Design and mathematical modelling of a synthetic symbiotic ecosystem

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Abstract: Artificial microbial ecosystems have been increasingly used to understand principles of ecology. These systems offer unique capabilities to mimic a variety of ecological interactions that otherwise would be difficult to study experimentally in a reasonable period of time. However, the elucidation of the genetic bases for these interactions remains a daunting challenge. To address this issue, we have designed and analysed a synthetic symbiotic ecosystem in which the genetic nature of the microbial interactions is defined explicitly. A mathematical model of the gene regulatory network in each species and their interaction through quorum sensing mediated intercellular signalling was derived to investigate the effect of system components on cooperative behaviour. Dynamic simulation and bifurcation analysis showed that the designed system admits a stable coexistence steady state for sufficiently large initial cell concentrations of the two species. The steady-state fraction of each species could be altered by varying model parameters associated with gene transcription and signalling molecule synthesis rates. The design also admitted a stable steady state corresponding to extinction of the two species for low initial cell concentrations and stable periodic solutions over certain domains of parameter space. The mathematical analysis was shown to provide insights into natural microbial ecosystems and to allow identification of molecular targets for engineering system behaviour.

1 Introduction

Artificial microbial ecosystems are frequently used to explore ecological theories due to their structural simplicity, compositional flexibility, long-term accessibility and experimental efficiency [1, 2]. When designed and constructed appropriately, artificial microbial ecosystems can exhibit complex behaviours that are observed in a variety of large-scale ecological systems, such as classic rock-paper-scissors interactions [3] and temporal and spatial distributions of biodiversity [4]. If the components of an artificial microbial ecosystem are manipulated, the consequence of altering system complexity can be further explored. Such an approach has been successfully used to determine the relationship between food-chain length, population level and productivity [5]. The rapidly evolvable nature of microbial ecosystems also enables efficient exploration of ecosystem evolution and dynamical behaviour. One such example is the evolution of an artificial predator–prey microbial ecosystem in which rapid evolution changed the population dynamics of the system [6]. Although the use of these artificial systems for exploration of large-scale ecosystems remains controversial, microbial ecosystems are widely viewed as powerful tools for validating general ecological theories [1, 7].

Despite numerous applications of artificial microbial ecosystems to illuminate ecological mechanisms, the genetic bases of these mechanisms remain largely unknown. In traditional ecological research, detailed molecular understanding of system interactions is generally inaccessible because of ecosystem complexity and the lack of efficient genetic tools [8]. Nevertheless, developing an understanding of the genetic bases of ecological interactions can provide significant insights into system structure and community behaviour, and even the evolution of ecosystems [9–12]. Although unraveling the genetic causes of complex behaviours in large-scale ecological systems remains extremely challenging, the rapid increase in genetic information for many microbial organisms enables limited genetic analysis of artificial microbial ecosystems and provides initial experimental data for developing genetic theories corresponding to established ecological theories [13, 14]. Therefore appropriately designed artificial microbial ecosystems with known and well-defined genetic backgrounds would represent valuable tools for ecological research [15, 16].

Recently, the construction of artificial genetic circuits has been increasingly used to illuminate the design principles of complex biological systems [17, 18]. In addition to simple designs that possess switching abilities, complex interactions such as population regulation and pattern formation have been demonstrated in artificial genetic circuits [19–21]. Such synthetic biological methods provide clear genetic origins for the microbial interactions, and they potentially can be used to explore the genetic bases of complex behaviours in natural ecosystems that are difficult to investigate by genetic analysis of artificial microbial ecosystems. Moreover, the components of artificial genetic circuits can be engineered to alter the network outputs, allowing the evolution of artificial microbial ecosystems to be explored [22]. By focusing on simplification of natural genetic networks, synthetic biology can provide unique and powerful insights into the design principles of complex biological systems. A high degree of experimental control allows the use of components rich in kinetic and

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biochemical information, the choice of genetic systems suitable for manipulation and the exploration of the role of each component individually and collectively. Well-defined genetic networks resulting from synthetic biology are amenable to detailed mathematical modelling and analysis, a property not shared by real genetic networks due to their complexity [23]. There is evidence that complex interactions in microbial ecosystems can be drastically altered by introducing a small number of mutations, sometimes even a single mutation [10, 12], indicating that synthetic biology could be a useful and efficient strategy for constructing artificial microbial ecosystems with complex interactions [15].

We report the conceptual design and model-based analysis of an artificial symbiotic ecosystem based on an engineered genetic network to provide a genetic basis for two-species mutualistic ecosystems that exhibit interactions similar to those observed in a variety of natural ecosystems [24]. Unlike a recent implementation in yeast which requires high initial cell densities of both species to establish the symbiotic interaction [15], our bacterial design allows triggering of the symbiotic interaction by species growing from low cell densities to a certain critical cell density. In addition, a number of mutant components of the designed ecosystem are available, allowing fine tuning of the symbiotic interaction for specific applications [25, 26]. The mathematical analysis provides insights into natural microbial ecosystems and allows identification of molecular targets for engineering system behaviour.

