

# Mixed growth curve data do not suffice to fully characterize the dynamics of mixed cultures

Eva Balsa-Canto<sup>a,1</sup>, Javier Alonso-del-Real<sup>b</sup>, and Amparo Querol<sup>b</sup>

In PNAS, Ram et al. (1) present an approach to decipher competition in mixed cultures of biotechnological interest. The approach—devoted to those cases in which competitive behavior depends on cellular densities—is intended to characterize mixed-culture individual dynamics by fitting the mixed-culture optical density (OD) data to a nonlinear competition Lotka–Volterra (NLV) model (2, 3).

Here we show, with experimental data and numerical examples, that the method does not guarantee convergence to the real individual dynamics.

From a theoretical point of view, it would be in principle possible to estimate competition parameters ( $c_i$ ) from mixed-culture OD data (i.e., parameters are structurally identifiable) (4, 5).

However, the method faces other difficulties in practice due to poor practical identifiability (6). We illustrate, with various examples, that the success of the method strongly depends on 1) the actual values of the individual growth and competition parameters, 2) the experiment design, that is, the initial densities, the duration of the experiment, and the number and location of sampling times, and 3) the data uncertainty.

First, we considered the individual and mixed growth of 2 yeast species (*Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii*) following the experimental approach in ref. 7. Fig. 1 shows that the mixed OD procedure predicts that both cells coexist, *S. kudriavzevii* outcompeting *S. cerevisiae*, while the data reveal that *S. cerevisiae* excludes *S. kudriavzevii*.

To analyze when and how the method may fail, we can use the NLV model nullclines (8). The relative

position of the nullclines determines the steady state of the system ( $N_1^*$ ,  $N_2^*$ ). One species excludes the other ( $N_i^* = K_i$ ,  $N_j^* = 0$ ) if nullclines do not intersect while stable or unstable coexistence appears when nullclines intersect each other.

Remarkably, multiple combinations  $N_1(t_s)$  and  $N_2(t_s)$  result in the same mixed-culture OD  $S(t_s)$  at a given sampling time  $t_s$ . Therefore, different individual steady states may result in the same mixed steady state ( $S^* = N_1^* + N_2^*$ ), that is, in the same mixed-culture OD at steady state (as illustrated in Fig. 2). Moreover, the individual steady states  $N_i^*$  are compatible with various  $c_i$  values and/or initial conditions. Therefore, even if the method recovers the steady state, it may fail to reproduce the dynamics toward it.

As a consequence, depending on the initial conditions, the number and location of sampling times and the duration of the experiment in many cases cannot be easily distinguished, particularly when real competition parameters are in the vicinity of the boundaries between coexistence and exclusion. Indeed, errors on the parameter estimates can transform coexistence into exclusion or the other way around. Data uncertainty increases the chances of failure. Fig. 2 B–D present some examples.

We performed the computations with the toolbox AMIGO2 (9). Scripts, further results, and details are included in documentation available at <https://sites.google.com/site/amigo2toolbox/examples> (10).

Summing up, despite its appeal, the measurement of mixed-culture OD does not suffice, and the need to account for the individual densities—at least at one well-located sampling time—remains.

<sup>a</sup>(Bio) Process Engineering Group, Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas (IIM-CSIC), 36208 Vigo, Spain; and <sup>b</sup>Grupo de Biología de Sistemas en Levaduras de Interés Biotecnológico, Instituto de Agroquímica y Tecnología de los Alimentos, Consejo Superior de Investigaciones Científicas (IATA-CSIC), 46980 Paterna, Valencia, Spain

Author contributions: E.B.-C. and A.Q. designed research; E.B.-C. and J.A.-d.-R. performed research; E.B.-C. analyzed data; J.A.-d.-R. performed the experiments; A.Q. designed the experiments; and E.B.-C., J.A.-d.-R., and A.Q. wrote the paper.

The authors declare no competing interest.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

Data deposition: Scripts, further results, and details are included in documentation available at <https://sites.google.com/site/amigo2toolbox/examples>.

<sup>1</sup>To whom correspondence may be addressed. Email: ebalsa@iim.csic.es.

