

Supplemental Material for “Proteome allocation influences population dynamics in microbial communities”

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I. STRAINS

The *Escherichia coli* strains used in our experiment were built from two strains, bAG11 and bAG13, with genetic background MG1655. Strains bAG11 and bAG13 express from the genome respectively, a red fluorescent protein and a yellow fluorescent protein with constitutive promoters. The genotype of strains bAG11 and bAG13 is shown in Table S.1. Then, the strains used in our experiment were obtained by transforming strains bAG11 and bAG13 with different plasmids. In particular:

- bAG16 (strain 3 in the Main Text), was built by transforming bAG11 with a plasmid containing the ampicillin resistance cassette and the yellow fluorescent protein Venus YFP under the control of the *trc* promoter, a hybrid of the *trp* and *lac* promoters
- bAG17 (strain 1 in the Main Text), was built by transforming strain bAG13 with a plasmid containing the ampicillin resistance cassette and the red fluorescent protein mCherry under the control of the *trc* promoter
- bAG24 and bAG25 (respectively strain 4 and 2) in the Main Text, were built by transforming, respectively, strain bAG13 and bAG11 with a plasmid containing the ampicillin resistance cassette, which was obtained by removing from the plasmid carried by bAG17 the inducible red fluorescent protein using traditional cloning.

Since bAG16 and bAG17 carry a plasmid with an additional fluorescent protein repressed by *lacI*, these strains can be induced to produce the additional fluorescent protein by adding Isopropyl β -D-1-thiogalactopyranoside (IPTG) to the culture medium. IPTG is a molecular mimic of allolactose that binds to the lac repressor encoded by *lacI*

Strain name	Background strain	Genotype
bAG11	MG1655	attTN7::pRpsL_mKate2Hyb
bAG13	MG1655	attTN7::pRNA1_mVenus

TABLE S.1: Genotype information on the *E. coli* strains from which we have built the ones used in our experiment.

allowing the expression of genes promoted by the *trc* promoter; because IPTG cannot be metabolized by *E. coli*, its concentration remains constant during our experiment and is unaltered by bacterial growth. Finally, all strains (except the ancestors bAG11 and bAG13) carry the AmpR resistance cassette which makes the two strains resistant to ampicillin, which we added to the medium to prevent contamination and plasmid loss.

II. IPTG CALIBRATION

We measured how strongly the fluorescence intensity of individual cells, a proxy for the total amount of fluorescent protein produced, varied as a function of the IPTG concentration. To do so, we inoculated strains bAG16 (strain 3 in the main text) and bAG17 (strain 4 in the main text) in a 96-well plate containing M63 minimal medium with ampicillin, 1% w/v glucose and the following concentrations of IPTG: $0\mu\text{M}$, $15\mu\text{M}$, $30\mu\text{M}$, $45\mu\text{M}$, $60\mu\text{M}$, $75\mu\text{M}$, $90\mu\text{M}$, $105\mu\text{M}$ (six technical replicates per concentration, per strain). The plate was incubated at 30°C for 4 hours with constant shaking at 1050rpm, after which we measured it at the flow cytometer to compute the mean fluorescence intensity of cells due to the induced fluorescent proteins at the various concentrations of IPTG (Figure S.3). While for strain bAG16 the relationship between the IPTG concentration and the fluorescent intensity of the cells is well fitted by a straight line, for bAG17 a quadratic function is necessary to reproduce the data. We used the fits shown in Figure S.3 to express the data shown in Figure 1 as functions of the fluorescence intensity of the cells (measured in the arbitrary units returned by the flow cytometer).

III. EXPERIMENTAL PROTOCOL

The competition assays discussed in the main text were performed as follows:

1. The four strains bAG16, bAG17, bAG24, bAG25 were cultured overnight from the stock culture in M63 medium with 1% w/v glucose and ampicillin. Then, the strains were mixed to perform two competition assays, i.e. bAG16+bAG24 (strain 3+4) and bAG17+bAG25 (strain 1+2), aiming for 50:50 relative frequencies.
2. The mixtures were inoculated in a 96-well plate that contained M63 medium with 1% w/v glucose and ampicillin at eight different IPTG concentrations: $0\mu\text{M}$, $15\mu\text{M}$, $30\mu\text{M}$, $45\mu\text{M}$, $60\mu\text{M}$, $75\mu\text{M}$, $90\mu\text{M}$, $105\mu\text{M}$ (six technical replicates per concentration and per strain combination).
3. The well plate was covered with a porous rayon film that allowed gas exchange and was cultured for 24 hours at 30°C with constant shaking at 1050rpm.
4. After 24 hours, the plate was reinoculated in a new 96-well plate with fresh medium (with the appropriate concentrations of IPTG in each well) with a dilution factor of 100. The new plate was cultured for another cycle at 30°C for 24 hours with constant shaking at 1050rpm, while the old one was diluted with a dilution factor of 2000 to be analyzed at the flow cytometer.

A. Time series

Figures S.4 to S.7 show the time series of the relative abundances of the strains in the two competition assays discussed in the main text. To compute the growth/death rate of the relative abundances, each curve (corresponding to one of the six replicas of that treatment) was fitted with a linear function.