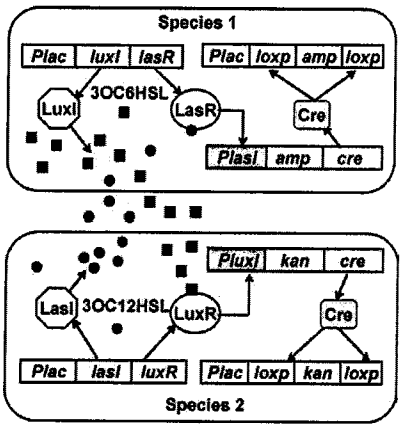

Fig. 1 *Molecular design of a synthetic microbial system for mimicking symbiosis in natural ecosystems*

Species 1 has kanamycin and chloramphenicol-resistant genes and species 2 has ampicillin and chloramphenicol-resistant genes in their genetic backgrounds (not shown)

At low cell densities, both species grow in the presence of ampicillin and kanamycin due to constitutive expression of the *amp* and *kan*-genes. When the two cell densities reach their threshold values, Cre-*loxP* site-specific gene recombination is activated and the LuxI–LuxR and LasI–LasR quorum-sensing systems are triggered

Thereafter, the two species become mutually dependent through *amp* expression regulated by the P_{LasI} promoter in species 1 and *kan* expression regulated by the P_{LuxI} promoter in species 2

Species can survive only if the cell densites are maintained above their threshold values

2 Results

2.1 System design

The synthetic ecosystem consists of two bacterial species and mimics natural symbiosis by making the survival of each species completely dependent on the cell density of the other species. We employed the bacterial quorum-sensing mechanism and Cre-*loxP* site-specific recombination to design the system. A typical quorum-sensing system is composed of three components, a LuxI homologue that synthesises a diffusible acyl homoserine lactone (AHL) signalling molecule and a LuxR homologue that activates the third component, a P_{luxI}-type promoter, when the concentrations of AHL exceed a threshold level [27]. Because accumulation of signalling molecules is proportional to the cell population density, quorum sensing allows cell density-dependent gene activation. This mechanism has been employed to design artificial genetic networks to perform complicated functions [19, 21, 28]. The Cre-*loxP* site-specific recombination mechanism is generally composed of a Cre enzyme and two *loxP* DNA fragments [29]. The Cre enzyme specifically recognises the *loxP* DNA sequences and catalyses their homologous recombination. If two *loxP* sequences are arranged in a reverse orientation, the recombination leads to the removal of DNA sequence between the two *loxP* sites. This mechanism allows for irreversible gene activation, a property that is desired for the design of the two-species ecosystem.

Fig. 1 shows the proposed design where *E. coli* cells containing different plasmids represent the two species. Species 1 constitutively produces the LuxI 3-oxo-C6-HSL (3-oxo-hexanoyl homoserine lactone) synthase, the LasR transcriptional activator and the ampicillin resistance protein (β-lactamase) whose gene (*amp*) is flanked by two *loxP* sites. In addition, a P_{lasI} promoter regulates expression of the *amp* and *cre* genes. In species 2, the LasI 3-oxo-C12-HSL (3-oxo-dodecanoyl homoserine lactone) synthase, the LuxR transcriptional activator and the kanamycin resistance protein whose gene (*kan*) is flanked by two *lox*P sites are expressed constitutively. A P_{luxI} promoter regulates expression of the *kan* and *cre* genes. Species 1 has a *kan* gene expressed constitutively in its background and species 2 has an analogous *amp* gene (not shown).

At low cell densities, both species can grow in the presence of ampicillin and kanamycin due to their constitutive expression of the *amp* and *kan* genes. When the two cell densities reach their threshold values, activation of both quorum-sensing systems leads to expression of the Cre-*loxP* site-specific recombinase, which recognises the *loxP* sites and deletes the *amp* and *kan* genes in species 1 and species 2, respectively. More specifically, the diffusible signalling molecules 3-oxo-C6-HSL synthesised by LuxI in species 1 and 3-oxo-C12-HSL synthesised by LasI in species 2 reach their threshold concentrations to form the complexes 3-oxo-C6-HSL–LasR in species 1 and 3-oxo-C12-HSL–LuxR in species 2. Subsequent activation of *amp* and *cre* gene expression in species 1 regulated by the P_{lasI} promoter (via the 3-oxo-C6-HSL-LasR complex) and of *kan* and *cre* gene expression in the species 2 regulated by the P_{luxI} promoter (via the 3-oxo-C12-HSL–LuxR complex) induce site-specific gene recombination, resulting in deletion of the *loxP*-flanked *amp* in the species 1 and *kan* in the species 2. Thereafter, both species become completely dependent on the expression of *amp* and *kan* genes that are regulated by P_{lasI} and P_{luxI}, respectively, in the two species, which in turn requires the concentrations of the signalling molecules to be maintained at sufficiently high levels. Once the quorum-sensing circuits are triggered, the two species become completely dependent on each other and failure to maintain a sufficiently high cell density of one species will lead to the extinction of both species.

