RESEARCH ARTICLE

Modeling population dynamics in a microbial consortium under control of a synthetic pheromone-mediated communication system

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Microbial consortia can be used to catalyze complex biotransformations. Tools to control the behavior of these consortia in a technical environment are currently lacking. In the present study, a synthetic biology approach was used to build a model consortium of two *Saccharomyces cerevisiae* strains where growth and expression of the fluorescent marker protein EGFP by the receiver strain is controlled by the concentration of α -factor pheromone, which is produced by the emitter strain. We have developed a quantitative experimental and theoretical framework to describe population dynamics in the model consortium. We measured biomass growth and metabolite production in controlled bioreactor experiments, and used flow cytometry to monitor changes of the subpopulations and protein expression under different cultivation conditions. This dataset was used to parameterize a segregated mathematical model, which took into account fundamental growth processes, pheromone-induced growth arrest and EGFP production, as well as pheromone desensitization after extended exposure. The model was able to predict the growth dynamics of single-strain cultures and the consortium quantitatively and provides a basis for using this approach in actual biotransformations.

KEYWORDScell-cell communication system, co-culture, mathematical model, pheromone, *Saccharomyces cerevisiae*

1 | INTRODUCTION

Biotechnological applications, especially in the field of metabolic engineering and synthetic biology, are dominated by pure culture strategies [1]. Despite the well-documented success of pure cultures, this strategy is challenged by the increasing complexity of biotechnological processes, for example, bioconversion of low-cost substrates into high-value products. In this context, pure cultures may be outperformed by consortia [2–6]. Compared with pure cultures, consortia

have several important benefits. The metabolic burden on production organisms can be alleviated by distributing different tasks to individual host strains. The modularization of biosynthetic pathways also enables the utilization of different intracellular environments, the reduction of undesired metabolic crosstalk between different pathway modules, individual optimization of pathway modules and exploitation of complex substrates [2]. However, the application of consortia in metabolic engineering and synthetic biology projects started only recently [2].

The primary challenge in using consortium approaches is to maintain a constant community composition despite differences in doubling time of the community members. In nature, consortia are stabilized by direct or indirect communication between the community members which is brought

Abbreviations: BI, budding index; CCA, cell cycle arrest; EGFP, enhanced green fluorescent protein; FSC, forward scatter; PR, pheromone response; RFI, relative fluorescent units; *S. cerevisiae*, *Saccharomyces cerevisiae*; tRFP, turbo red fluorescent protein.

about by, for example, cell-cell contact, exchange of signaling molecules and cross-feeding of essential nutrients [5]. Cross-feeding approaches were applied to synthetic consortia, but these strategies only promote stable consortia at intermediate densities [7] and are prone to the evolution of ‘cheaters’, that are subpopulations which consume the benefit provided by others but fail to provide the return benefit [3]. To circumvent these problems, we have recently suggested to engineer growth-controlling synthetic cell-cell communication systems using signal molecules [4,8]. This approach could serve as a novel strategy to control complex production processes which, for example, utilize substrate availability as a trigger element to switch on/off cell-cell communication, thereby adapting the cellular behavior to optimize process flow and product yields [8,9].

In this context, the mating pheromone system of the yeast *Saccharomyces cerevisiae* is of particular interest. *S. cerevisiae* forms haploid cells of two different mating types, **a** or α , each secreting a specific pheromone, **a** or α -factor, respectively. Both cell types carry mating-type specific G-protein coupled receptors embedded in the plasma membrane, which recognize pheromones of the complementary cell type. Pheromone binding activates the pheromone response (PR) pathway which triggers Far1-dependent cell cycle arrest in G1, Ste12-dependent transcription of mating-associated genes and culminates in the formation of a mating projection that is termed ‘shmoo’ [10,11].

Since the PR in *S. cerevisiae* is well characterized at both the genetic and molecular levels, it can be readily engineered to elicit a desired cellular response. For example, deletion of the *bar1* gene encoding an aspartyl protease prevents degradation of α -factor and renders *MATa* cells more sensitive to the pheromone. Inversely, strains of the **a** or α mating type can be rendered insensitive to pheromone by inactivation of the receptor proteins Ste2 and Ste3, respectively [11]. In addition, deletion of the *far1* gene (encoding a CDK inhibitor) prevents cell cycle arrest in response to mating pheromone whilst a Ste12-dependent transcriptional response can be maintained [12].

In the present study, we investigated the population dynamics of a two-strain consortium under control of an engineered pheromone-based communication system. The emitter strain constitutively produced the α -factor mating pheromone, which triggered entry into cell cycle arrest and/or expression of a fluorescent protein in the receiver strain. We have studied growth of these strains in pure cultures in the presence and absence of synthetic α -factor, and in co-cultivation experiments. Pure culture experiments served to parameterize a quantitative mathematical model, which particularly accounted for the pheromone-dependent entry into cell cycle arrest and expression of a fluorescent marker protein. Using this model, the behavior of a two-strain consortium was simulated and found to be in good agreement with experimental

PRACTICAL APPLICATION

Consortium approaches benefit from the modularization of biosynthetic labor between the community members in order to increase product yields through the reduction of metabolic burden for each strain. However, the main disadvantage of consortium-based designs is a lack of available tools to control the behavior and performance of individual subpopulations and of the entire consortium. We implemented an artificial cell-cell communication system by using mating pheromones in a consortium that consists of two haploid *S. cerevisiae* strains. We demonstrated that the behavior of one strain can be changed *via* pheromones secreted by the other strain. This accomplishment provides a basis for advancing the reliability and performance of consortium-based biosynthesis.

