Supplementary material for research article

Tunable population dynamics in a synthetic filamentous co-culture

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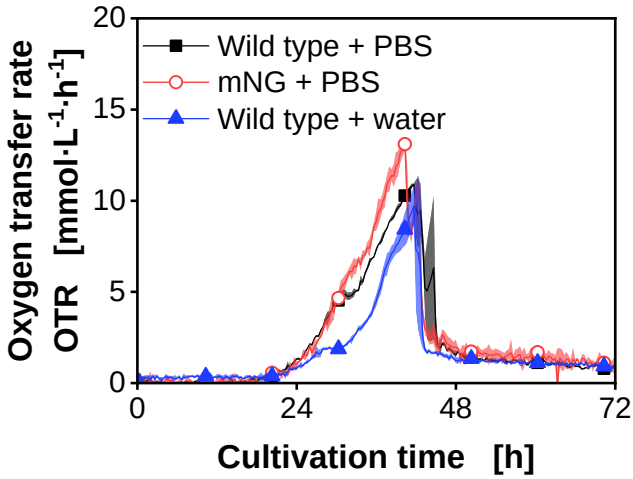

Figure S1. Oxygen transfer rates for axenic cultivations of *Streptomyces coelicolor* A3(2) wild type from figure 2 and S3 and *Streptomyces coelicolor* A3(2) mNG from Figure 4. Spore stocks were prepared either with phosphate-buffered saline (PBS) or deionized water. For clarity, only a representative amount of data points is indicated by the corresponding symbols. Culture conditions: 48-well round well plate, N = 3, X_0 = 10^6 spores·mL^{-1}, V_L = 1000 µL, n = 800 rpm, d_0 = 3 mm, T = 30 °C, LNP medium.