We developed a conceptual model of the proposed system by coupling kinetic equations for the gene regulatory network to extracellular mass balances on the two species and a single growth limiting substrate through modified Monod growth expressions (see Supplementary information). Due to the lack of experimentally based parameter values, the model was most suitable for qualitative predictions about the effects of individual molecular processes on overall system behaviour. We were particularly interested in the following two questions. Can the designed system exhibit behaviours observed in natural microbial ecosystems? Can system behaviour be altered by manipulating molecular parameters that can be changed by well-established genetic engineering methods such as directed evolution [30]?

2.2 Effect of the initial cell densities

The synthetic ecosystem was designed such that the quorum-sensing circuits are triggered and the two species become mutually dependent when the cell density of each species achieves its threshold value. Within the mathematical model, these threshold values represent the initial cell densities of the two species. The dynamic behaviour of the synthetic ecosystem was strongly dependent on the threshold values, with a stable coexistence steady state established for sufficiently large initial cell densities (Fig. 2*a*) and a stable extinction steady state observed for low initial cell densities (Fig. 2*b*). The stable coexistence state indicated that both species can survive in an open environment in which resources are continuously supplied, providing theoretical validation of the ecosystem design. Although the faster growing species 2 dominated with a much larger steady-state cell density, species 1 was able to survive due to the engineered symbiotic mechanism. In the absence of such cellular interactions, the coexistence steady state is generically unstable and the faster growing species survives at the expense of the slower growing species [24]. The stable extinction steady state demonstrated that the design was capable of describing mutual extinction and emphasised the importance of engineering the quorum-sensing circuits such that Cre-*loxP* site-specific gene recombination occurs at sufficiently large cell densities to support coexistence. The two solutions obtained were reflected in the signalling molecule concentrations (insets of Fig. 2), which were proportional to the cell densities.

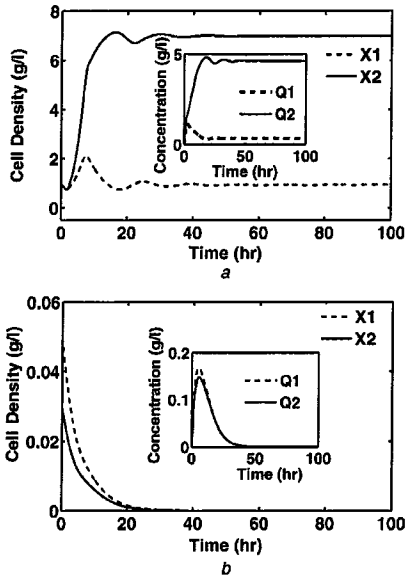

Fig. 2 *Effect of the initial cell densities on the dynamics of the synthetic ecosystem*

a Stable coexistence steady-state solution was obtained for relatively large initial cell densities $X_1(0) = X_2(0) = 1$ (nominal values)
The faster growing species 2 dominated, but the slower growing species 1 was able to survive due to the engineered symbiotic mechanism
b Stable extinction steady-state solution was obtained for small initial cell densities $X_1(0) = 0.05$ and $X_2(0) = 0.03$

2.3 Effect of model parameters

The effect of model parameter variations on system behaviour was investigated to identify promising molecular targets for genetic engineering. Bifurcation analysis was used to determine the sensitivity of each parameter with respect to key system outputs, including the domain of attraction of the coexistence steady state and the resulting fraction of the slower growing species 1. Following preliminary screening of each parameter, we focused our analysis on the synthesis rates of the two signalling molecules (f_{Q_1} and f_{Q_2}) and the expression rates of the two antibiotic resistance proteins (f_{A_1} and f_{A_2}) as these parameters might be altered experimentally by manipulating gene expression or by engineering the transcriptional activators LuxR and LasR [25, 26]. Bifurcation analysis with respect to either f_{Q_1} or f_{Q_2} revealed a unique threshold value above which the coexistence steady state was stable (Fig. 3). The coexistence state was separated from the stable extinction state by an unstable steady state corresponding to low cell densities, suggesting that the domain of attraction of the coexistence state was large compared with that of the extinction state. This behaviour was observed for nominal values of the

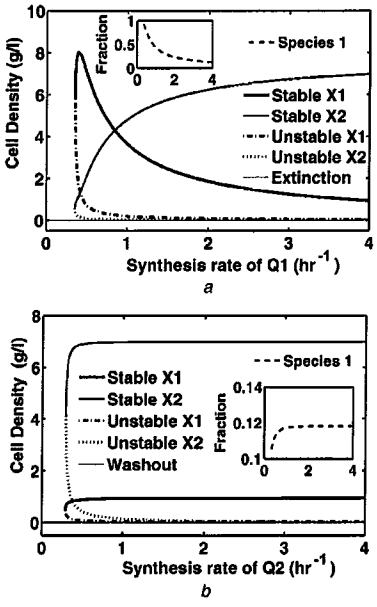

Fig. 3 *Bifurcation analysis with respect to the synthesis rates of the signalling molecules where the nominal values were* $f_{Q_1} = f_{Q_2} = 4\ h^{-1}$

Locally stable coexistence state was produced above a threshold value of the signalling molecule synthesis rate of the slower growing species, and the extinction steady state was globally stable below this threshold value
Fraction of slower growing cells varied significantly with the synthesis rate of Q_1 *a*, whereas negligible variations occurred with the synthesis rate of Q_2 *b*

signalling molecule synthesis rates, which produced a stable extinction state only for very small initial cell densities (Fig. 2). The extinction state was globally stable below the threshold values, in which case signalling molecule synthesis was insufficient to sustain the symbiotic mechanism.