First published December 24, 2019.

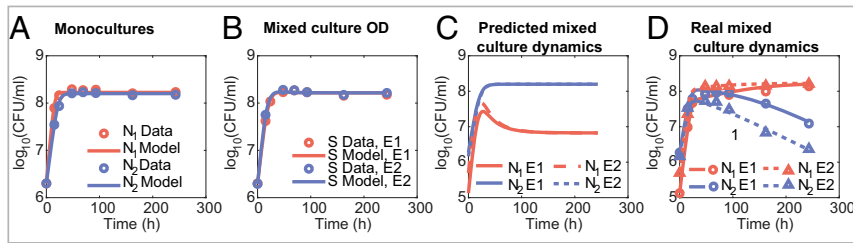


Fig. 1. Individual and mixed growth of 2 yeast species: *S. cerevisiae* and *S. kudriavzevii*. (A) The best fit to the data for the individual cultures. (B) The best fit to the mixed OD data corresponding to 2 different experiments (E1 and E2) with different initial relative densities. (C) The individual dynamics as predicted by the mixed OD method. (D) The individual dynamics as recovered by a qPCR analysis and the corresponding fit to the NLV model. This example shows that the mixed OD approach Ram et al. (1) propose would lead to the wrong conclusion that both species coexist and *S. kudriavzevii* outcompetes *S. cerevisiae*, while qPCR data reveal exclusion of *S. kudriavzevii* in both experiments.