IV. THE MODEL

A. Derivation of Eqs (4) to (6b)

In our minimal model, the temporal evolution of the strain's biomass is given by $\dot{m}_\sigma = m_\sigma \eta_\sigma \varphi_\sigma$, so that $m_\sigma(t) = m_\sigma(0) \exp(\eta_\sigma \varphi_\sigma t)$. By using the fact that $\varphi_\sigma(1 + \gamma_\sigma) = \Phi_\sigma$ (Eq (3)) and the assumption that $\eta_1 = \eta_2 = \eta$ and $\gamma_1 = \gamma_2 = \gamma$ (since all the strains share the same background, i.e. the ancestor strains have the same fitness as shown in Figures S.1 and S.2), we obtain:

$$m_\sigma(t) = m_\sigma(0) \exp\left(\frac{\eta}{1 + \gamma} \Phi_\sigma t\right). \quad (\text{S.1})$$

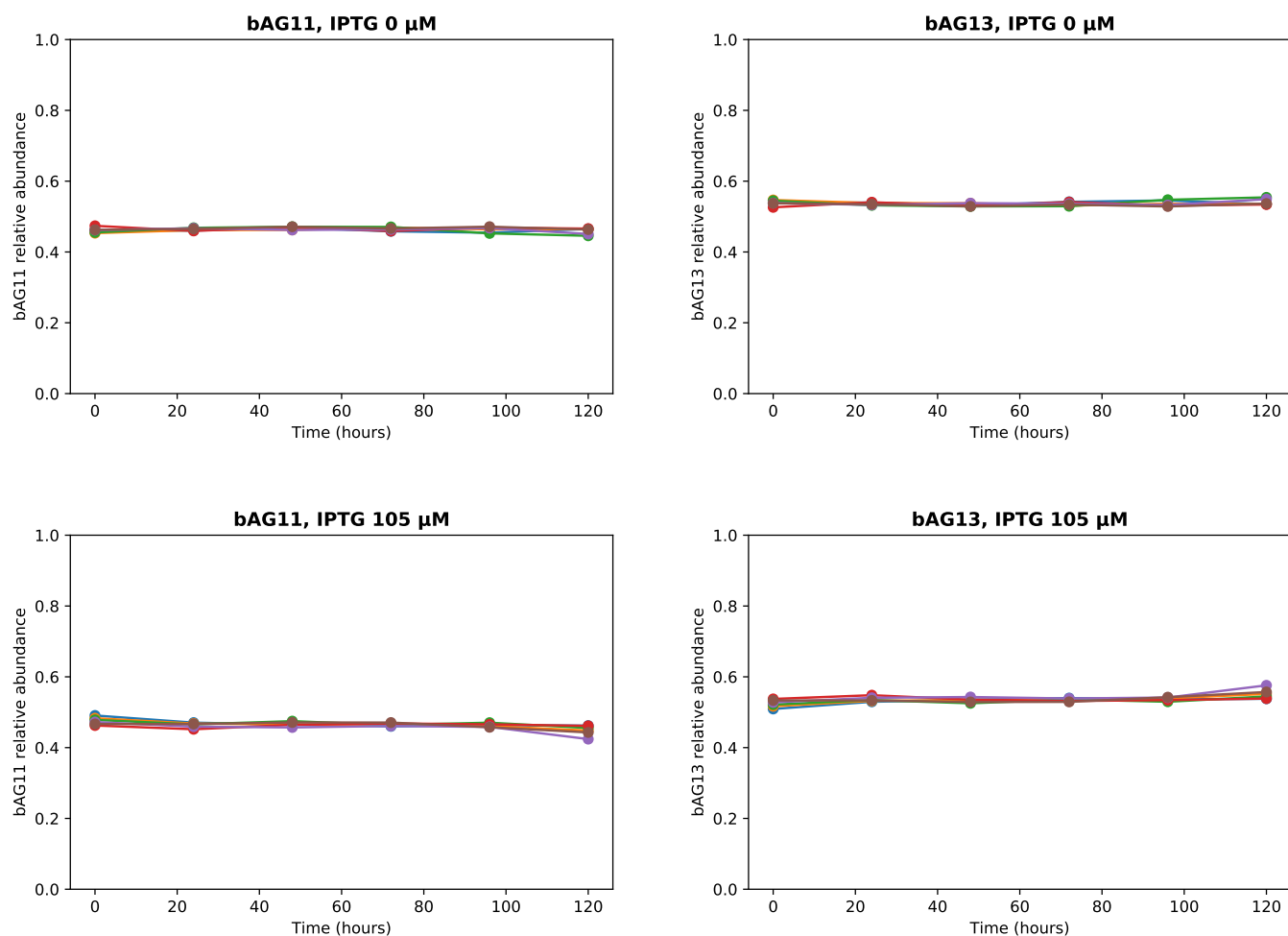


FIG. S.1: Competition assays between strains bAG11 and bAG13. These assays were done with the same experimental protocol shown in III (without adding ampicillin to the culture medium since strains bAG11 and bAG13 do not carry any plasmid and therefore are not ampicillin resistant). As we can see, the relative abundances of the two strains remain constant and so both have the same fitness.