For some applications of the synthetic ecosystem, the ability to engineer system behaviour to achieve coexistence states with different fractions of the two species may be desirable. Once the threshold cell densities of the species are reached and the quorum-sensing circuits are triggered, the faster growing species will continue proliferating at a maximal rate determined by the available resources subject to the constraint that the cell density of the slower growing species remains above the threshold value necessary to maintain the mutualistic interaction. Relatively large Q_1 synthesis rates produced a mixture dominated by the faster growing species 2 (inset of Fig. 3*a*) as a low threshold cell density of the slower growing species 1 was sufficient for coexistence. By contrast, a mixture dominated by the slower growing species was obtained for small Q_1 synthesis rates because a much larger threshold cell density of species 1 was required to establish the symbiosis. The impact of the Q_2 synthesis rate on the population fraction was negligible (inset of Fig. 3*b*) because the cell density of the faster growing species was determined by the available resources and the cell density of the slower growing species was determined by its threshold density, neither of which changed with varying Q_2 synthesis rate. Therefore the signalling molecule synthesis rate of the slower growing species (f_{Q_1}) appears to be a promising molecular target for engineering, as the fraction of the slower growing species could be substantially varied (0.1–0.9) by reducing f_{Q_1} an order of magnitude from its nominal value. Analogous results were obtained for the expression rates of the two antibiotic resistance proteins, with the coexistence state stable above threshold values and the population fraction depending strongly on the expression rate of the kanamycin resistance protein A_2, necessary for the survival of the slower growing species (Fig. 6, supplementary information).

Due to their strong impact on steady-state system behaviour, the signalling molecule synthesis rate of the slower growing species (f_{Q_1}) and the antibiotic resistance protein expression rate of the faster growing species (f_{A_2}) were chosen for combined dynamic analysis. Two-parameter bifurcation analysis revealed that the parameter space was divided into two regions (Fig. 4). The coexistence and extinction states were both locally stable for relatively large f_{Q_1} and f_{A_2} values, with larger domains of attraction from different initial cell densities observed for the coexistence state as the f_{Q_1} and f_{A_2} values were further displaced from the dividing line. The extinction state was globally stable for small f_{Q_1} and f_{A_2} values below the dividing line. We performed simulation tests with four different combinations of the two-model parameters to examine their combined effect on system dynamics (insets in Fig. 4). In the coexistence region, the dynamic responses depended strongly on the parameter values, suggesting that the synthetic ecosystem can be experimentally tuned for different applications. For all simulations, the cell density of the slower growing species exhibited a characteristic overshooting behaviour before settling to its equilibrium value. This transient behaviour allowed both species to proliferate rapidly before the system settled to a coexistence state where the cell density of the slower growing species assumed a small value sufficient to maintain the mutualistic interaction. By capturing the effect of these potentially adjustable model parameters on the existence and stability

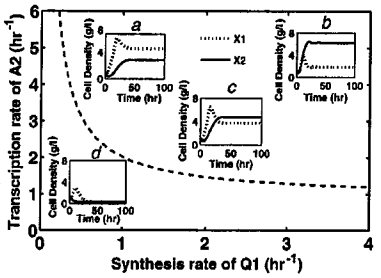

Fig. 4 *Parametric space analysis for the signalling molecule synthesis rate of the slower growing species (f_{Q_1}) and the antibiotic resistance protein expression rate of the faster growing species (f_{A_2})*

Curve divides two domains where the extinction state was globally stable (below the curve) and the coexistence and extinction states were locally stable (above the curve)

Insets show dynamic responses of the cell densities for four parameter sets (h^{-1}): *a* $f_{Q_1} = 1, f_{A_2} = 4$; *b* $f_{Q_1} = 4, f_{A_2} = 4$; *c* $f_{Q_1} = 2, f_{A_2} = 2$; *d* $f_{Q_1} = 1, f_{A_2} = 1$

of the coexistence state, the parametric analysis provided essential design guidelines for implementing the synthetic ecosystem.

2.3 Existence of periodic behaviour

Bifurcation analysis was performed with each model parameter to determine if the proposed design was structurally capable of exhibiting other dynamic behaviours with implications for complex natural ecosystems. We were particularly interested in stable periodic solutions, which would correspond to dynamic coexistence between the two species. For sufficiently small values of the growth saturation constant of the faster growing species 2 (K_2), we discovered that the model could produce simple periodic solutions (Fig. 5). The coexistence steady state was largely resistant to variations at large K_2 values, indicating some system robustness to the growth rate of species 2. However, a supercritical Hopf bifurcation occurred and small amplitude oscillations appeared when K_2 was reduced well below its nominal value. Oscillation amplitudes in the cell densities increased monotonically beyond the bifurcation point with significant oscillations apparent at $K_2 = 0.05$ (inset of Fig. 5). This oscillatory behaviour was a direct consequence of the reduced resource requirement when the faster growing species proliferated at its maximal rate, which was possible because of the small