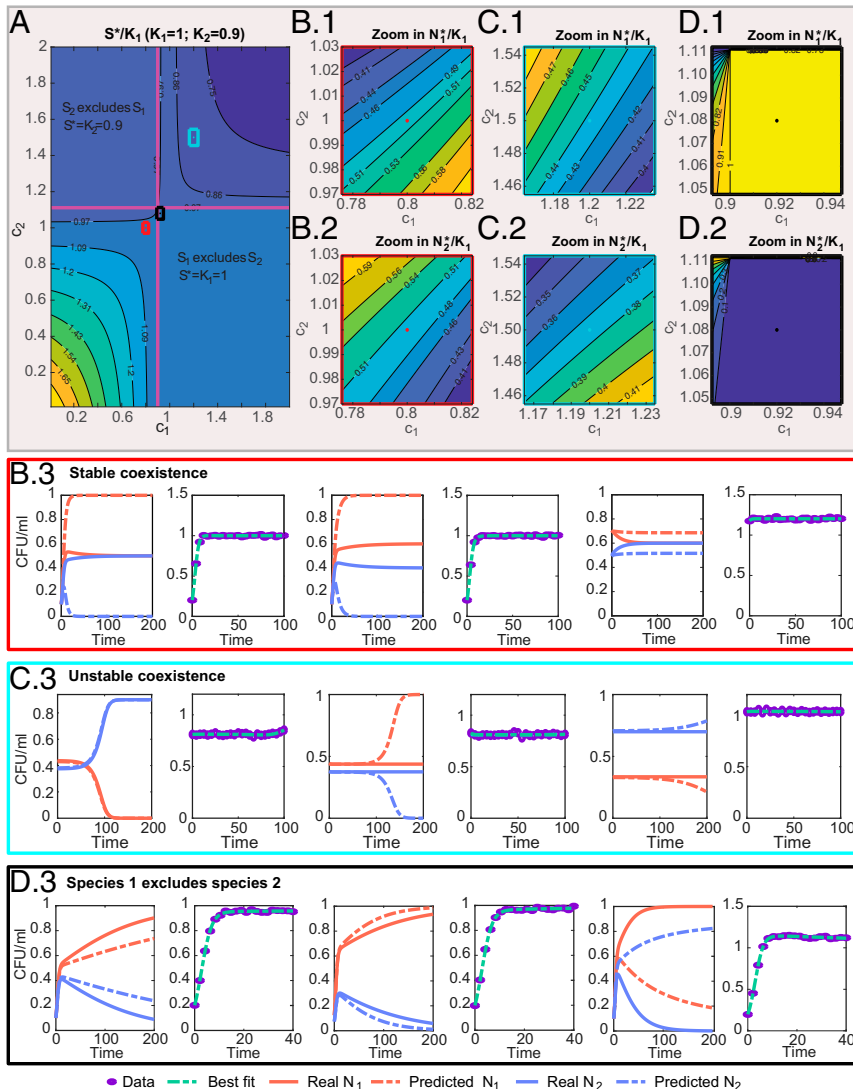


Fig. 2. Numerical illustrative examples showing a case in which  $r_1 = 0.5$ ,  $K_1 = 1$ ,  $v_1 = 1$  and  $r_2 = 0.45$ ,  $K_2 = 0.9$ ,  $v_2 = 1$ . Numerical experimental data were generated with a 1% SD experimental noise. (A) The steady state of the mixed system ( $S^*/K_1$ ) for a given range of values for the parameters  $c_1$  and  $c_2$ . (B.1 and B.2, C.1 and C.2, and D.1 and D.2) The distribution of the individual steady states corresponding to a specific box within the coexistence, exclusion, and unstable coexistence regions (red, black, and cyan boxes, respectively). Each box corresponds to a  $\pm 3\%$  variation around a given parameter value. Note that while the boxes in A show almost no modifications over the  $S^*/K_1$  values, B, C, and D show multiple steady-state values compatible with the same mixed-culture steady state. (B.3, C.3, and D.3) Several illustrative examples of how the mixed OD method may fail to predict the actual dynamics of the system, even if the individual parameters  $r_i$ ,  $K_i$ , and  $v_i$  are precisely known. B.3 shows, for example, that 2 different coexistence scenarios evolving toward  $S^* = 1$  may be confused with an exclusion scenario in which  $S^*$  also equals 1; besides, coexistence scenarios may also be confused as shown in the last example. C.3 presents the difficulties to recover unstable coexistence cases, even if experiments are sufficiently long. D.3 illustrates that even if exclusion can be recovered, the predicted dynamics may be faster or slower than the actual dynamics. The last example in the row shows, again, the confusion between exclusion and coexistence. The accompanying information presents the consequences of shortening the experiments for these particular examples.

- 
- 1 Y. Ram et al, Predicting microbial growth in a mixed culture from growth curve data. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 14698–14707 (2019).
  - 2 R. Dilao, T. Domingos, A general approach to the modelling of trophic chains. *Ecol. Model.* **132**, 191–202 (2000).
  - 3 S. P. Otto, T. Day, *A Biologist's Guide to Mathematical Modeling in Ecology and Evolution* (Princeton University Press, 2007).
  - 4 O.-T. Chis, J. R. Banga, E. Balsa-Canto, Structural identifiability of systems biology models: A critical comparison of methods. *PLoS One* **6**, e27755 (2011).
  - 5 T. S. Ligon et al., Genssi 2.0: Multi-experiment structural identifiability analysis of sbml models. *Bioinformatics* **34**, 1421–1423 (2018).
  - 6 O.-T. Chis, A. F. Villaverde, J. R. Banga, E. Balsa-Canto, On the relationship between sloppiness and identifiability. *Math. Biosci.* **282**, 147–161 (2016).
  - 7 J. Alonso-del Real, M. Lairón-Peris, E. Barrio, A. Querol, Effect of temperature on the prevalence of *saccharomyces non cerevisiae* species against a *S. cerevisiae* wine strain in wine fermentation: Competition, physiological fitness, and influence in final wine composition. *Front. Microbiol.* **8**, 150 (2017).
  - 8 J. D. Murray, *Mathematical Biology. I: An Introduction* (Springer-Verlag, ed. 3, 2002).
  - 9 E. Balsa-Canto, D. Henriques, A. Gabor, J. R. Banga, AMIGO2, a toolbox for dynamic modeling, optimization and control in systems biology. *Bioinformatics* **32**, 3357–3359 (2016).
  - 10 E. Balsa-Canto, J. Alonso-del-Real, A. Querol, Microbial competitive behaviour in mixed-cultures: Parameter estimation of Nonlinear Lotka-Volterra models. AMIGO2. <https://sites.google.com/site/amigo2toolbox/examples>. Deposited 25 September 2019.