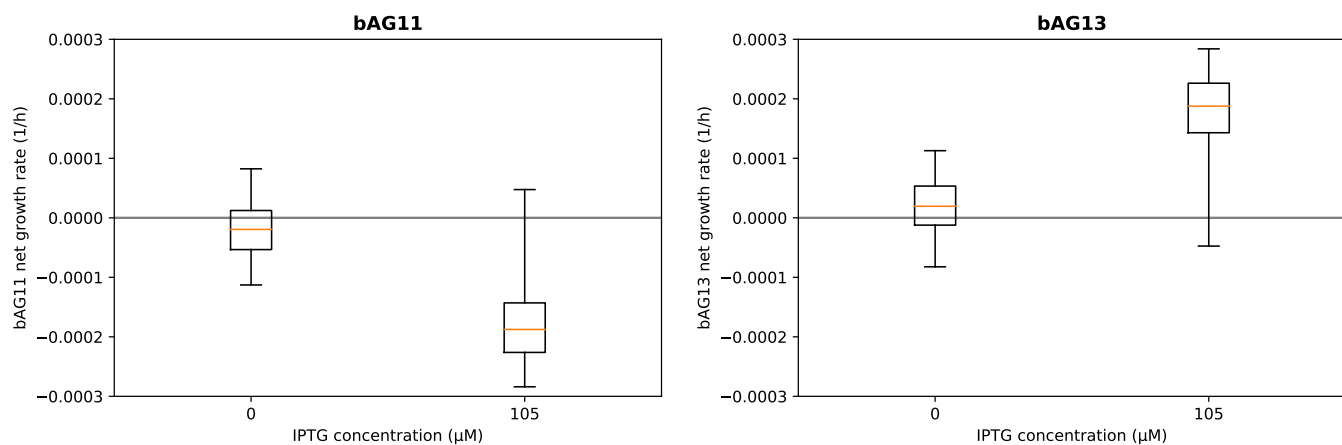


FIG. S.2: Boxplot of the growth rates (computed as a linear fit of the time series shown in Figure S.1) for the different IPTG concentrations.

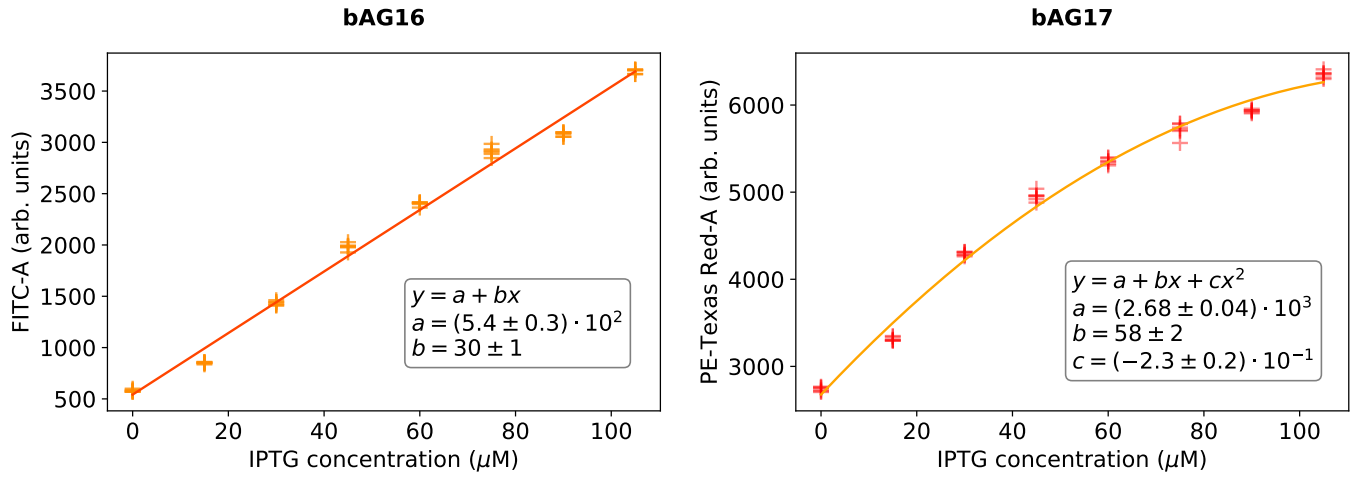


FIG. S.3: Results of the IPTG calibration. Each cross in the plot represents one of the six replicas of that treatment, and was obtained by computing the mean value of the fluorescence from the flow cytometry data. As we can see, while the relationship between IPTG concentration and yellow fluorescence for bAG16 is well fitted by a linear function, for bAG17 a quadratic one is necessary.

