

# Supplementary Material for:

## Cheating promotes coexistence in a two-species one-substrate culture model

<sup>1</sup>Xenophontos, Constantinos<sup>1,2</sup>, Harpole, W. Stanley<sup>2,3,4</sup>, Küsel, Kirsten<sup>1,2</sup>, Clark, Adam Thomas<sup>2,3,5</sup>

1. Institute of Biodiversity, Friedrich Schiller University Jena, Jena 07743, Germany
2. German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, 04103 Leipzig, Germany
3. Department of Physiological Diversity, Helmholtz-Centre for Environmental Research (UFZ), 04318 Leipzig, Germany
4. Institute of Biology, Martin Luther University of Halle-Wittenberg, 06120 Halle, Germany
5. Institute of Biology, Karl-Franzens University of Graz, 8010 Graz, Austria

---

<sup>1</sup>Correspondence  
Constantinos Xenophontos  
Friedrich Schiller University Jena  
Institute of Biodiversity  
Dornburger Str. 159  
07743 Jena  
Germany

[constantinos.xenophontos@uni-jena.de](mailto:constantinos.xenophontos@uni-jena.de)

Figure S1

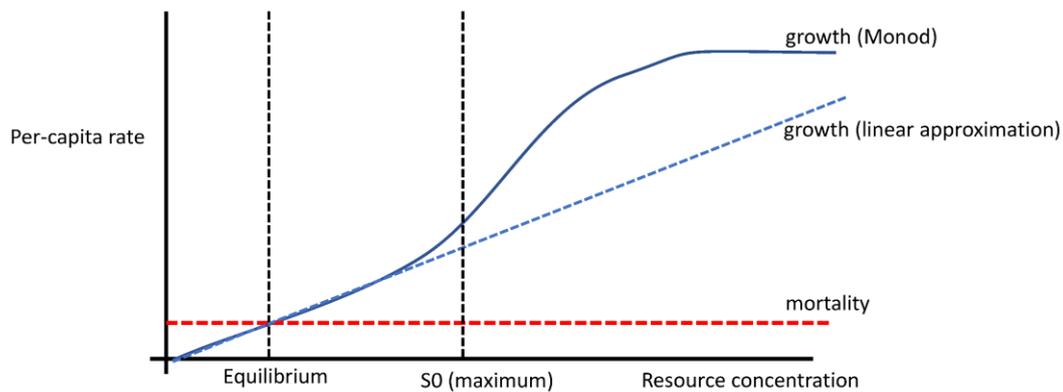


Figure S1: Modelling species growth in a closed system culture with a finite substrate source. While the simulated species experience linear growth, infinite resources are not assumed in the model. This is because there is 1) a maximum total resource in the system ( $S_0$ ), and 2) a dynamic within the system that limits growth (i.e., per-capita mortality is fixed, whereas growth declines linearly as resource becomes scarce). This can be conceptualised as a Monod system, where the maximum possible resource in the system is small enough that species growth rate never gets past the steep slope of the s-shaped curve. For the range of resource values between 0 and  $S_0$  (i.e., the maximum resource in the system), dynamics are almost identical between the linear approximation, and the Monod function. In the model, we use the linear function because it greatly simplifies the equilibria, without qualitatively changing the results. We solved the system of equations using the symbolic equation solver Maxima (Maxima 2014), and then checked the solutions after the fact for accuracy, to ensure that the derivative was indeed zero. These two facts - that  $S_0$  is finite, and that per-

capita growth rates decline as resource becomes scarce, but mortality does not, is why finite equilibria can be calculated for our system.

## References

Maxima. 2014. Maxima, a Computer Algebra System. Version 5.34.1.  
<https://maxima.sourceforge.io/>.

| Table S1. Model variables and parameters. |  |        |  |
|---|--|--------|--|
| Variable                                  | Definition   |        |  |
| $N_E$                                     | Biomass concentration of the enzyme producer species (mg biomass L <sup>-1</sup> solution)   |        |  |
| $N_C$                                     | Biomass concentration of the cheater species (mg biomass L <sup>-1</sup> solution)   |        |  |
| $E$                                       | Enzyme concentration (K mg enzyme L <sup>-1</sup> solution) - see below for definition of K  |        |  |
| $R_E$                                     | Resource concentration in the vicinity of the enzyme producer (mg resource L <sup>-1</sup> solution)   |        |  |
| $R_C$                                     | Resource concentration in the vicinity of the cheater (mg resource L <sup>-1</sup> solution)   |        |  |
| $S$                                       | Substrate concentration (mg substrate L <sup>-1</sup> solution)  |        |  |
| Parameter                                 | Definition   | Value  | Source   |
| $r$                                       | Species intrinsic growth rate per units resource (hour <sup>-1</sup> / (mg resource L <sup>-1</sup> solution))                                       | 1.66   | Wang <i>et al.</i> , 2010<br>Lessard, 2013<br>+  |
| $m$                                       | Species mortality rate (hour <sup>-1</sup> )   | 0.11   | Wang <i>et al.</i> , 2010  |
| $e$                                       | Growth rate reduction associated with enzyme production (hour <sup>-1</sup> )  | 0.0005 | Frankena <i>et al.</i> , 1988<br>Halter <i>et al.</i> , 2019<br>ESS value from our model |
| $m_z$                                     | Rate of enzyme breakdown (hour <sup>-1</sup> )   | 1.05   | Busto <i>et al.</i> , 1996<br>§  |
| $g$                                       | Rate at which the enzyme produces resources from substrate, in terms of K mg of enzyme (hour <sup>-1</sup> / (K mg enzyme L <sup>-1</sup> solution)) | 138.01 | Wang <i>et al.</i> , 2012<br>Premalatha <i>et al.</i> , 2015<br>⌘                        |
| $q$                                       | Units of resource per unit of biomass (mg resource mg <sup>-1</sup> biomass)   | 0.65   | Wortel <i>et al.</i> , 2018  |