TABLE 1 *S. cerevisiae* strains

Name	Genotype	Origin
BY4741 $\Delta bar1$	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 YIL015w::kanMX4</i>	Euroscarf
<i>P_{FIG1}-EGFP</i>	BY4741 $\Delta bar1$ <i>TYR1::FIG1-EGFP</i>	This study
$\Delta far1$ <i>P_{FIG1}-EGFP</i>	BY4741 $\Delta bar1$ <i>YJL157c::hphMX6 TYR1::FIG1-EGFP</i>	This study
BY4741 $\Delta ste2$	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 YFL026w::kanMX4</i>	Euroscarf
$\Delta ste2$ <i>P_{FIG1}-EGFP</i>	BY4741 $\Delta ste2$ <i>TYR1::FIG1-EGFP</i>	This study
BY4742 $\Delta ste3$	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 YKL178c::kanMX4</i>	Euroscarf
<i>P_{GPD1}-tRFP</i>	BY4742 $\Delta ste3$ <i>TYR1::GPD1-tRFP</i>	This study

data. Our study provides a quantitative framework for predicting growth of yeast consortia under the control of a pheromone-based communication system.

2 | MATERIALS AND METHODS

2.1 | Strains and media

The *S. cerevisiae* strains BY4741 $\Delta bar1$, BY4741 $\Delta ste2$ and BY4742 $\Delta ste3$ (Table 1) were purchased from Euroscarf

(Frankfurt, Germany). To enable pheromone-inducible expression of fluorescent proteins, the Open Reading Frame (ORF) encoding enhanced green Fluorescent Protein (EGFP) was placed downstream of the pheromone-responsive *FIG1* promoter (P_{FIG1}) and upstream of the yeast *CYC1* terminator, and the expression cassette was integrated into the pFA6a-natMX6 vector (Supporting Information Figure S3) [13]. The entire fragment encompassing the pheromone-inducible expression cassette and the natMX6 marker was PCR-amplified and transformed into the corresponding yeast strains to replace the authentic *TYR1* locus, thereby creating the strains BY4741 $\Delta bar1 P_{FIG1}$ -EGFP and BY4741 $\Delta ste2 P_{FIG1}$ -EGFP. To generate yeast cells constitutively expressing the turbo Red Fluorescent Protein (tRFP), the corresponding ORF was placed downstream of the yeast *GPD1* promoter (P_{GPD1} ; and upstream of the *CYC1* terminator) and integrated into pFA6a-natMX6 (Supporting Information Figure S4). The corresponding fragment (constitutive expression cassette and natMX6 marker) was transformed into yeast, again replacing the *TYR1* locus to yield BY4742 $\Delta ste3 P_{GPD1}$ -tRFP. Finally, the yeast *FAR1* gene was deleted using the hphMX6 cassette [13] to create BY4741 $\Delta bar1 \Delta far1 P_{FIG1}$ -EGFP.

For bioreactor cultivations, a defined mineral medium, which contained glucose as the growth-supporting carbon source [14], essential supplements for auxotrophic markers (per liter: 135 mg histidine, 335 mg leucine, 320 mg lysine, 90 mg methionine, 200 mg tyrosine, 105 mg uracil) and additional amino acids (per liter: 100 mg glutamic acid, 50 mg phenylalanine, 375 mg serine, 200 mg threonine) to ensure optimal growth of BY strains [15], was used. 0.1 vol% Antifoam A (Sigma-Aldrich, USA) was added to prevent foaming.

2.2 | Growth conditions

Cultivations were carried out in a 1 L stirred bioreactor (Multifors, Infors HT, Switzerland) at 900 rpm, 30°C and a constant air flow of 0.5 L min⁻¹ adjusted by a mass flow controller (Aalborg, USA). The O₂ and CO₂ content in the exhaust gas was measured by an EL3020 gas analyzer (ABB, Germany). The pH was kept at 5.5 *via* automatic addition of 2 M NaOH. The reactor was filled with salts dissolved in 500 mL water and autoclaved for 20 min at 121°C. All other media compounds were transferred to the bioreactor in form of sterile-filtered stock solutions, resulting in a liquid volume of 800 mL [14].

The inoculum was developed in two steps. First, an Erlenmeyer flask with YPD medium (10 g yeast extract, 20 g peptone, 18 g glucose per liter) was prepared, inoculated with a frozen culture of the corresponding yeast strain (stored at -80°C in 50 vol% glycerol) and shaken for 24 h with 180 rpm at 30°C. In a second step, another Erlenmeyer flask

was set up containing the same medium as used for the cultivation in a stirred tank reactor, but buffered with 27.3 g L⁻¹ 2-(N-morpholino)ethanesulfonic acid hydrate at pH 5.5, and inoculated with an adequate aliquot of the previous preculture, to give an initial optical density at 600 nm (OD₆₀₀) of 0.2. This preculture was harvested after 8 h to 10 h ensuring a population exhibiting exponential growth on glucose and used for inoculation of the bioreactor with an initial OD₆₀₀ of 0.2. For co-culture experiments, an initial OD₆₀₀ = 0.1 was used for each strain. In pure cultures, the pheromone response was induced after 3 h of cultivation *via* synthetic α -factor (Mol-surf, Germany) added to a final concentration of 1 μ M.