Therefore, the relative abundances of the two strains will evolve in time as:

$$f_1(t) = \frac{m_1(t)}{m_1(t) + m_2(t)} = \left\{ 1 + \frac{m_2(0)}{m_1(0)} \exp \left[\frac{\eta}{1+\gamma} (\Phi_2 - \Phi_1) t \right] \right\}^{-1} \quad (\text{S.2a})$$

$$f_2(t) = \frac{m_2(t)}{m_1(t) + m_2(t)} = \left\{ 1 + \frac{m_1(0)}{m_2(0)} \exp \left[\frac{\eta}{1+\gamma} (\Phi_1 - \Phi_2) t \right] \right\}^{-1}. \quad (\text{S.2b})$$

Expanding these expressions for small t we get:

$$f_1(t) \sim f_1(0) - f_1(0)f_2(0) \frac{\eta}{1+\gamma} (\Phi_2 - \Phi_1) t \quad (\text{S.3a})$$

$$f_2(t) \sim f_2(0) - f_1(0)f_2(0) \frac{\eta}{1+\gamma} (\Phi_1 - \Phi_2) t, \quad (\text{S.3b})$$

and by deriving with respect to t :

$$\Lambda_1 = \frac{df_1}{dt} = f_1(0)f_2(0) \frac{\eta}{1+\gamma} (\Phi_1 - \Phi_2) \quad (\text{S.4a})$$

$$\Lambda_2 = \frac{df_2}{dt} = f_1(0)f_2(0) \frac{\eta}{1+\gamma} (\Phi_2 - \Phi_1), \quad (\text{S.4b})$$

where Eq (S.4a) is exactly Eq (4), and we see that $\Lambda_2 = -\Lambda_1$. If we now write $\Phi_1 = \Phi_1^{(0)} - \varphi_U$, Eqs (S.4a) and (S.4b) become:

$$\Lambda_1 = \underbrace{f_1(0)f_2(0) \frac{\eta}{1+\gamma} (\Phi_1^{(0)} - \Phi_2)}_{\ell} - \underbrace{f_1(0)f_2(0) \frac{\eta}{1+\gamma} \varphi_U}_{\lambda} \quad (\text{S.5a})$$

$$\Lambda_2 = - \underbrace{f_1(0)f_2(0) \frac{\eta}{1+\gamma} (\Phi_1^{(0)} - \Phi_2)}_{\ell} + \underbrace{f_1(0)f_2(0) \frac{\eta}{1+\gamma} \varphi_U}_{\lambda}, \quad (\text{S.5b})$$

which is Eq (5) with the definitions given in Eqs (6a) and (6b).

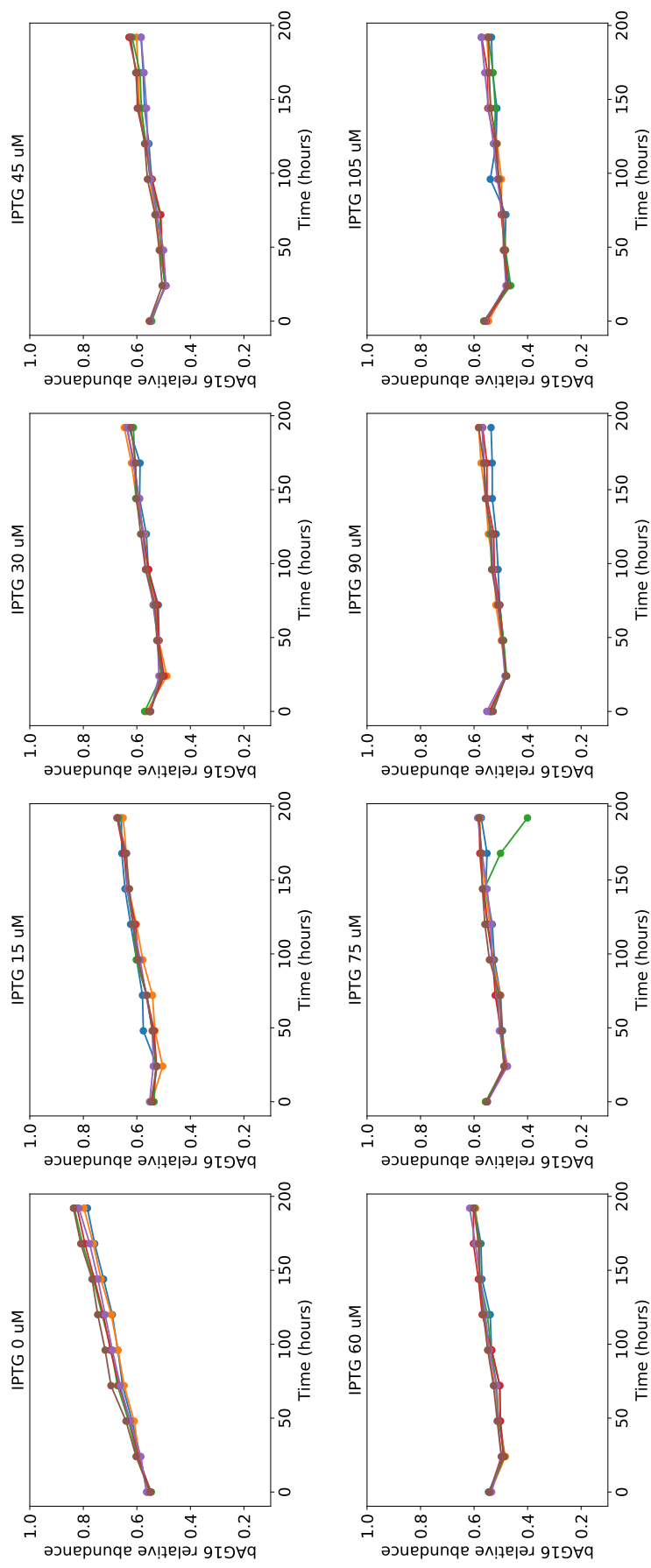


FIG. S.4: Time series of the relative abundance of strain bAG16 in the competition assay bAG16+bAG24. Each curve represent one of the six replicas of the treatment.

