (Caisson Labs, K003) and 34 μg/mL chloramphenicol (Duchefa Biochemie, C0113). Green or red fluorescence of each strain was confirmed by fluorescence microscopy (Nikon Eclipe Ti, S1 Figure).

**Growth and competition experiments.** All experiments were performed at30°C. Strains were inoculated into 3 ml LB+Cap+Kan and grown overnight with shaking. Saturated overnight cultures were diluted into fresh media so that the initial OD was detectable above the OD of media alone (1:1-1:20 dilution rate). In experiment B, to avoid a lag phase, cultures were pre-grown until the exponential growth phase was reached as determined by OD measurements (4-6 h). Cells were then inoculated into 100 μL LB+Cap+Kan in a 96-well flat-bottom microplate (Costar):

* 32 wells contained a monoculture of the GFP-labeled strain
* 30 wells contained a monoculture of the RFP-labeled strain
* 32 wells containing a mixed culture of both GFP- and RFP-labeled strains
* 2 wells contained only growth medium

The cultures were grown in an automatic microplate reader (Tecan infinite F200 Pro), shaking at 886.9 RPM, until they reached stationary phase. OD595 readings were taken every 15 minutes with continuous shaking between readings.

Samples were collected from the incubated microplate at the beginning of the experiment and once an hour for 6-8 hours: 1-10 µL were removed from 4 wells (different wells for each sample), and diluted into cold PBS buffer (DPBS with calcium and magnesium; Biological Industries, 02-020-1). These samples were analyzed with a fluorescent cell sorter (Miltenyi Biotec MACSQuant VYB). GFP was detected using a 488nm/520(50)nm FITC laser. RFP was detected with a 561nm/615(20)nm dsRed laser. Samples were diluted further to eliminate "double" event (events detected as both "green" and "red" due to high cell density) and noise in the cell sorter (2).

**Data analysis.** Fluorescent cell sorter output data was analyzed using R (20) with the *flowPeaks* package that implements an unsupervised flow cytometry clustering algorithm (21). Growth curve data were analyzed using *Curveball*, a new open-source software written in Python (18) that implements the approach presented in this manuscript. The software includes both a programmatic interface (API) and a command line interface (CLI), and therefore does not require programming skills. The source code makes use of several Python packages: NumPy (22), SciPy (23), Matplotlib (24), Pandas (25), Seaborn (26), LMFIT (27), Scikit-learn (28), and SymPy (29).

**Fitting growth models.** To fit growth models (eq. 2) to growth curve density data we used the least-squares non-linear curve fitting procedure in SciPy’s *least\_squares* function (23). We then calculate the Bayesian Information Criteria (BIC) of several nested models, defined by fixing some of the growth parameters (see Appendix 1, **Table S1**, **Figure S2**). BIC is given by:

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where is the number of model parameters, is the number of data points, are the time points, is the optical density at time point , and is the expected density at time point according to the model. We selected the model with the lowest BIC (30, 31). Other metrics for model selection can be used, but BIC was chosen for its simplicity and flexibility.

**Fitting exponential models.** The following represents a common approach for estimating growth rates from growth curve data, and was used as a benchmark for our new approach (see **Figure 1** and black dashed lines in **Figure 4** and **Figure 5**). A polynomial is fitted to the mean of the growth curve data *N(t)*. The time of maximum growth rate *tmax* is found by differentiating the fitted polynomial and finding the maximum of the derivative. Values *a* and *b* are found such that *f(t)=b+at* describes a tangent line at the point of maximum growth (*tmax, N(tmax)*).The intercept *b* and the slope *a* are interpreted as the initial density *N0=eb* and the growth rate *r=a* in an exponential growth model *N(t)=N0ert* (*N0* is usually disregarded).

**Fitting competition models.** To fit competition models (eq. 3) we used the Nelder-Mead simplex method (also called downhill simplex method) from SciPy’s *minimize* function (23) to find the competition parameters that minimize the difference between (eq. 3) and the total OD of mixed cultures. and were calculated using numerical integration of eq. 3 with SciPy’s *odeint* function (23).

**Selection coefficients estimation.** Selection coefficients were estimated from pairwise competition results using where and are the predicted frequencies of the strains and is time (1). The resulting *st* values were then averaged across time. Note that these estimates can depend on the experimental conditions, such as duration, media, temperature, and strain composition.

**Data availability.** Data deposited on *figshare* (doi:10.6084/m9.figshare.3485984; *for editors and reviewers only:* <https://figshare.com/s/b08c6b975779e03ec48e>).

**Code availability**. Source code will be available upon publication at <https://github.com/yoavram/curveball> ; an installation guide, tutorial, and documentation will be available upon publication at <http://curveball.yoavram.com>. *For editors and reviewers only:* installation guide, tutorial, documentation, and source code is available at <https://curveball.netlify.com> (password: *dh5alpha*).