2.3 | Analytics

2.3.1 | Biomass and metabolites

The biomass dry weight was determined by separating the biomass from the liquid phase *via* centrifugation, washing the pellet two times, and drying it at 103°C. It is worth mentioning that the correlation between OD₆₀₀ and biomass concentration loses its linearity while cells form shmoo tips, therefore, measuring the biomass directly under conditions that trigger shmoo formation is mandatory. The average cell weight of a population cultivated as pure culture was determined by $\bar{m} = X/N$, with the total biomass concentration X and the total cell concentration N . To calculate the biomass of the different subpopulations in a co-culture, the relation $X_{FSC,i} = \bar{m}_{FSC,i} N_i$ was used, where i refers to the subpopulation and $\bar{m}_{FSC,i}$ to the average cell weight determined *via* forward scatter (FSC) of the flow cytometer (see below). For the determination of $\bar{m}_{FSC,i}$ a correlation between the arithmetic mean of FSC of the cell population $\overline{FSC}$ and the cell weight $\bar{m}$ was introduced. Subsequently, an exponential function was fitted to these data points (Supporting Information Figure S1), which results in Eq. (1) with the coefficients $a = 1.73 \times 10^{-12}$ g and $b = 0.0127$.

$$\bar{m}_{FSC,i} = a \cdot \exp\left(b \cdot \overline{FSC}_i\right) \quad (1)$$

Then $X_{FSC,i}$ was used to calculate the biomass ratio of the different subpopulations which in turn was multiplied with the measured total biomass concentration X to obtain X_i .

The concentration of ethanol and glycerol was analyzed by HPLC as described in [16] using an RI detector. Glucose was quantified as reducing sugar by the DNS method [14].

2.3.2 | Fluorescence and cell concentration

Fluorescence and cell concentration measurements were carried out using a flow cytometer (Cyflow Cube 8, Partec GmbH, Germany). Cell suspensions were sonicated (10 s, 40%, Topas UDS751, Germany) to disperse cell aggregates and diluted with phosphate buffered saline (PBS) prior to analysis. The EGFP fluorescence was measured utilizing

excitation at 488 nm and emission at 565–615 nm (FL2). Changes in fluorescence were followed by recording the arithmetic mean of 100 000 events *via* FL2. Subsequently, this value was subtracted by the arithmetic mean of the basal fluorescence, resulting in a value that was defined as the fluorescence of EGFP in an average cell. The cell concentration N was determined by analyzing 200 μL of the sample.

2.3.3 | Cell cycle analysis

For cell cycle analysis, the protocol as described in [17] was modified. In short: to ensure optimal flow properties in the flow cytometer, approximately 2×10^6 cells were harvested by centrifugation ($8000 \times g$, 6 min, 4°C), resuspended in 300 μL water, then fixed with 700 μL ethanol and stored at -20°C for at least 12 h. Subsequently, cells were washed with TRIS buffer (50 mM TRIS, pH 7.5), centrifuged and resuspended in TRIS buffer containing 0.2 mg mL^{-1} RNase A (Merck, Germany). After 4 h of incubation at 37°C , cells were washed with 1 mL TRIS buffer, sonicated at low intensity and incubated with 5 μL 0.5 nM SYTOX Green (Invitrogen, USA) for 10 min at room temperature in the dark. For analysis, 100 000 particles of the prepared samples were recorded using the FL2 detector. The resulting peaks representing cells with a single or duplicated genome are assigned to the G_1 and G_2 , respectively. G_1 is associated with bud formation, therefore, the fraction $G_2/(G_1 + G_2)$ represents the budding index [18].

2.3.4 | Cell morphology analysis

Morphological changes were analyzed *via* FSC in a flow cytometry analysis. Fresh cell samples were sonicated and diluted with PBS prior to recording 100 000 particles. For simplicity, the arithmetic mean of the FSC was used as an equivalent for the cell volume and cell weight likewise.

3 | RESULTS AND DISCUSSION

To investigate the behavior of two-strain consortia that are under control of a pheromone-based communication system, we constructed a receiver strain of mating type **a** which was rendered hypersensitive to α -factor by deletion of *bar1* and which expressed EGFP under the control of the pheromone-responsive *FIG1* promoter. Growth of the receiver strain (BY4741 $\Delta bar1 P_{FIG1}$ -EGFP) was first studied in pure cultures in the presence or absence of synthetic α -factor to quantify the physiological and morphological response to the pheromone. In the next step, the receiver strain was cultivated in presence of the emitter strain which constitutively secreted α -factor and which was insensitive to pheromone due to the deletion of *ste3*. To distinguish both strains in mixed cultures, emitter cells (BY4742 $\Delta ste3 P_{GPD1}$ -tRFP) constitutively expressed a red fluorescent marker protein from the

GPD1 promoter. All cultivation experiments were carried out in controlled bioreactors to obtain quantitative information on the cellular physiology and population dynamics. Our investigations were restricted to the respiro-fermentative growth phase on glucose because PR is repressed after depletion of this carbon source [19].

3.1 | Pure culture experiments

To describe the growth behavior of the receiver strain, cells are classified as proliferating (PRO) or cell-cycle-arrested cells (CCA). When cultivating the receiver strain without addition of α -factor, the population followed the typical patterns for diauxic growth of *S. cerevisiae*. Cells proliferated on glucose with a maximal growth rate of $\mu_{max}^{PRO} = 0.42 \text{ h}^{-1}$ and maintained an average cell weight of $\bar{m}_{PRO} = 1.5 \times 10^{-11} \text{ g cell}^{-1}$. Other kinetic parameters are shown in Table 2.

In the subsequent experiment, synthetic α -factor was added to an exponentially growing culture after 3 h of cultivation to a final concentration of 1 μM . Due to α -factor-mediated PR, cell cycle arrest and shmoo formation were initiated and the exponential increase of the cell concentration ceased approximately 1 h after addition of the pheromone (Figure 2A). Accordingly, the budding index (BI), which correlates to the fraction of cells with a duplicated genome, decreased during the same time span until all cells were in the G_1 phase (Figure 2D). The apparent lag time between addition of α -factor and manifestation of the cell cycle arrest at the population level can be explained by the unsynchronized growth of the population and the fact that cells had to complete the cell cycle before becoming arrested upon entry into G_1 phase.