|       |   |      |   |
|-------|---|------|---|
| $q_z$ | Resource concentration of K mg enzyme, relative to resource concentration of 1 mg biomass<br>(K mg enzyme mg <sup>-1</sup> resource)/(mg biomass mg <sup>-1</sup> resource)   | 1    | By definition of K  |
| $d$   | Resource diffusion rate (hour <sup>-1</sup> )   | 0.1  | *   |
| $a$   | Rate at which substrate in the system is made biologically available (hour <sup>-1</sup> )  | 0.01 | McDowell <i>et al.</i> , 1988<br>McFee <i>et al.</i> , 1995<br>Kalbitz <i>et al.</i> , 2000 |
| $S_0$ | Total initial substrate concentration in the system (mg substrate L <sup>-1</sup> solution)   | 1.25 | McDowell <i>et al.</i> , 1988<br>McFee <i>et al.</i> , 1995<br>Kalbitz <i>et al.</i> , 2000 |
| $q_s$ | Conversion of substrate to resource (mg substrate mg <sup>-1</sup> resource)  | 1    | By definition of S  |
| $K$   | Scaling constant used to simplify the presentation and simulation of our model system. K is chosen such that K mg enzyme have the same resource content as 1 mg of biomass, i.e. such that the conversion coefficient $q_z = 1$ . | 0.53 |   |

+ To estimate  $r$ , we took the maximum growth rate for the species from Wang et al. 2010 ( $r = 2.08$ ) and set this equal to the growth rate achieved in our system at the maximum possible resource concentration (i.e.,  $S_0$ ), such that maximum growth rate =  $2.08 = r S_0$ , and  $r = 2.08/S_0 = 2.08/1.25 = 1.66$ . This value for  $r$  represents the growth rate per unit resource in our system.

α To estimate rate  $g$  for the scaled enzyme concentration  $E$ , we standardized the reported optimised enzyme production rate from Premalatha et al., 2015 (i.e., 72.63 mg L<sup>-1</sup>, based reported maximum enzyme yield estimated at equilibrium for the maximum possible resource concentration, i.e.,  $S_0$ ) by the constant  $K$  (i.e., the resource content of enzymes relative to biomass). This yielded a production rate for the “standardised” enzyme units that we use in the remainder of the model (i.e., in  $K$  mg enzyme L<sup>-1</sup>).

§ For  $m_z$  in our model, we assume that enzyme concentration and therefore dead enzyme concentration is negligible as a resource, compared to the availability of the main nutrient resource.

\* In our model, if  $d = 0$ , then we assume that there is no diffusion (e.g. enzymes and resources stay inside the cell). Otherwise, as  $d$  approaches infinity, the assumption is that diffusion is very fast (e.g. cheaters and producers mixed in dense culture).

## References

- Busto, N. Ortega, and M. Perez-Mateos. 1996. Location, kinetics and stability of cellulases induced in *Trichoderma reesei* cultures. *Bioresource technology* 57:187–192.
- Frankena, J., H. W. Van Verseveld, and A. H. Stouthamer. 1988. Substrate and energy costs of the production of exocellular enzymes by *Bacillus licheniformis*. *Biotechnology and bioengineering* 32:803–812.
- Halter, W., S. Michalowsky, and F. Allgöwer. 2019. Extremum seeking for optimal enzyme production under cellular fitness constraints. Pages 2159–2164 2019 18th European Control Conference (ECC).
- Kalbitz, K., S. Solinger, J.-H. Park, B. Michalzik, and E. Matzner. 2000. Controls on the dynamics of dissolved organic matter in soils: A review. *Soil science* 165:277.
- Lessard, J. C. (2013) 'Chapter Eleven - Growth Media for *E. coli*', in Lorsch, J. (ed.) *Methods in Enzymology*. Academic Press, pp. 181–189. doi: 10.1016/B978-0-12-420067-8.00011-8.
- McDowell, W. H., and G. E. Likens. 1988. Origin, Composition, and Flux of Dissolved Organic Carbon in the Hubbard Brook Valley. *Ecological monographs* 58:177–195.
- McFee, W. W., J. M. Kelly, B. E. Herbert, and P. M. Bertsch. 1995. Characterization of Dissolved and Colloidal Organic Matter in Soil Solution: A Review. *Carbon Forms and Functions in Forest Soils*. Soil Science Society of America.
- Premalatha, N., N. O. Gopal, P. A. Jose, R. Anandham, and S.-W. Kwon. 2015. Optimization of cellulase production by *Enhydrobacter* sp. ACCA2 and its application in biomass saccharification. *Frontiers in microbiology* 6:1046.
- Wang, G., W. M. Post, M. A. Mayes, J. T. Frerichs, and J. Sindhu. 2012. Parameter estimation for models of ligninolytic and cellulolytic enzyme kinetics. *Soil biology & biochemistry* 48:28–38.
- Wang, P., L. Robert, J. Pelletier, W. L. Dang, F. Taddei, A. Wright, and S. Jun. 2010. Robust growth of *Escherichia coli*. *Current biology*: CB 20:1099–1103.
- Wortel, M. T., E. Noor, M. Ferris, F. J. Bruggeman, and W. Liebermeister. 2018. Metabolic enzyme cost explains variable trade-offs between microbial growth rate and yield. *PLoS computational biology* 14:e1006010.