**Figure reproduction**. Data was analyzed and figures were produced using a Jupyter notebook (32) that will beavailable as a supporting file and at <https://github.com/yoavram/curveball_ms>. *For editors and reviewers only:* the notebook is available at <https://dl.dropboxusercontent.com/u/1578682/supp.ipynb>.

# Acknowledgments

We thank Y. Pilpel, D. Hizi, I. Françoise, I. Frumkin, O. Dahan, A. Yona, T. Pupko, A. Eldar, I. Ben-Zion, E. Even-Tov, H. Acar, J. Friedman, J. Masel, M.W. Feldman, and E. Rosenberg, for helpful discussions and comments, and L. Zelcbuch, N. Wertheimer, A. Rosenberg, A. Zisman, F. Yang, E. Shtifman Segal, I. Melamed-Havin, and R. Yaari for sharing materials and experimental advice.

This research has been supported in part by the Israel Science Foundation 1568/13 (LH) and 340/13 (JB), the Minerva Center for Lab Evolution (LH), Manna Center Program for Food Safety & Security, the Israeli Ministry of Science & Technology, and Stanford Center for Computational, Evolutionary and Human Genomics (YR), TAU Global Research and Training Fellowship in Medical and Life Science and the Naomi Foundation (MB), and the European Research Council (FP7/2007-2013)/ERC grant 340087 (JB).

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# Appendices

## Appendix 1: Monoculture model

We derive our growth models from a resource consumption perspective (14, 33). We denote by the density of a limiting resource, and by the density of the cell population, both in total mass per unit of volume.

We assume that the culture is well-mixed and homogeneous and that the resource is depleted by the growing cell population without being replenished. Therefore, the intake of resources occurs when cells meet resource via a mass action law with resource uptake rate . Once inside the cell, resources are converted to cell mass at a conversion rate of . Cell growth is assumed to be proportional to , whereas resource intake is proportional to a power of cell density, . We set .

We can describe this process with differential equations for and :

|  |  |  |
| --- | --- | --- |
|  |  | (A1a)  (A1b) |

These equations can be converted to equations in and :

,

which yields

|  |  |  |
| --- | --- | --- |
|  |  | (A2a)  (A2b) |

with .

To solve this system, we use a conservation law approach by setting (34). We find that *M* is constant

,

and we can substitute in eq. A2b to get

|  |  |  |
| --- | --- | --- |
|  | . | (A3) |

Substituting again , and defining , we get

|  |  |  |
| --- | --- | --- |
|  | , | (A4) |

which is the Richards differential equation (35), with the maximum population density *K* and the specific growth rate at low density.

We solve eq. A4 via eq. A3, which is a logistic equation and therefore has a known solution. Setting the initial cell density we have

.

Eq. A4 is an autonomous differential equation ( doesn't explicitly depend on ). To include a lag phase, Baranyi and Roberts (12) suggested to add an adjustment function , which makes the equation non-autonomous (explicitly dependent on ):

|  |  |  |
| --- | --- | --- |
|  | . | (A5) |

Baranyi and Roberts suggested a Michaelis-Menten type of function (36)

,

which has two parameters: *q0* is the initial physiological state of the population, and *m* is rate at which the physiological state adjusts to growth conditions. Integrating gives

.

Therefore, integrating eq. A5 produces eq. 2.

The term in eq. A5 is used to describe the deceleration in the growth of the population as it approaches the maximum density . When , the deceleration is the same as in the standard logistic model and the density at the time of the maximum population growth is half the maximum density, . When or , the deceleration is slower or faster, respectively, and the density at the time of the maximum growth rate is (see Richards 1959, which uses different variables: ).

We use six forms of the Baranyi-Roberts model (S2 Figure, S1 Table). The full model is described by eq. 2 and has six parameters. A five-parameter form of the model assumes , such that the curve is symmetric like in the standard logistic model, but still incorporates the adjustment function and therefore includes a lag phase. Another five-parameter form has both rate parameters set to the same value (), which was suggested to make the fitting procedure more stable (36, 37). A four-parameter form has both of the previous constraints, setting and (36). Another four-parameter form of the model has no lag phase, with 1, which yields the Richards model (35), also called the -logistic model (38), or the generalized logistic model. This form of the model is useful in cases where there is no observed lag phase: either because the population adjusts very rapidly or because it was already adjusted prior to the growth experiment, possibly by pre-growing it in fresh media before the beginning of the experiment. The last form is the standard logistic model, in which and .