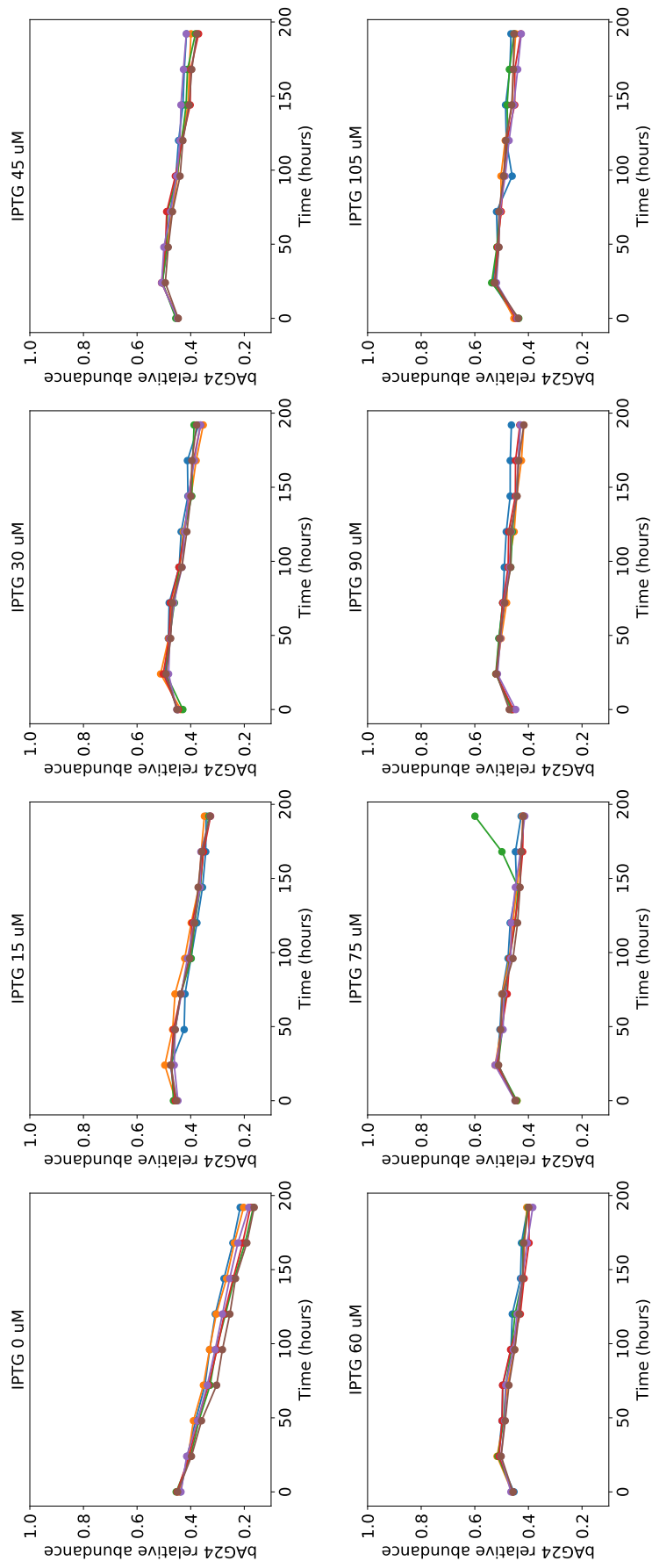


FIG. S.5: Time series of the relative abundance of strain bAG24 in the competition assay bAG16+bAG24. Each curve represent one of the six replicas of the treatment.