Even though the cell concentration of the receiver strain remained constant after exposure to α -factor, biomass concentration continued to increase. This was the result of an increase in cell weight of the G_1 -arrested cells (Figure 2B and C) which is associated with shmoo tip formation. The specific growth rate of cell-cycle-arrested cells was significant lower than for proliferating cells ($\mu_{max}^{CCA} = 0.23 \text{ h}^{-1}$ compared to $\mu_{max}^{PRO} = 0.42 \text{ h}^{-1}$). Cell-cycle-arrested cells consumed glucose more slowly (Figure 2E) and exhibited a smaller biomass yield when compared to proliferating cells ($Y_{X/S}^{CCA} = 0.11 \text{ g g}^{-1}$, $Y_{X/S}^{PRO} = 0.15 \text{ g g}^{-1}$). The lower biomass yield was accompanied by increased formation of byproducts such as glycerol (Figure 2G), which supports recent findings [20]. The secretion of glycerol is a characteristic element of the PR because the High-Osmolarity-Glycerol (HOG) pathway is activated as a consequence of reduced internal osmolarity in shmooing cells [21].

The respiration coefficient (RQ) was not affected by the decreased growth rate of the cell-cycle-arrested cells, which is in line with constant ethanol yields of proliferating and arrested cells $Y_{E/S}$ ($Y_{E/S}^{PRO} = Y_{E/S}^{CCA} = Y_{E/S}$; Figure 2I). This

TABLE 2 Kinetic parameters for the co-culture model of the receiver (BY4741 $\Delta bar1 P_{FIG1}$ -EGFP) and the emitter strain (BY4742 $\Delta ste2 P_{GPD1}$ -tRFP)

Parameter	Receiver PRO	Receiver CCA	Emitter
μ	0.42 h ⁻¹	0.23 h ⁻¹	0.39 h ⁻¹
$Y_{X/S}$	0.15 g g ⁻¹	0.11 g g ⁻¹	0.13 g g ⁻¹
$Y_{E/S}$	0.53 g g ⁻¹	0.53 g g ⁻¹	0.44 g g ⁻¹
$Y_{G/S}$	0.04 g g ⁻¹	0.11 g g ⁻¹	0.05 g g ⁻¹
$Y_{af/X}$	—	—	22 nmol g ⁻¹
$\frac{1}{m}^{PRO*}$	1.5×10^{-11} g cell ⁻¹	1.5×10^{-11} g cell ⁻¹	1.5×10^{-11} g cell ⁻¹
π_{EGFP}	—	36 RFI h ⁻¹ cell ⁻¹	—
r_{+1}	—	1.96 h ⁻¹	—
r_{-1}	—	1.96 h ⁻¹	—

result is in contradiction to data of Williams et al. [20] who observed a temporally decreasing RQ, which was interpreted as a shift towards a more respiratory metabolism upon cell cycle arrest; this finding should, however, not be overrated because of their unreliable RQ determination.

Upon exposure to α -factor, the *FIG1* promoter is activated and drives the EGFP expression in the engineered receiver cells. Accordingly, an increasing fluorescence intensity was observed at approximately 1 h after addition of pheromone (Figure 2H). This time delay of 1 h was consistent with slow maturation of EGFP and the time required to switch on pheromone-dependent gene expression [22]. Assuming a linear correlation between fluorescence intensity and the amount of EGFP per cell, the increase of accumulated EGFP was also linear (Figure 2H). The fact that the total amount of EGFP did not increase exponentially suggests that the EGFP synthesis rate is independent of the cell weight.

Due to the low growth rate of arrested cells (μ^{CCA}), a linear fit could also be adjusted to cell weight data, however, single cell analysis performed by Colman-Lerner et al. [23] revealed exponential growth in volume. To test the influence of the growth rate on EGFP production, we examined EGFP formation in a $\Delta far1$ knock-out strain that does not arrest in G₁ phase in response to mating pheromones [12] and which maintains its maximal growth rate for proliferation $\mu_{max}^{PRO} = 0.42$ h⁻¹, obtaining identical values for the slope of EGFP as the receiver strain (Supporting Information Figure S2). This indicates that the synthesis of EGFP is in this context independent of cell size and growth rate.

3.2 | Model development

Based on the results of the pure culture experiments, we developed a mathematical model to predict the behavior of a population composed of receiver and emitter cells that communicate *via* extracellular pheromones. The model scheme is depicted in Figure 1 and pertains to the pheromone-controlled changes of cell phenotypes and EGFP production in a co-culture of *S. cerevisiae* BY4741 $\Delta bar1 P_{FIG1}$ -EGFP

(receiver, index: rec) and BY4742 $\Delta ste2 P_{GPD1}$ -tRFP (emitter, index: emi) cells. Emitter cells secrete diffusible α -factor, which is detected by the receiver cells. The pheromone triggers transition of receiver cells from the proliferating state (PRO) to the cell-cycle-arrested state (CCA). This transition can be reversed under certain conditions (see below). EGFP is only produced by cell-cycle-arrested receiver cells. Since the average mass $\bar{m}_{rec}$ of receiver cells is not constant, biomass concentration X_i^j of all cell types is expressed as the product of cell concentration N_i^j and respective average cell mass $\bar{m}_i^j$ according to Eq. (2).

$$X_i^j = N_i^j \bar{m}_i^j \quad (2)$$

where i represents the strain (rec or emi) and j the phenotype (PRO or CCA).