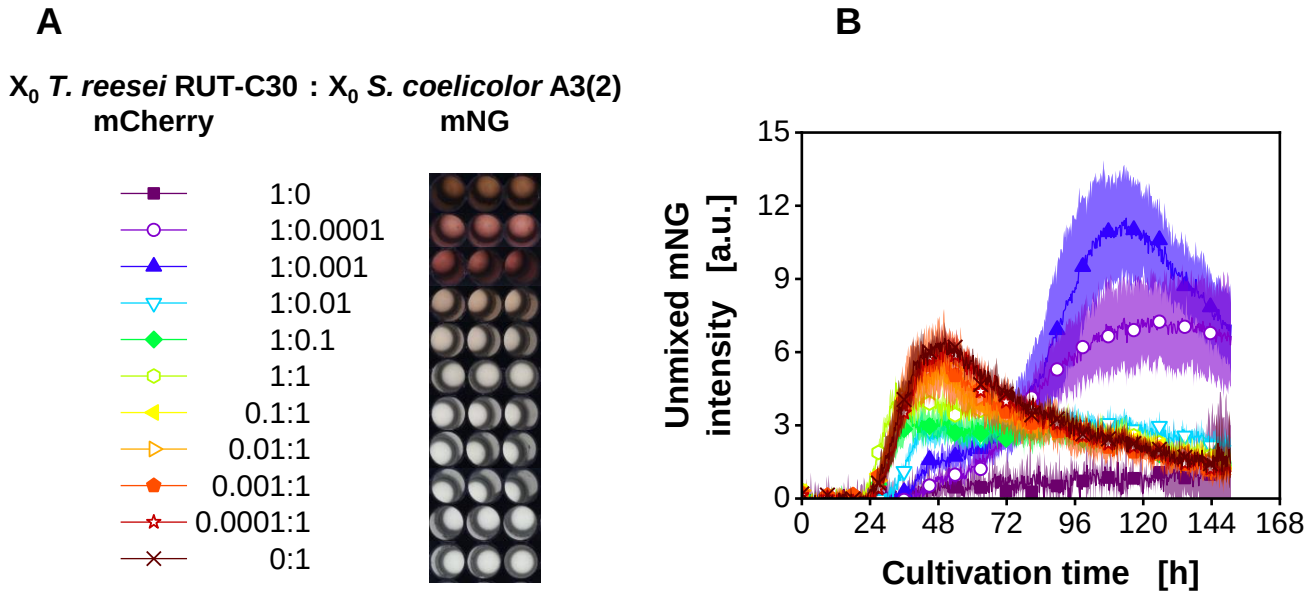

Figure S2. Co-cultivations of *Trichoderma reesei* RUT-C30 mCherry and *Streptomyces coelicolor* A3(2) mNG. Inoculation ratios were varied, as given relating to the standard inoculation size of $X_0 = 10^6$ spores·mL^{-1}. The spore stock of *S. coelicolor* was prepared with phosphate-buffered saline. (**A**) Macroscopic pictures of the culture broth after termination of the cultivation. (**B**) Umixed mNG intensity signal from data obtained at channel 1 (Excitation: 480; Emission: 520 nm) and channel 2 (Excitation: 450; Emission: 520 nm). For clarity, only a representative amount of data points is indicated by the corresponding symbols in (A). Culture conditions: 48-well round well plate, N = 3, V_L = 1000 µL, n = 800 rpm, d_0 = 3 mm, T = 30 °C, LNP medium.

Method - Unmixing of green fluorescence signals

The method reported by Lichten et. al 2014 was used for spectral unmixing of the online fluorescence signal corresponding to the mNeonGreen (mNG) tag [1]. This method was validated and adapted for co-cultures by Palacio-Barrera et al. 2022 [2]. Online green fluorescence signals of the mNG tag were unmixed using data of two channels: Data of Channel 1, corresponds to a Green Fluorescent Protein (or GFP-like) dominated signal (Excitation: 480; Emission: 520 nm). Data of Channel 2, corresponds to an autofluorescence dominated signal (Excitation: 450; Emission: 520 nm), which is mainly attributed to the biogenic fluorophore riboflavin. Measurements were performed with a gain of 100. Detailed description of method adaption can be found in the work of Palacio-Barrera et al. 2022 [2].

Discussion - Unmixing of green fluorescence signals

It can be observed that the trends for the unmixed signal (Figure S1) of all traces are very similar, when compared to the green fluorescence signal obtained from channel 1 and portrayed in Figure 3B. The fluorescence values are lower, since here, only the signal that comes from the mNeonGreen tag is considered. Herewith, there is a drop of the unmixed fluorescence after 52 h, in the axenic culture of *S. coelicolor* mNG as well as the co-cultures with low *T. reesei* to *S. coelicolor* ratio (Brown to green lines). As the mentioned co-cultures were unpigmented, we hypothesize that after 52 h, there was either biomass death, or proteolytic degradation. Likewise, we suggest, that as in these co-cultures production of fluorescence proteins was discontinued very early, photobleaching probably occurred. The mentioned drop in unmixed fluorescence is not pH related. The pKa of mNeonGreen is 5.7 and in these cases, the final pH of the broth was above 6.7 (Table S1). In contrast, for the co-cultures with the two highest *T. reesei* to *S. coelicolor* ratios, which are highly pigmented, a drop in the unmixed signal is visible at the end of the culture. We suggest that onset of pigment formation is the main cause of this drop, as already reported by Finger et al. 2022 and Palacio-Barrera et al. 2022 [1,3]. This exemplary process of unmixing the mNG fluorescence signal as specific biomass measure for *S. coelicolor* from the green autofluorescence signal shows for this co-culture that the overall green autofluorescence signal also gives a good relative account for the biomass of *S. coelicolor* (compare Figure S1B with Figure 3B of main text). Since it was not possible in all used Biolector devices to measure two independent green fluorescence channels simultaneously as is required for applying the unmixing process, we only present the total green fluorescence as a relative signal for *S. coelicolor* biomass for all other experiments of this manuscript.

Table S1. Final pH of broth for *T. reesei* mCherry : *S. coelicolor* mNG co-cultures and axenic controls

T. reesei mCherry : *S. coelicolor* mNG	Final pH
1:0	5.81 ± 0.01
1:0.0001	5.78 ± 0.02
1:0.001	5.90 ± 0.01
1:0.01	6.74 ± 0.02
1:0.1	6.75 ± 0.01
1:1	6.73 ± 0.01
0.1:1	6.75 ± 0.00
0.01:1	6.74 ± 0.00
0.001:1	6.74 ± 0.01
0.0001:1	6.72 ± 0.00
0:1	6.74 ± 0.01

References - Unmixing of green fluorescence signals

[1] C.A. Lichten, R. White, I.B.N. Clark, P.S. Swain, Unmixing of fluorescence spectra to resolve quantitative time-series measurements of gene expression in plate readers, BMC Biotechnology 14 (2014) 11. https://doi.org/10.1186/1472-6750-14-11.