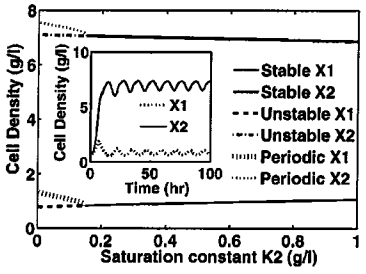

Fig. 5 *Bifurcation analysis with respect to the growth saturation constant of the faster growing species (K_2)*

Stable coexistence steady state was observed at larger K_2 values, including the nominal $K_2 = 0.5$

Supercritical Hopf bifurcation occurred as K_2 was reduced, producing a stable coexistence state characterised by sustained oscillations

Oscillation amplitudes increased as K_2 was reduced further, with significant oscillations produced at $K_2 = 0.05$ (inset)

growth saturation constant ($(\mu S/(K+S) \sim \mu$). Under these conditions, species 2 could continue maximal growth at high cell densities, leading to rapid consumption of resources and subsequent decrease in the cell density of species 1. Once the species 1 cell density dropped below its threshold value, the concentration of the signalling molecule Q_1 was no longer sufficient to support the survival of species 2. The subsequent decrease in the species 2 cell density was accompanied by an increase in available resources, which allowed species 1 to achieve a sufficiently large cell density to maintain the mutualistic interaction. Consequently, periodic solutions were only observed for small growth saturation constants and large cell densities of the faster growing species 2. Although oscillatory dynamics might not be observed in many natural microbial ecosystems, our results demonstrate that the architecture of the designed ecosystem can lead to rather complicated and unanticipated dynamic behaviours.

3 Discussion

Symbiosis has been observed in complex ecosystems including many microbial ecosystems, and its importance in the evolution of higher organisms has been hypothesised [24]. As is common in ecology, the genetic bases of such positive interactions are rarely addressed due to ecosystem complexity and the lack of efficient genetic tools. Despite the increasing availability of microbial genome sequences, the need to correlate this genomic information to biological function remains a daunting task as understanding whole genome sequences is frequently necessary to identify the genetic basis [12]. Synthetic biology has emerged as an alternative for performing this task [11, 15, 31]. The identification of unknown cooperative mechanisms of microbial ecosystems using this rational engineering approach remains challenging. However, synthetic biology allows the genetic origins of designed phenotypes to be investigated directly and efficiently. Once a microbial ecosystem based on artificial genetic circuits is established, its dynamic behaviours can be readily altered by engineering the components of the circuits using directed evolution or combinatorial assembly [25, 32]. The resulting synthetic ecosystems might be useful for understanding the evolution and design principles of natural microbial ecosystems [6].

We have used the bacterial quorum-sensing mechanism to design a two-species symbiotic ecosystem in which the genetic origin of the mutualistic interaction was clearly defined to illustrate the usefulness of synthetic biology in microbial ecology. Our design uses Cre-*loxP* site-specific recombination to establish the cell densities of the two species before triggering the engineered quorum-sensing circuits. Thereafter, quorum-sensing regulated gene expression was active and the two species became mutually dependent. A nonlinear dynamic model was developed and used to analyse system behaviour, especially the existence of stable states in which the two species coexist despite differences in their substrate dependent growth rates. The cell densities at which quorum sensing was triggered represented initial conditions in the model, and we showed that sufficiently large initial cell densities were required for the coexistence steady state to be stable. Otherwise, the system evolved to the extinction steady state where both species perished.

Bifurcation analysis showed that system behaviour was strongly impacted by the signalling molecule synthesis rate of the slower growing species and the antibiotic resistance protein expression rate of the faster growing species, because these model parameters largely determined the minimal cell density of the slower growing species required to sustain the mutualistic interaction. The two parameters had substantial effects on both the dynamic response and the achievable steady-state population ratio, suggesting that they are suitable molecular targets for engineering system behaviour. We also showed that the growth saturation parameter of the faster growing species could strongly impact system behaviour, with a periodic solution corresponding to stable but dynamic coexistence between the two species observed for sufficiently small parameters values. Such oscillatory behaviour might be beneficial for studying certain natural ecosystems, but would be undesirable in most industrial-scale fermentations. Although the saturation parameter is an intrinsic growth property of a microbial organism and cannot be directly affected through our genetic design, different parameter values could be obtained by using mutant strains or different microbial organisms for system implementation. The information revealed by mathematical modelling is valuable for implementing the synthetic ecosystem to achieve desired symbiotic behaviours.

Mutualism is a consequence of the long-term evolution of mixed microbial cultures under a variety of environmental conditions [33]. As such, the dynamic behaviour of a symbiotic ecosystem is expected to change as the environment varies [34]. Mathematical analysis of the designed synthetic ecosystem revealed that the evolution of certain system components is most effective for robustly altering ecosystem behaviour. Rate processes associated with these system components, which include the synthesis rates of intercellular signalling molecules, would be expected to vary strongly with the prevailing environmental conditions. Such hypotheses suggest that the designed two-species ecosystem could be useful for exploring the evolution of natural microbial ecosystems experimentally. The predicted dynamic behaviours and the derived hypotheses remain to be validated experimentally. Nevertheless, the insights revealed by this synthetic biological approach may enhance our understanding of mutualism and its evolution.