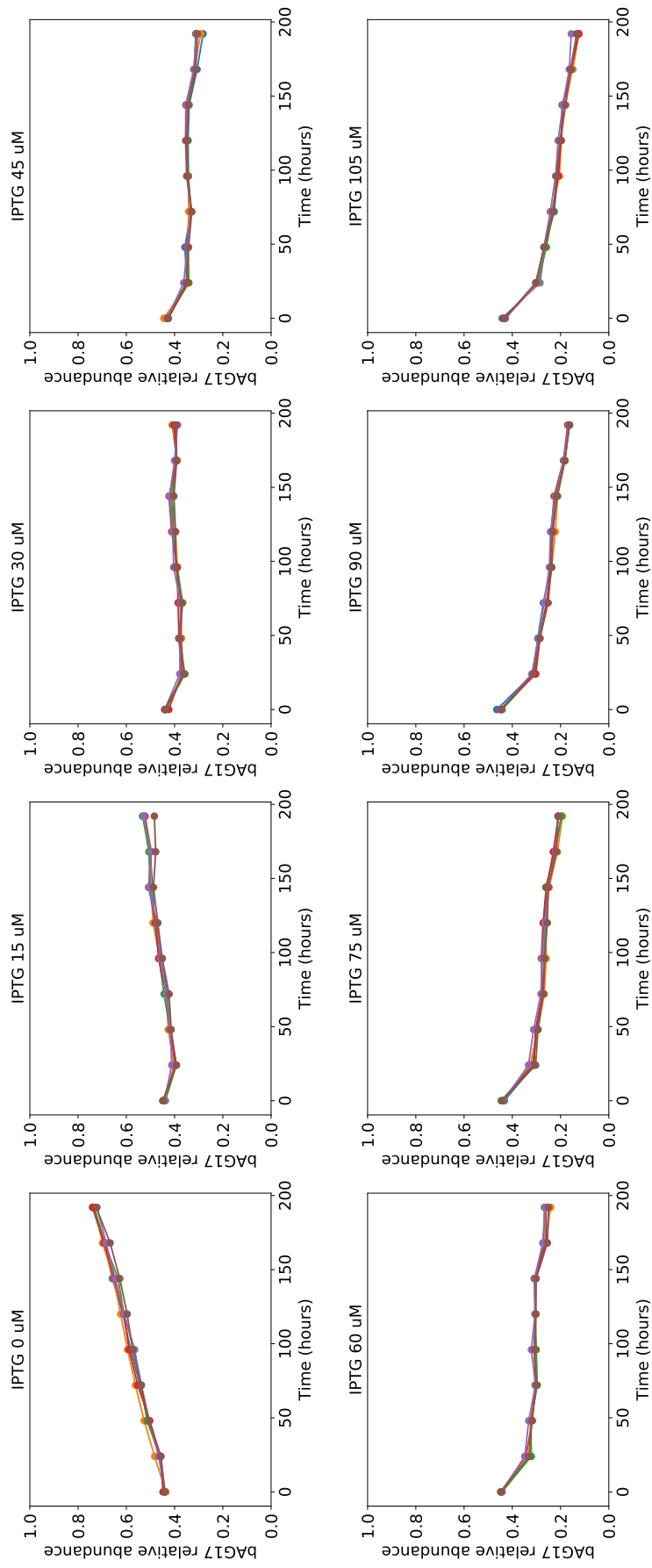


FIG. S.6: Time series of the relative abundance of strain bAG17 in the competition assay bAG17+bAG25. Each curve represent one of the six replicas of the treatment.

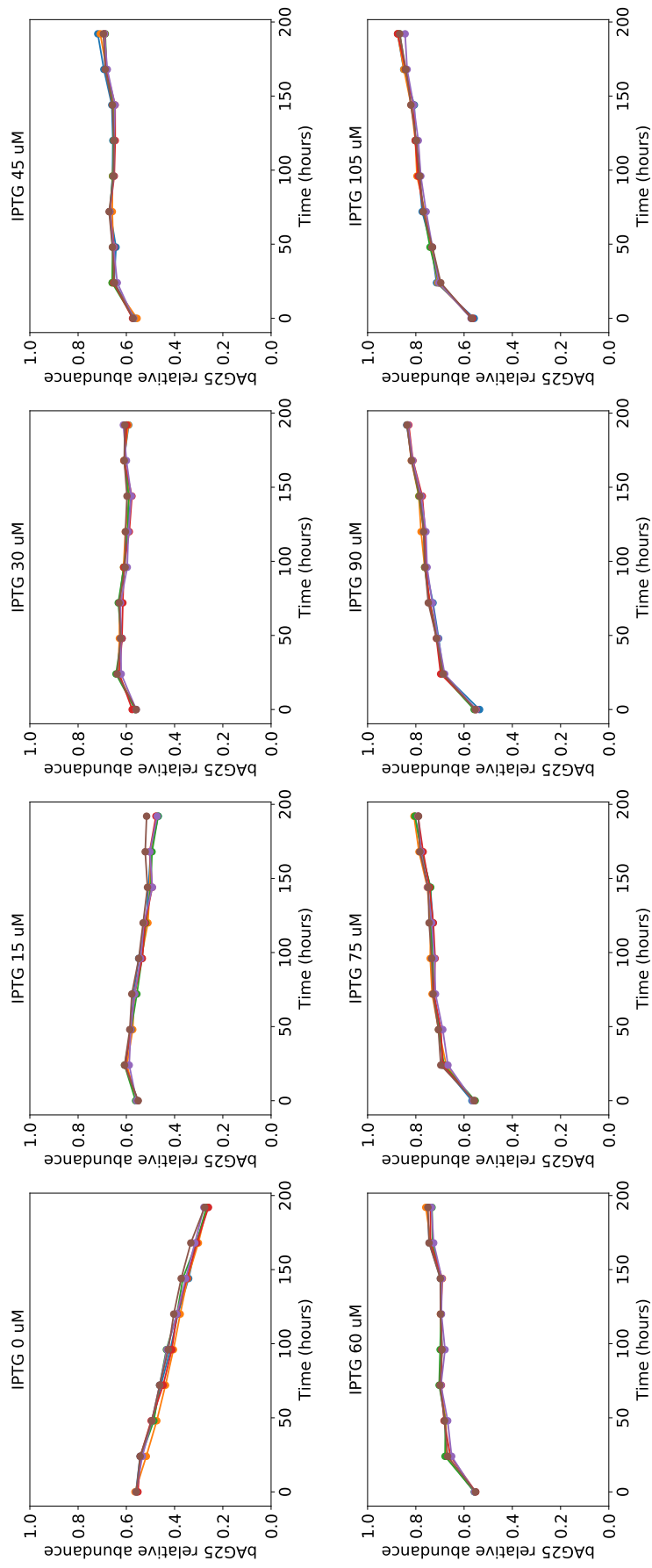


FIG. S.7: Time series of the relative abundance of strain bAG15 in the competition assay bAG17+bAG25. Each curve represent one of the six replicates of the treatment.