3.2.1 | Mass balance equations for extracellular metabolites

Our pure culture experiments have revealed that arrested cells consume glucose and produce glycerol at different rates compared to proliferating cells. Therefore, mass balance equations of these extracellular compounds (concentrations of: X , biomass; S , glucose; E , ethanol; G , glycerol; α , α -factor) account for these differences by assigning specific yield coefficients Y_i^j to each cell type:

$$\frac{dS}{dt} = -\frac{\mu_{rec}^{PRO} X_{rec}^{PRO}}{Y_{X/S,rec}^{PRO}} - \frac{\mu_{rec}^{CCA} X_{rec}^{CCA}}{Y_{X/S,rec}^{CCA}} - \frac{\mu_{emi}^{PRO} X_{emi}^{PRO}}{Y_{X/S,emi}^{PRO}} \quad (3)$$

$$\begin{aligned} \frac{dE}{dt} = & \frac{\mu_{rec}^{PRO} Y_{E/S,rec}^{PRO} X_{rec}^{PRO}}{Y_{X/S,rec}^{PRO}} + \frac{\mu_{rec}^{CCA} Y_{E/S,rec}^{CCA} X_{rec}^{CCA}}{Y_{X/S,rec}^{CCA}} \\ & + \frac{\mu_{emi}^{PRO} Y_{E/S,emi}^{PRO} X_{emi}^{PRO}}{Y_{X/S,emi}^{PRO}} \end{aligned} \quad (4)$$

FIGURE 1 Segregated model for a co-culture of the receiver (BY4741 $\Delta bar1 P_{FIG1}$ -EGFP) and the emitter strain (BY4742 $\Delta ste2 P_{GPD1}$ -tRFP). The biomass of the receiver strain consists of the two phenotypes proliferation and cell cycle arrest. The biomass of a phenotype in turn is the number of the respective cells multiplied by their average cell weight. The pheromone response of the α -haploid receiver strain is activated by α -factor, which is secreted by the α -haploid emitter strain. The pheromone response includes cell cycle arrest, shmoo formation and synthesis of EGFP. The emitter has no α -factor receptors and cannot respond to the α -factor secreted by the receiver strain

$$\frac{dG}{dt} = \frac{\mu_{rec}^{PRO} Y_{G/S,rec} X_{rec}^{PRO}}{Y_{X/S,rec}^{PRO}} + \frac{\mu_{rec}^{CCA} Y_{G/S,rec} X_{rec}^{CCA}}{Y_{X/S,rec}^{CCA}} + \frac{\mu_{emi}^{PRO} Y_{G/S,emi} X_{emi}^{PRO}}{Y_{X/S,emi}^{PRO}} \quad (5)$$

$$\frac{d\alpha f}{dt} = \mu_{emi}^{PRO} Y_{\alpha f/X} X_{emi}^{PRO} \quad (6)$$

Specifically, $Y_{X/S}$ is the biomass yield coefficient on glucose, $Y_{E/S}$, $Y_{G/S}$ and $Y_{\alpha f/X}$ are the product yield coefficients of ethanol, glycerol and α -factor, respectively. The former two parameters have been determined in the pure culture experiments while $Y_{\alpha f/X}$ was calculated based on data sets of [24]. The specific growth rate μ was calculated using the Monod equation:

$$\mu_i^j = \mu_{max,i}^j \frac{S}{S + K_S} \quad (7)$$

where μ_{max} represents the maximal growth rate and K_S the substrate affinity (calculated with data from [25]).

3.2.2 | Initiation of the pheromone response

As summarized in Figure 1, the presence of pheromone triggers the transition of proliferating receiver cells to the cell-cycle-arrested phenotype and the expression of EGFP. However, after arrested receiver cells have been exposed to α -factor for a certain period of time, they become desensitized to the pheromone and re-enter the proliferating state [26]. In the mathematical model, initiation of these processes is

captured by discrete switch functions denoted $\delta_{PRO \rightarrow CCA}$, δ_{FIG1} , and $\delta_{CCA \rightarrow PRO}$, respectively. All switch functions are under the control of α -factor constitutively produced by the emitter strain. Once α -factor concentration exceeds a threshold concentration ($\alpha f_{PR,crit}$) of 0.1 nM [23], expression of pheromone responsive genes is triggered. The associated time point is stored in the variable t_{PR} according to the following equation.

$$t_{PR} = \begin{cases} \emptyset, & \text{if } \alpha f < \alpha f_{PR,crit} \\ t, & \text{if } \alpha f \geq \alpha f_{PR,crit} \cap t_{PR} = \emptyset \end{cases} \quad (8)$$

The transcriptional response culminates after a certain delay t_{del} in the manifestation of the observable phenotypes cell cycle arrest and EGFP expression. The delay time can be interpreted as the consequence of protein maturation or the response time of a regulatory cascade and can therefore vary depending on the specific process. According to the experimentally characterized response of receiver cells to synthetic α -factor, the delay time for cell cycle arrest was set to 0.3 h and the delay time for EGFP expression to 1 h. Accordingly, the switch functions for the initiation of cell cycle arrest and expression of EGFP are defined by Eqs. (9) and (10), respectively.

$$\delta_{PRO \rightarrow CCA} = \begin{cases} 0, & \text{if } t < t_{PR} + 0.3 \text{ h} \\ 1, & \text{if } t \geq t_{PR} + 0.3 \text{ h} \cap \delta_{CCA \rightarrow PRO} = 0 \end{cases} \quad (9)$$

$$\delta_{FIG1} = \begin{cases} 0, & \text{if } t < t_{PR} + 1 \text{ h} \\ 1, & \text{if } t \geq t_{PR} + 1 \text{ h} \cap \delta_{CCA \rightarrow PRO} = 0 \end{cases} \quad (10)$$