The synthetic ecosystem could also have potential applications in industrial fermentation. Bioremediation involves mixed cultures in which two or more species compete for common growth-limiting substrates in a continuous bioreactor. Because species typically have different substrate dependent growth rates, the coexistence steady state is unstable and the fastest growing species eventually dominates while the slower growing species are washed out of the bioreactor [35, 36]. Rather than implement complex operational strategies such as separation and recycle of the slower growing species, our proposed synthetic design offers the possibility of developing a self-stabilising system through cellular engineering. Our mathematical analysis suggests that desired populations of the microbial species could be obtained through molecular engineering of key system components, such as the signalling molecule synthesis rate of the slower growing species.

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Supplementary Information

A dynamic model of the synthetic ecosystem was developed by combining kinetic equations for the gene regulatory network (Figure 1) with mass balance equations on each species. The gene regulation model was derived from a simplified reaction scheme (Table 1) that captured intracellular events following Cre-*loxP* site-specific gene recombination, at which time the species become mutually dependent. The intracellular model was based on the following assumptions: (1) constant synthesis rates for LuxI, LuxR, LasI, and LasR as their genes are constitutively expressed through the activation of P_{lac} in the presence of IPTG; (2) synthesis rates of 3-oxo-C6-HSL and 3-oxo-C12-HSL proportional to the concentrations of the corresponding synthases; (3) saturation kinetics for the expression rates of *amp* and *kan* regulated by P_{lasI} and P_{luxI} , respectively; and (4) fast diffusion of 3-oxo-C6-HSL and 3-oxo-C12-HSL such that their intracellular and extracellular concentrations are uniform.

The gene regulatory model was derived from the reaction scheme in Table 1. The differential equations for the LuxI, LasR, LasI and LuxR proteins were derived under the assumption of constant synthesis rates:

$$\begin{aligned}
\frac{dL_1}{dt} &= f_{L1}X_1 - d_{L1}\frac{L_1}{X_1}X_1 - DL_1 \\
\frac{dR_1}{dt} &= f_{R1}X_1 - d_{R1}\frac{R_1}{X_1}X_1 - f_{C1}\frac{R_1}{X_1}Q_2X_1 + d_{C1}\frac{C_1}{X_1}X_1 - DR_1 \\
\frac{dL_2}{dt} &= f_{L2}X_2 - d_{L2}\frac{L_2}{X_2}X_2 - DL_2 \\
\frac{dR_2}{dt} &= f_{R2}X_2 - d_{R2}\frac{R_2}{X_2}X_2 - f_{C2}\frac{R_2}{X_2}Q_1X_2 + d_{C2}\frac{C_2}{X_2}X_2 - DR_2
\end{aligned} \tag{1}$$

The system variables and model parameters are defined in Tables 2 and 3, respectively. The degradation and complex formation rates were expressed in terms

of intrinsic protein concentrations, except for the signaling molecules for which the intracellular and extracellular concentrations were uniform due to the assumption of fast diffusion. Each equation contained a final term corresponding to material removal by external flow, which was expressed in terms of the dilution rate D. Differential equations for the signaling molecules were written as:

$$\begin{aligned}\frac{dQ_1}{dt} &= f_{Q1}\frac{L_1}{X_1}X_1 - d_{Q11}Q_1X_1 - d_{Q12}Q_1X_2 - f_{C2}\frac{R_2}{X_2}Q_1X_2 + d_{C2}\frac{C_2}{X_2}X_2 - DQ_1 \\ \frac{dQ_2}{dt} &= f_{Q2}\frac{L_2}{X_2}X_2 - d_{Q21}Q_2X_1 - d_{Q22}Q_2X_2 - f_{C1}\frac{R_1}{X_1}Q_2X_1 + d_{C1}\frac{C_1}{X_1}X_1 - DQ_2\end{aligned} \quad (2)$$

The first term on the RHS of these equations resulted from the assumption that the signaling molecule synthesis rates were proportional to the concentrations of the corresponding synthases. The remaining terms account for degradation inside the two species, reversible formation of the complexes, and material removal. Correspondingly, the differential equations for the two complexes were:

$$\begin{aligned}\frac{dC_1}{dt} &= f_{C1}\frac{R_1}{X_1}Q_2X_1 - d_{C1}\frac{C_1}{X_1}X_1 - DC_1 \\ \frac{dC_2}{dt} &= f_{C2}\frac{R_2}{X_2}Q_1X_2 - d_{C2}\frac{C_2}{X_2}X_2 - DC_2\end{aligned} \quad (3)$$

Because the expression rates of the ampicillin and kanamycin resistance proteins were assumed to follow saturation kinetics, the differential equations for these protein concentrations were:

$$\begin{aligned}\frac{dA_1}{dt} &= f_{A1}\frac{C_1/X_1}{a_1 + C_1/X_1}X_1 - d_{A1}\frac{A_1}{X_1}X_1 - DA_1 \\ \frac{dA_2}{dt} &= f_{A2}\frac{C_2/X_2}{a_2 + C_2/X_2}X_2 - d_{A2}\frac{A_2}{X_2}X_2 - DA_2\end{aligned} \quad (4)$$