B. A more detailed model

Our minimal model makes a very strong assumption, i.e. that the strains are always in exponential growth. In our experiment this was not the case, since the density of the cells saturated well before the following re-inoculation in fresh medium was made (i.e., 24 hours).¹ Here we show that by into account the fact that cells can reach saturation before the following re-inoculation, our results remain unchanged.

A model better suited to describe the experiments would be as follows. The temporal dynamics of biomass and glucose concentration between two consecutive dilutions satisfies:

$$\dot{m}_\sigma = m_\sigma \eta_\sigma r(c) \varphi_\sigma \quad \sigma = 1, 2 \quad (\text{S.6a})$$

$$\dot{c} = -r(c)(m_1 \eta_1 \varphi_1 + m_2 \eta_2 \varphi_2), \quad (\text{S.6b})$$

where $c(t)$ is the concentration of glucose at time t and $r(c) = c/(c+K)$ is Monod's function. This model is somewhat similar to MacArthur's consumer-resource model (P. Chesson, *Theor. Pop. Biol.* **37**, 26 (1990)), with the difference that there is no mortality term in Eq (S.6a): between two consecutive dilutions, the biomass m_σ of strain σ will grow as long as there is glucose available, and because $r(0) = 0$ the strains will stop growing (i.e. they will enter the stationary phase) once glucose runs out.

We now make the following approximation: we assume that, after every reinoculation, glucose is initially abundant (i.e. $r \sim 1$) and that the transition of $r(c)$ from 1 to 0 as c decreases is abrupt, which happens if K is sufficiently small. In other words, we assume that K is sufficiently small so that $r(c) \sim 1$ until a given time T (the instant at which glucose is completely depleted), when $r(c)$ abruptly goes to zero (i.e., $r(c(t)) \sim \Theta(T-t)$ with Θ the Heaviside's step function). This means that after a reinoculation m_σ will grow exponentially for a time interval of length T , after which it will stop until the next dilution. If we still set $\eta_1 = \eta_2 = \eta$ and $\gamma_1 = \gamma_2 = \gamma$ and call D the dilution factor between reinoculations, we have that the biomass $m_\sigma^{(N)}$ of strain σ at the N -th dilution is ($m_\sigma^{(0)}$ being the biomass at the initial inoculation):

$$m_\sigma^{(1)} = m_\sigma^{(0)} \exp\left(\frac{\eta}{1+\gamma} \Phi_\sigma T\right) \quad (\text{S.7a})$$

$$m_\sigma^{(2)} = \frac{m_\sigma^{(1)}}{D} \exp\left(\frac{\eta}{1+\gamma} \Phi_\sigma T\right) = \frac{m_\sigma^{(0)}}{D} \exp\left(2 \frac{\eta}{1+\gamma} \Phi_\sigma T\right) \quad (\text{S.7b})$$

$$m_\sigma^{(3)} = \frac{m_\sigma^{(2)}}{D} \exp\left(\frac{\eta}{1+\gamma} \Phi_\sigma T\right) = \frac{m_\sigma^{(0)}}{D} \exp\left(3 \frac{\eta}{1+\gamma} \Phi_\sigma T\right) \quad (\text{S.7c})$$

⋮

$$m_\sigma^{(N)} = \frac{m_\sigma^{(0)}}{D^{N-1}} \exp\left(N \frac{\eta}{1+\gamma} \Phi_\sigma T\right). \quad (\text{S.7d})$$

Therefore, the relative abundances of the two strains at the N -th dilution will be:

$$f_1^{(N)}(t) = \left\{ 1 + \frac{m_2^{(0)}}{m_1^{(0)}} \exp\left[N \frac{\eta}{1+\gamma} (\Phi_2 - \Phi_1) T\right] \right\}^{-1} \quad (\text{S.8a})$$

$$f_2^{(N)}(t) = \left\{ 1 + \frac{m_1^{(0)}}{m_2^{(0)}} \exp\left[N \frac{\eta}{1+\gamma} (\Phi_1 - \Phi_2) T\right] \right\}^{-1}, \quad (\text{S.8b})$$

¹ The typical growth rate of the strains, estimated from growth curves measured in the same experimental conditions used for the competition assays, is 0.3 1/h. The competition assays started from a cellular density of $\sim 8 \cdot 10^6$ cells/mL, thus if the growth was exponential the density after 24 hours would have been $\sim 1.4 \cdot 10^{10}$, which is much higher than the typical density ($\sim 10^9$ cells/mL) that *E. coli* cells reach at saturation. With a growth rate of 0.3 1/h, the time needed to reach a cellular density that is hundredfold the initial one (and therefore the time needed to reach saturation after a re-inoculation) is approximately 15.4 h.

and computations analogous to those shown in section IV A lead to:

$$\Lambda_1 = f_1^{(0)} f_2^{(0)} \frac{\eta}{1 + \gamma} (\Phi_1 - \Phi_2) \quad (\text{S.9a})$$

$$\Lambda_2 = f_1^{(0)} f_2^{(0)} \frac{\eta}{1 + \gamma} (\Phi_2 - \Phi_1) , \quad (\text{S.9b})$$

where this time the “derivative” was taken with respect to N .