Transition of cell-cycle-arrested cells to the proliferating state is initiated according to Eq. (11) after cells were arrested during the time span t_{CCA} .

$$\delta_{CCA \rightarrow PRO} = \begin{cases} 0, & \text{if } t < t_{PR} + t_{CCA} \\ 1, & \text{if } t \geq t_{PR} + t_{CCA} \end{cases} \quad (11)$$

It is of note that release from cell cycle arrest was not observed in pure culture experiments, presumably because the very high concentration of synthetic α -factor prevented desensitization of receiver cells to the pheromone. Therefore, the parameter t_{CCA} was not inferred from experimental data but was adapted to fit results of the simulation to experimental data in co-culture experiments (see below).

3.2.3 | Population dynamics and average cell mass

Population dynamics: Our model distinguishes the cell types proliferating receiver cells, cell-cycle-arrested receiver cells and proliferating emitter cells (Figure 1), whose cell concentrations are denoted N_{rec}^{PRO} , N_{rec}^{CCA} and N_{emi}^{PRO} , respectively. Proliferating emitter and receiver cells grow with the specific growth rate μ_i^{PRO} that only depends on substrate availability (Eq. (7)). During the PR, expression rate of the CDK inhibitor Far1 increases in the receiver cells, causing accumulation of this regulator in the nucleus and a stop of cell cycle progression [26]. In our model, the cell cycle arrest is accounted for by converting cells from the phenotype proliferation, N_{rec}^{PRO} , to cells of the phenotype cell cycle arrest, N_{rec}^{CCA} , with the transfer rate r_{+1} (Eqs. (12) and (13)) after the switch function $\delta_{PRO \rightarrow CCA}$ is activated in response to pheromone (Eq. (9)). Inversely, cells are transferred from the cell-cycle-arrested state to the proliferating state with rate r_{-1} after the switch function $\delta_{CCA \rightarrow PRO}$ is activated (Eq. (11)). Rate r_{-1} was set to the same value as r_{+1} so that all cells spend the same amount of time in the cell-cycle-arrested state. The growth state of the emitter strain is not affected by α -factor. Accordingly, population dynamics of the three cell types are captured by the balance equations (Eqs. (12) – (14))

$$\frac{dN_{rec}^{PRO}}{dt} = \mu_{rec}^{PRO} N_{rec}^{PRO} - \delta_{PRO \rightarrow CCA} r_{+1} N_{rec}^{PRO} + \delta_{CCA \rightarrow PRO} r_{-1} N_{rec}^{CCA} \quad (12)$$

$$\frac{dN_{rec}^{CCA}}{dt} = \delta_{PRO \rightarrow CCA} r_{+1} N_{rec}^{PRO} - \delta_{CCA \rightarrow PRO} r_{-1} N_{rec}^{CCA} \quad (13)$$

$$\frac{dN_{emi}^{PRO}}{dt} = \mu_{emi}^{PRO} N_{emi}^{PRO} \quad (14)$$

Average cell mass: As shown in our experiments, the average mass of the arrested ($\bar{m}_{rec}^{CCA}$) and proliferating ($\bar{m}_{rec}^{PRO}$)

receiver cells is not constant but depends on their cultivation history. Receiver cell populations that did not undergo PR have an average mass ($\bar{m}_{rec}^{PRO*}$) that is equal to the constant average mass of proliferating emitter cell populations. $\bar{m}_{rec}^{PRO*}$ can be considered the lowest cell mass that can be attained by the receiver strains. When receiver cells become arrested in the presence of pheromone, they continue to grow at the specific rate μ_{rec}^{CCA} and can become up to four times heavier than their proliferating counterparts (Figure 2). Thus, comparatively light proliferating receiver cells that just enter the CCA state will decrease the average weight of the arrested cell population. Inversely, heavy CCA cells, which change to the proliferating state, will increase the average cell mass of the proliferating population. Furthermore, the extent by which the average cell mass in a population is changed by the addition of cells from the opposite growth state (CCA or PRO) depends on the relative size of the two populations (*e.g.*, few light proliferating cells that are added to a large population of heavy cell-cycle-arrested cells will not significantly change the average mass in the CCA population). Therefore, the rate by which the average mass in a population changes due to the addition of cells of the opposite growth state is weighted by the ratio of the population sizes, resulting in the following balance equations which describe the changes of the average cell mass in the population of all three cell types.

$$\frac{d\bar{m}_{rec}^{PRO}}{dt} = \delta_{CCA \rightarrow PRO} \frac{r_{-1} N_{rec}^{CCA}}{N_{rec}^{PRO}} (\bar{m}_{rec}^{CCA} - \bar{m}_{rec}^{PRO}) + \mu_{rec}^{PRO} (\bar{m}_{rec}^{PRO*} - \bar{m}_{rec}^{PRO}) \quad (15)$$

$$\frac{d\bar{m}_{rec}^{CCA}}{dt} = \delta_{PRO \rightarrow CCA} \mu_{rec}^{CCA} \bar{m}_{rec}^{CCA} + \frac{r_{+1} N_{rec}^{PRO}}{N_{rec}^{CCA}} (\bar{m}_{rec}^{PRO} - \bar{m}_{rec}^{CCA}) \quad (16)$$

$$\frac{d\bar{m}_{emi}^{PRO}}{dt} = 0 \quad (17)$$

3.2.4 | Dynamics of EGFP expression

The EGFP concentration per cell was measured by flow cytometry and is expressed in arbitrary units as relative fluorescence intensity (RFI). The average EGFP concentration per cell ($\bar{EGFP}$) refers to the average cell-specific EGFP concentration in the entire receiver cell population, which was defined by the absence of the red fluorescence signal that served as the label for emitter cells. As shown in the pure culture of the receiver strain, the synthesis of EGFP was detected approximately 1 h after addition of α -factor