[2] A. Palacio-Barrera, I. Schlembach, M. Finger, J. Büchs, M. Rosenbaum, Reliable online measurement of population dynamics for filamentous co-cultures, Microbial Biotechnology (2022).

[3] M. Finger, F. Sentek, L. Hartmann, A. Palacio-Barrera, I. Schlembach, M. Rosenbaum, J. Büchs, Insights into *Streptomyces coelicolor* A3(2) growth and pigment formation with high-throughput online monitoring, Engineering in Life Science (2022).

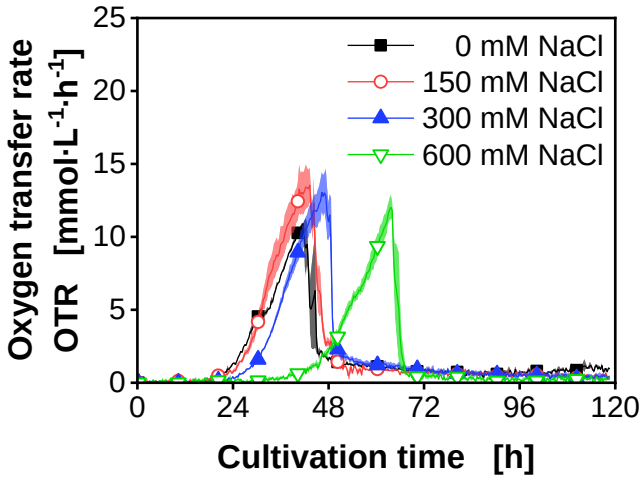

Figure S3. Oxygen transfer rates for axenic cultivations of *Streptomyces coelicolor* A3(2) wild type with varying amounts of added sodium chloride. For clarity, only a representative amount of data points is indicated by the corresponding symbols. Culture conditions: 48-well round well plate, N = 3, X_0 = 10^6 spores·mL^{-1}, V_L = 1000 µL, n = 800 rpm, d_0 = 3 mm, T = 30 °C, LNP medium.

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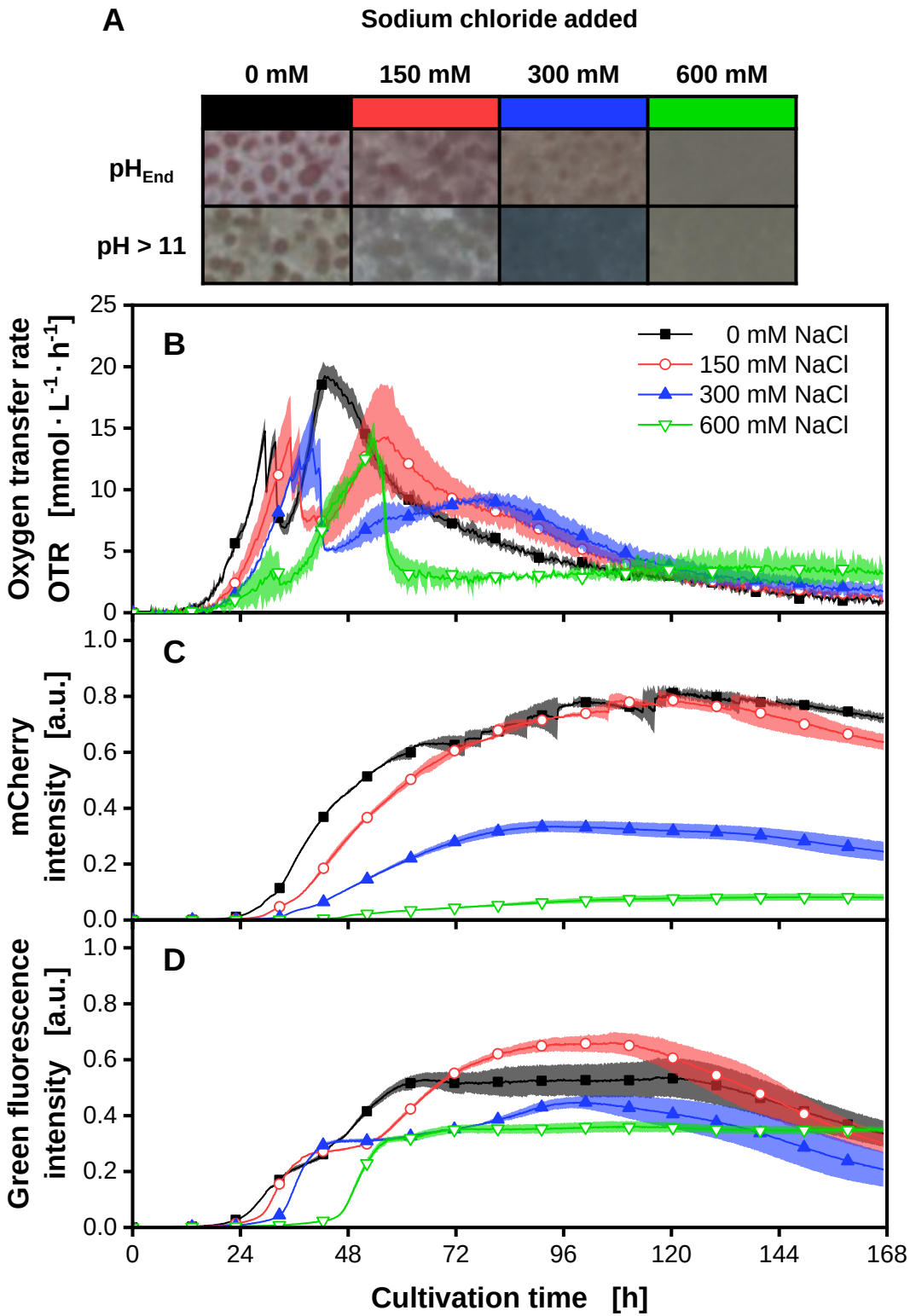
A
Sodium chloride added
0 mM
150 mM
300 mM
600 mM
pH$_{End}$
pH > 11
B
0 mM NaCl
150 mM NaCl
300 mM NaCl
600 mM NaCl
Oxygen transfer rate OTR [mmol · L^{-1} · h^{-1}]
C
mCherry intensity [a.u.]
D
Green fluorescence intensity [a.u.]
Cultivation time [h]