The extracellular phase was modeled with standard differential equations for two species that compete for a common growth limiting resource in a well-mixed environment with equal supply and removal rates D:

$$\frac{dX_1}{dt} = \mu_1 X_1 - DX_1$$
$$\frac{dX_2}{dt} = \mu_2 X_2 - DX_2 \qquad (5)$$
$$\frac{dS}{dt} = D(S_0 - S) - \frac{\mu_1 X_1}{Y_1} - \frac{\mu_2 X_2}{Y_2}$$

Monod kinetics were used to describe the substrate dependence of the growth rates. The gene regulatory model was coupled to the extracellular model by modifying the Monod kinetics to capture the growth inhibition that results when the antibiotic resistance protein concentrations drop below their threshold values:

$$\mu_1 = \frac{\mu_{m1} S}{K_1 + S} \frac{(\overline{A}_1 / X_1)^n}{1 + (\overline{A}_1 / X_1)^n}, \quad \overline{A}_1 = \frac{A_1}{\rho_1}$$
$$\mu_2 = \frac{\mu_{m2} S}{K_2 + S} \frac{(\overline{A}_2 / X_2)^n}{1 + (\overline{A}_2 / X_2)^n}, \quad \overline{A}_2 = \frac{A_2}{\rho_2} \qquad (6)$$

where ρ_j is the threshold concentration value for the protein, and n is a integer power that determines the strength of the inhibitory effect. The complete model was comprised of 13 coupled differential equations (1)-(5) with the growth rate expressions (6), and parameter values chosen to be within reasonable ranges. A complete set of nominal parameter values is provided in Table 3. Because the gene regulation model captured system behavior following Cre-*loxP* site-specific gene recombination, initial conditions for the two cell densities were interpreted as variables whose values were determined by triggering of the quorum sensing circuits. The effects of these initial conditions and key model parameter values on system behavior were investigated by dynamic simulations and bifurcation analysis using XPPAUTO.

Tables

Table 1. The reactions included in the gene regulatory model. L_1: LuxI, L_2: LasI, R_1: LasR, R_2: LuxR, Q_1: 3-oxo-C6-HSL, Q_2: 3-oxo-C12-HSL, C_1: LasR-3-oxo-C12-HSL complex, C_2: LuxR-3-oxo-C6-HSL complex, A_1: Amp, A_2: Kan, φ: degradation product. Table 3 contains a complete list of the system variables.

Species 1	Species 2
$DNA \rightarrow L_1 \rightarrow \varphi$	$DNA \rightarrow L_2 \rightarrow \varphi$
$DNA \rightarrow R_1 \rightarrow \varphi$	$DNA \rightarrow R_2 \rightarrow \varphi$
$L_1 \rightarrow Q_1 \rightarrow \varphi$	$L_2 \rightarrow Q_2 \rightarrow \varphi$
$R_1 + Q_2 \rightleftharpoons C_1$	$R_2 + Q_1 \rightleftharpoons C_2$
$DNA + C_1 \rightarrow A_1 \rightarrow \varphi$	$DNA + C_2 \rightarrow A_2 \rightarrow \varphi$

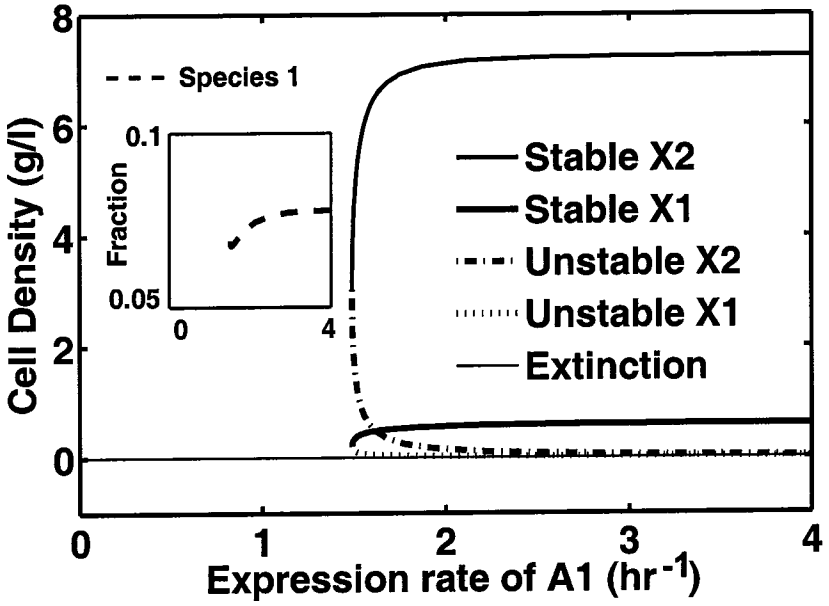
Table 2. System variables and their definitions.