FIGURE 2 Comparison of pure cultures of receiver strain (BY4741 $\Delta bar1$ P_{FIG1} -EGFP) without (- αf) and with 1 μM α -factor (+ αf) added at 3 h (vertical dotted line). Symbols represent experimental and lines simulated data, except for panel (I) where lines represent experimental datasets. (A) cell concentration; (B) cell weight; (C) biomass concentration; (D) BI; (E) glucose; (F) ethanol and (G) glycerol concentration; (H) EGFP per cell; (I) experimental RQ

(Figure 2H). This time delay is captured by the above described switch function δ_{FIG1} . Once EGFP production has started, its cell-specific concentration increases linearly over time (Figure 2H). Therefore, it was concluded that EGFP synthesis in arrested receiver cells occurs at a constant rate (π_{EGFP}), which is independent of cell size and growth rate. Since only cell-cycle-arrested receiver cells produce EGFP, and because proliferation of receiver cells will dilute EGFP in the population, the corresponding EGFP synthesis and dilution rates have to be weighted against the total size of the receiver cell population. Thus, the following equation for the average EGFP concentration in the receiver cell population can be derived (Eq. (18)):

$$\frac{d\overline{EGFP}}{dt} = \delta_{FIG1} \frac{N_{rec}^{CCA}}{N_{rec}^{PRO} + N_{rec}^{CCA}} \pi_{EGFP} - \mu_{rec}^{PRO} \frac{N_{rec}^{PRO}}{N_{rec}^{PRO} + N_{rec}^{CCA}} \overline{EGFP} \quad (18)$$

3.3 | Co-culture experiments

After having studied growth and pheromone response of the emitter and receiver strains in pure culture experiments, we examined the population dynamics of two-strain consortia. Constitutive expression of the red fluorescent marker protein tRFP in the emitter cells served to distinguish the two strains by flow cytometry analysis. Both strains were inoculated at the same time at identical initial cell densities. The increase in cell numbers of the individual strains, substrate consumption and by-product formation, the changes in the average cell weight of receiver cells, and EGFP expression in the receiver cells were monitored and compared to the predictions of the mathematical model.

3.3.1 | Co-culture without pheromone response

In order to exclude the possibility that cell-cell interactions other than the PR affect growth of the consortium, we investigated the growth of a co-culture consisting of strains that are not responsive to mating pheromones. A ‘blind’ receiver (BY4741 $\Delta ste2$ P_{FIG1} -EGFP), lacking the pheromone

FIGURE 3 Co-culture of the 'blind' receiver (BY4741 $\Delta ste2 P_{FIG1}$ -EGFP) and the emitter strain (BY4742 $\Delta ste3 P_{GPD1}$ -tRFP). Symbols represent experimental and lines simulated data; black and gray represent the subpopulation of the 'blind' receiver and the subpopulation of the emitter, respectively. (A) cell concentration; (B) biomass concentration; (C) EGFP concentration

receptor Ste2, and the emitter strain (BY4742 $\Delta ste3 P_{GPD1}$ -tRFP) were cultivated in the same medium. We found that none of the strains exhibited any growth retardation and EGFP expression was completely absent. In addition, the model correctly predicted the evolution of cell densities and biomass concentrations when the PR was switched off in the simulation (Figure 3). From these observations, we concluded that there are no measurable interactions between emitter and receiver cells except for those brought about by the PR, and that the model adequately describes the basic growth dynamics of the two strains.

3.3.2 | Co-culture with pheromone response

When emitter and receiver strains were inoculated at the same time, proliferation of the receiver strain ceased at the very early stages of the cultivation (Figure 4A) while the emitter strain continued to proliferate exponentially without any noticeable perturbation. This observation was in agreement with the mathematical simulations which predicted that the threshold α -factor concentration of $\alpha f_{FIG1, crit} = 0.1$ nM [23] (Figure 4H) was surpassed already at 0.2 h after inoculation, thus, activating PR and thereby cell-cycle arrest of the receiver cells. Arrested receiver cells continued to grow in size, therefore making a significant contribution to the total biomass concentration in the reactor despite their comparatively small cell number (35.7% biomass compared to 21.9 % cells after 5 hours of cultivation, Figure 4C). Similarly, pheromone-induced EGFP production in the receiver cells started after approximately 1 hour of cultivation, resulting in a linear increase of the cell-specific average EGFP concentration (Figure 4G). The comparison of experimental and computational results during the first 5 hours of the cultivation indicated that the mathematical model predicts population dynamics, substrate consumption and product formation with good accuracy (Figure 4).

However, in contrast to the pure culture experiments, receiver cells re-entered the proliferating state after approximately 5 h of cultivation (Figure 4A), which was also witnessed by the gradual decrease of their average cell mass (Figure 4B). Concomitantly, intracellular EGFP accumulation stopped at approximately 150 RFI cell⁻¹ and average EGFP concentration in the receiver cell population decreased thereafter (Figure 4G). This apparent desensitization of receiver cells against the α -factor pheromone was not due to the exhaustion of glucose whose presence is required for the PR (Figure 4D). Therefore, another mechanism must have controlled pheromone sensitivity of the receiver cells. Previous studies of Strickfaden et al. [27] and Atay et al. [28] showed that α -factor arrested cells become resistant to the pheromone after extended exposure because cyclins accumulate over time in the nucleus, thereby suppressing the PR and facilitating transition of the arrested cells to the proliferating state. The duration of α -factor arrest depends on the concentration of the pheromone [12]. It is therefore reasonable to assume that we could not observe α -factor desensitization in pure culture experiments before glucose was depleted since α -factor concentration was very high ($\alpha f = 1000$ nM for the pure culture compared to $\alpha f = 7$ nM after 5 h for the co-culture).