Figure S4. Co-cultivations of *Trichoderma reesei* RUT-C30 mCherry and *Streptomyces coelicolor* A3(2) wild type with varying amounts of added sodium chloride. (**A**) Macroscopic pictures of the culture broth after termination of the cultivation. The pH was changed to a value above 11 to determine production of the blue pigment actinorhodin (second row in (A)). (**B**) Oxygen transfer rates. (**C**) mCherry intensity signals (Excitation: 587 nm; Emission: 610 nm). (**D**) Green fluorescence intensity signals (Excitation: 483 nm; Emission: 520 nm). For clarity, only a representative amount of data points is indicated by the corresponding symbols in (B) – (D). Culture conditions: 48-well round well plate, N = 3, X_0 = 10^6 spores·mL^{-1}, V_L = 1000 µL, n = 800 rpm, d_0 = 3 mm, T = 30 °C, LNP medium.

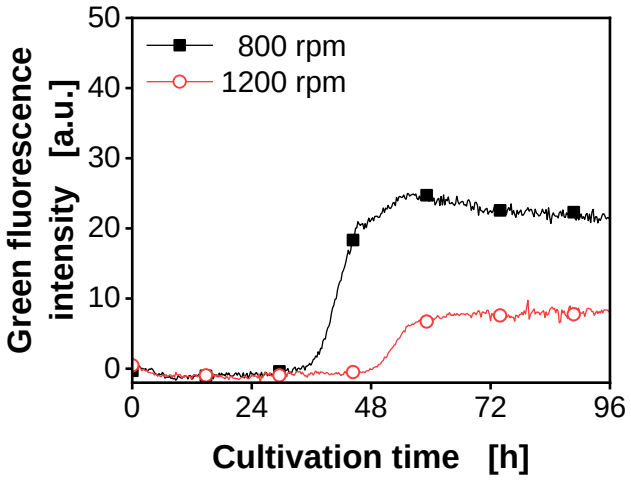

Figure S5. Biomass signal for axenic cultivations of *Streptomyces coelicolor* A3(2) wild type with shaking frequencies of 800 and 1200 rpm. The green fluorescence intensity signals (Excitation: 480 nm; Emission: 520 nm) were monitored. For clarity, only a representative amount of data points is indicated by the corresponding symbols. Culture conditions: 48-well round well plate, $X_0 = 10^6$ spores·mL^{-1}, V_L = 1000 µL, n = 800 rpm, d_0 = 3 mm, T = 30 °C, LNP medium.