Symbol	Definition	Units
L_1	LuxI concentration	g/l
R_1	LasR concentration	g/l
Q_1	3-oxo-C6-HSL concentration	g/l
C_1	LasR-[3-oxo-C12-HSL] complex concentration	g/l
A_1	Ampicillin resistant protein concentration	g/l
L_2	LasI concentration	g/l
R_2	LuxR concentration	g/l
Q_2	3-oxo-C12-HSL concentration	g/l
C_2	LuxR-[3-oxo-C6-HSL] complex concentration	g/l
A_2	Kanamycin resistant protein concentration	g/l
S	Substrate concentration	g/l
X_1	Cell density of species 1	g/l
X_2	Cell density of species 2	g/l

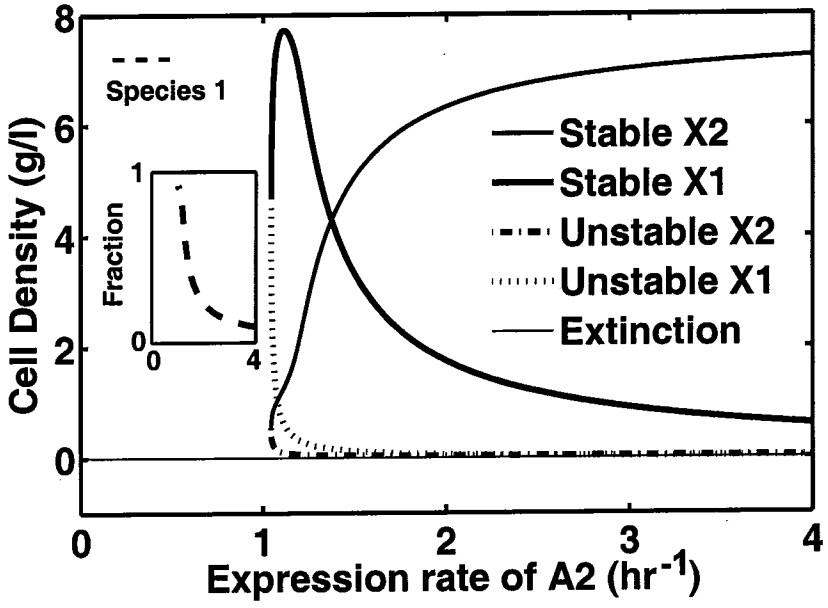
Table 3. Complete list of model parameters and their nominal values.

Parameter	Definition	Nominal Value
μ_{m1}	Maximum growth rate of species 1	0.5 hr^{-1}
K_1	Growth saturation constant of species 1	1 g/l
f_{L1}	Expression rate of L_1	3 hr^{-1}
d_{L1}	Degradation rate of L_1	6 hr^{-1}
f_{R1}	Expression rate of R_1	2 hr^{-1}
d_{R1}	Degradation rate of R_1	5 hr^{-1}
f_{Q1}	Synthesis rate of Q_1	4 hr^{-1}
d_{Q11}	Degradation rate of Q_1 inside species 1	0.5 l/g-hr
d_{Q12}	Degradation rate of Q_1 inside species 2	0.5 l/g-hr
f_{C1}	Formation rate of complex C_1	4 l/g-hr
d_{C1}	Dissociation rate of complex C_1	1 hr^{-1}
f_{A1}	Maximum expression rate of A_1	4 hr^{-1}
a_1	Expression saturation constant of A_1	1 g/l
d_{A1}	Degradation rate of A_1	1 hr^{-1}
ρ_1	Threshold concentration of A_1	1 g/l
μ_{m2}	Maximum growth rate of species 2	1.5 hr^{-1}
K_2	Growth saturation constant of species 2	0.5 g/l
f_{L2}	Expression rate of L_2	3 hr^{-1}
d_{L2}	Degradation rate of L_2	4 hr^{-1}
f_{R2}	Expression rate of R_2	2 hr^{-1}
d_{R2}	Degradation rate of R_2	5 hr^{-1}
f_{Q2}	Synthesis rate of Q_2	4 hr^{-1}
d_{Q21}	Degradation rate of Q_2 inside species 1	0.5 l/g-hr
d_{Q22}	Degradation rate of Q_2 inside species 2	0.5 l/g-hr
f_{C2}	Formation rate of complex C_2	3 l/g-hr
d_{C2}	Dissociation rate of complex C_2	1 hr^{-1}
f_{A2}	Maximum expression rate of A_2	4 hr^{-1}
a_2	Expression saturation constant of A_2	1 g/l
d_{A2}	Degradation rate of A_2	1 hr^{-1}
ρ_2	Threshold concentration of A_2	1 g/l
S_0	Concentration of supplied substrate	20 g/l
Y_1	Yield coefficient of species 1 with respect to substrate	0.5 g/g
Y_2	Yield coefficient of species 2 with respect to substrate	0.4 g/g
n	Strength of inhibitory effect	5
D	Dilution rate	0.2 hr^{-1}

Figure 6. Bifurcation analysis with respect to the transcriptional rates of the antibiotic resistance proteins where the nominal values were $f_{A1} = f_{A2} = 4$ hr^{-1}. The transcriptional rate of the antibiotic resistance protein A1 did not have a significant effect on the population fraction of the two species (a), while changes in the A2 transcriptional rates led to a substantial shift of the population fraction (b).



(a)



(b)