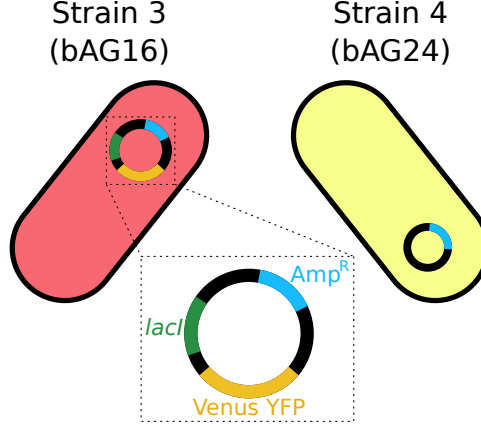


FIG. S.8: Schematic representation of the strains used in the second competition assay.

V. RESULTS OF EXPERIMENT WITH STRAINS 3 AND 4

A schematic representation of the second competition assay, i.e. bAG16+bAG24, is shown in Figure S.8. The results of this competition assay are shown in Figure S.9. We can first notice that in this case we always have $\Lambda_1 > 0$ and $\Lambda_2 < 0$, and so we are not able to span different regimes of the system (with only one possible outlier at $75\mu\text{M}$ IPTG, visible also in Figures S.4 and S.5). Additionally, the data points relative to the treatment without IPTG do not lie on the line described by all the other points; for this reason, in Figure S.9 we show the results of a linear fit both when the points relative to the treatment at $0\mu\text{M}$ IPTG are considered (light blue line) and when they are excluded (orange line). In both cases we still have that the slopes and intercepts of the lines are equal in magnitude but have opposite sign. Using the same assumptions outlined in the main text, we can estimate the order of magnitude of $\delta\Phi$ as $\delta\Phi \sim 0.3\%$ (more specifically in the two cases we obtain, respectively, $\delta\Phi \sim 0.35\%$ and $\delta\Phi \sim 0.26\%$).

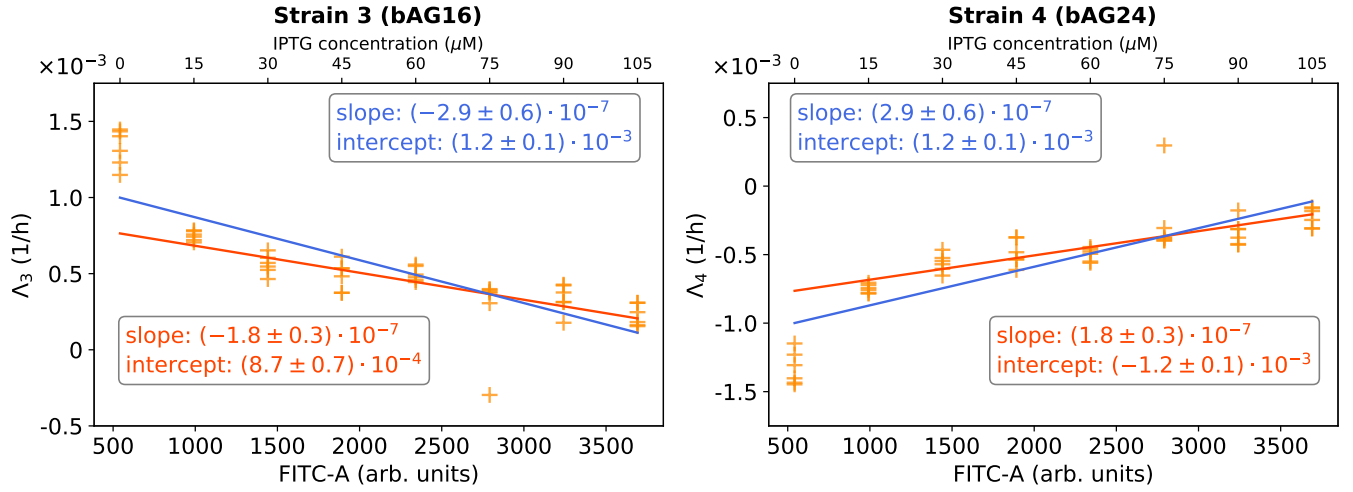


FIG. S.9: Results of the experiment done with strains bAG16 and bAG24, equivalent to Figure 3 in the Letter. As we can see, in this case we are not able to span all the regimes exhibited by the system since we always have $\Lambda_1 > 0$ and $\Lambda_2 < 0$ (with the noticeable exception of one outlier at $75\mu\text{M}$ IPTG). Furthermore, the data points relative to $0\mu\text{M}$ IPTG do not lie on the line described by all the other points, so we are showing the linear fits obtained both when these points are included (light blue curve) and when they are excluded (orange line).