## Appendix 2: Mixed culture model

We consider the case in which two species or strains grow in the same culture, competing for a single limiting resource, similarly to eq. A1:

|  |  |  |
| --- | --- | --- |
|  |  | (B1a)  (B1b)  (B1c) |

We define , and (where *j* is 1 when *i* is 2 and vice versa) and find that and is constant. We then substitute into the differential equations for . Denoting and , we get

|  |  |  |
| --- | --- | --- |
|  |  | (B2a)  (B2b) |

where . To get eq. 3 from eq. B2, we include a lag phase by adding the adjustment function ; see details in As in Appendix 1.

We get a similar result if the strains are limited by different resources and that both strains consume:

|  |  |  |
| --- | --- | --- |
|  |  | (B3a)  (B3b)  (B3c)  (B3d) |

Here, we notice first that and therefore is a constant. We then substitute in eqs. B3 and continue as above. This changes the definition of .

If the uptake rates depend on the resource rather than the strain then

|  |  |  |
| --- | --- | --- |
|  |  | (B4a)  (B4b) |

Then we define and and again continue as above.

# Supporting information

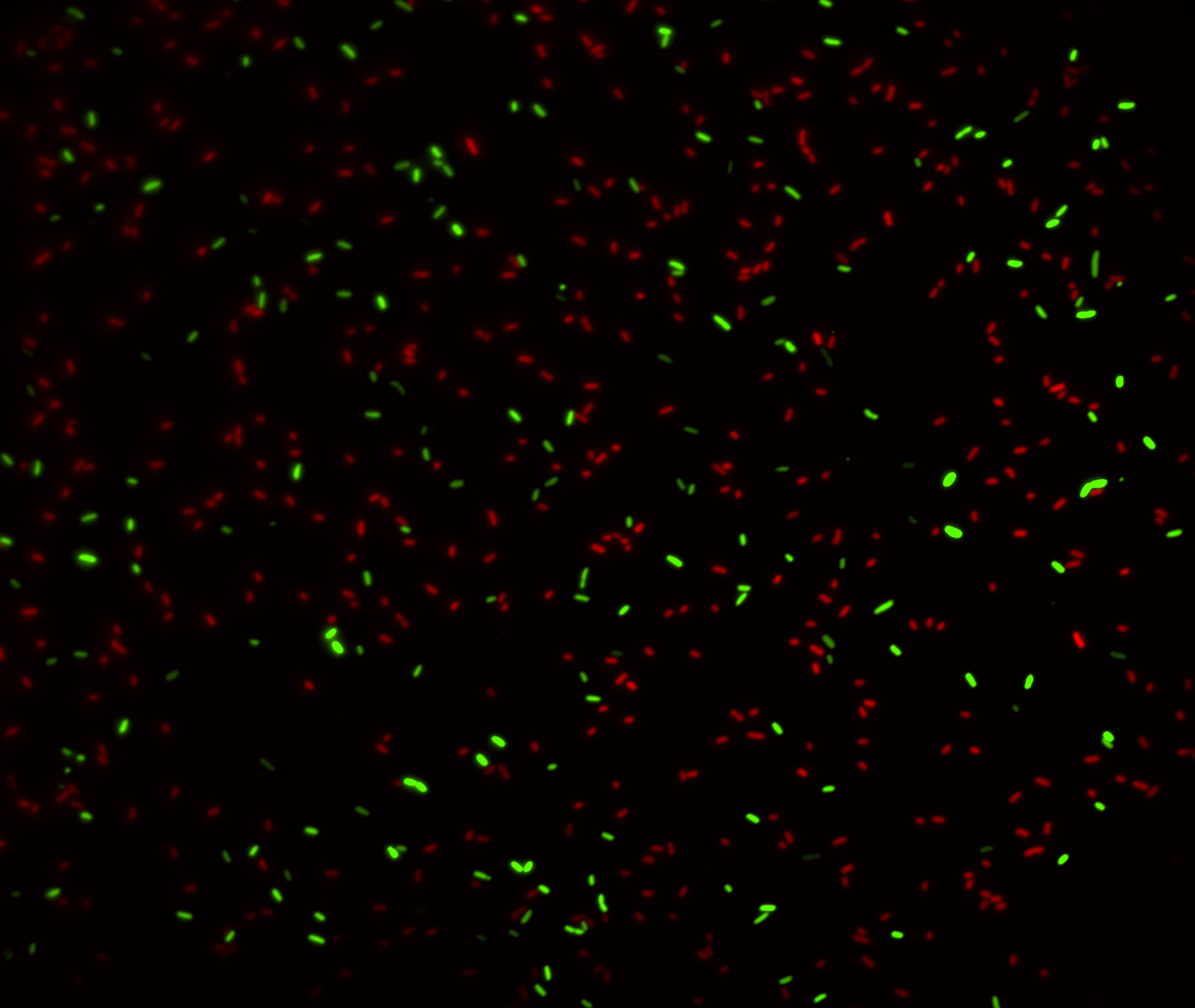


Figure S1. Fluorescence microscopy of *E. coli* strains carrying GFP or RFP. Image of a mixture of TG1-RFP (strain A1 and B1) and DH5α-GFP (strain A2 and B2) cells.

model_diagram.pdf

Figure S2. Growth models hierarchy. The Baranyi-Roberts model and five nested models defined by fixing one or two parameters. See Appendix 1 and Table S1 for more details.