Therefore, the mathematical model was extended to account for pheromone desensitization by introducing the switch function $\delta_{CCA \rightarrow PRO}$ (Eq. (11)) which released receiver cells from the arrest independent of the α -factor concentration after a residence time in the CCA state of $t_{CCA \rightarrow PRO} = 5$ h. As can be seen in Figure 4, the extended model predicts the evolution of most state variables of the consortium with good agreement. Only the average EGFP concentration in the receiver cell population was significantly underestimated for cultivation times longer than 7 hours. The fact that EGFP in the receiver cell population decreased more slowly than expected based on its proliferation-dependent dilution in the cells suggests that EGFP production ceased with a delay due to the slow maturation of EGFP [22].

FIGURE 4 Co-culture of the receiver (BY4741 $\Delta bar1$ P_{FIG1} -EGFP) and the emitter strain (BY4742 $\Delta ste2$ P_{GPD1} -tRFP). Symbols represent experimental and lines simulated data; black, gray and white representing the subpopulation of the receiver, the subpopulation of the emitter and the whole population, respectively. Solid and dashed lines represent simulations where cells can re-enter the cell cycle (activation of $\delta_{PRO \rightarrow CCA}$) and cannot ($\delta_{CCA \rightarrow PRO}$ remains deactivated), respectively. (A) cell concentration; (B) cell weight; (C) biomass concentration; (D) glucose; (E) ethanol and (F) glycerol concentration; (G) EGFP per cell; (H) simulated α -factor concentration based on experimental data published in [24]

4 | CONCLUDING REMARKS

The present study focused on investigating and modeling the pheromone response of *S. cerevisiae* as a novel system for artificial intra-species communication to control the behavior of individual subpopulations within consortia. It presents the quantitative analysis of the pheromone response of an α -haploid strain in pure and co-culture. Our modeling approach considers alterations of single cells and entire subpopulations. This segregation provides the possibility to upscale detailed single cell observations to a technically relevant level. The mathematical model includes the cell cycle arrest, metabolic and morphological changes as well as a synthetic module for the expression of EGFP via the pheromone-inducible promoter *FIG1*. These processes are activated when a defined α -factor threshold concentration is surpassed.

In the course of the co-cultivation, the pheromone response of the α -haploid receiver strain was induced by α -factor, which is constitutively produced by an α -haploid emitter strain. The simulation revealed that the pheromone response of the

receiver strain in pure culture (induced by synthetic α -factor) and co-culture is comparable as long as the cell cycle arrest is maintained.

After an extended period of exposure to the pheromone, cells of the receiver strain divide, which was also integrated in the model with a manually picked activation point. The synthesis of EGFP was observed to be independent of the growth rate and cell volume, therefore linear under the given conditions. This suggests that the expression capacity of *FIG1* is reached with moderate or even low but increasing pheromone concentrations. As a result, the total amount of EGFP produced by a single cell solely depends on time as long as the pheromone response cascade is activated.

The presented model can describe the trend of particular events of the pheromone response of *S. cerevisiae* and can be adopted for other yeasts or other ascomycetes with similar signaling systems, e.g. *Schizosaccharomyces pombe*. The general framework of the provided model is easily expandable to more than two organisms and interactions. Naturally, it can be adapted for communication-based pure culture studies, e.g., the positive feedback quorum sensing system with

density-dependent pheromone auto-induction developed by Williams et al. [29], to analyze critical parameters such as the cell concentration and pheromone synthesis rate. The general concept of the model results in a more comprehensive description of quantitative phenomena during the pheromone response, thereby assisting the development of microbial consortia as well as their reproducibility, reliability and controllability.

Nomenclature

E	[g L ⁻¹]	Concentration of ethanol
$\overline{\text{FSC}}_i$	[a.u.]	Arithmetic mean of FSC signals over the population
G	[g L ⁻¹]	Concentration of glycerol
K_S	[g L ⁻¹]	Half saturation constant of glucose
m_i^j	[g cell ⁻¹]	Mass of a single cell
N_i^j	[cell L ⁻¹]	Concentration of cells
r_{+1}	[h ⁻¹]	Transfer rate PRO → CCA
r_{-1}	[h ⁻¹]	Transfer rate CCA → PRO
S	[g L ⁻¹]	Concentration of glucose
t	[h]	Process time
X_i^j	[g L ⁻¹]	Concentration of biomass dry weight
Y_i^j	[g g ⁻¹]	Yield coefficient
αf	[nM]	Concentration of α -factor
Greek symbols		
μ_i^j	[h ⁻¹]	Specific growth rate
$\delta_{\text{PRO} \rightarrow \text{CCA}}$	[–]	Switch function for the transfer from PRO to CCA
$\delta_{\text{CCA} \rightarrow \text{PRO}}$	[–]	Switch function for the transfer from CCA to PRO
δ_{FIG1}	[–]	Switch function to activate the <i>FIG1</i> promoter system
π_{EGFP}	[RFI cell ⁻¹ h ⁻¹]	EGFP production rate
Indices		
i	[–]	Strain
j	[–]	Phenotype of the strain

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

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