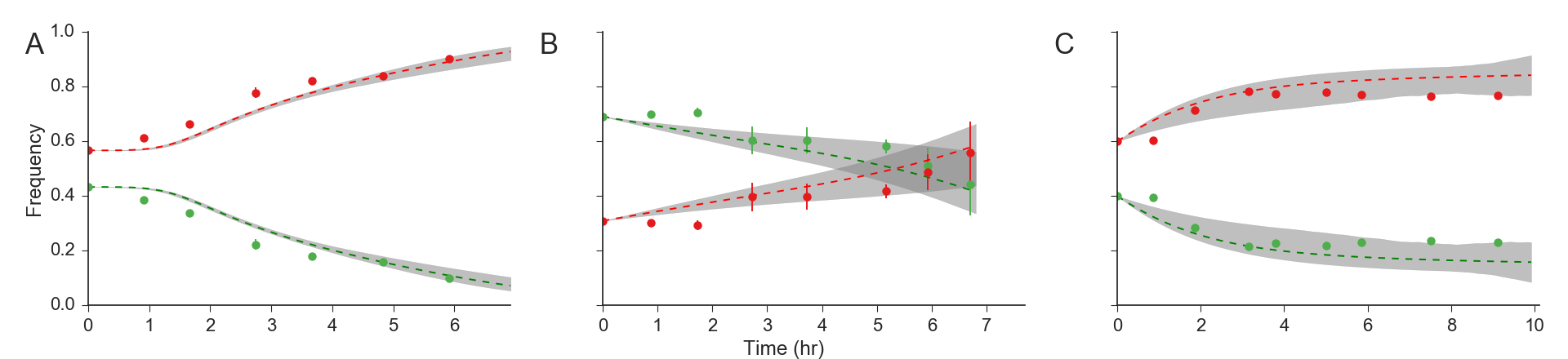


Figure S3. Mixed culture growth predictions with confidence intervals. See legend of Figure 5 for description of the markers and lines. The gray area shows the 95% confidence interval of the best-fit model, calculated using bootstrap (1,000 samples).

Table S1. Growth models.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model name | **# Parameters** | **Free Parameters** | **Fixed Parameters** | **References** |
| Baranyi Roberts 1994 | 6 |  | - | (12) |
| Baranyi 1997 | 5 |  |  | - |
| Baranyi Roberts 1994 | 5 |  |  | - |
| Richards 1959 | 4 |  |  | (35) |
| Baranyi 1997 | 4 |  |  | (36) |
| Logistic | 3 |  |  | (39) |

The table lists the growth models used for fitting growth curve data. All models are defined by eqs. 1 and 2, by fixing specific parameters. is the initial population density; is the maximum population density; is the specific growth rate in low density; is the deceleration parameter; is the initial physiological state; is the physiological adjustment rate. Note that when , the value of is irrelevant. See also the hierarchy diagram in **Figure S2** and a detailed discussion in **Appendix 1**.

**Table S2. Estimated growth parameters.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Experiment A | | | | Experiment B | | | | Experiment C | | | |
| *Strain*  *Parameter* | | **A1 (red)** | | **A2 (green)** | | **B1 (red)** | | **B2 (green)** | | **C1 (red)** | | **C2 (green)** | |
|  | 0.124 | | 0.125 | | 0.23 | | 0.286 | | 0.204 | | 0.188 | |
|  | 0.65 | | 0.528 | | 0.628 | | 0.619 | | 0.741 | | 0.633 | |
|  | 0.587 | | 0.376 | | 0.484 | | 0.304 | | 8 | | 8 | |
|  | 1\* | | 2.636 | | 1.491 | | 2.484 | | 0.164 | | 1\* | |
|  | 0.008 | | 0.032 | | **-\*** | | **-\*** | | 0.393 | | 0.039 | |
|  | 3.735 | | 0.937 | | **-\*** | | **-\*** | | 0.104 | | 0.188 | |

\* denotes fixed parameters.

- denotes invalid parameter values.

The table lists the growth models used for fitting growth curve data. All models are defined by eqs. 1 and 2, by fixing specific parameters.