Supplementary Information

Metabolic codependence gives rise to collective oscillations within biofilms

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I. Strains

All experiments were done using *Bacillus subtilis* NCIB 3610. The wild type strain was a gift from Wade Winkler (University of Maryland)[30], and all other strains were derived from it and are listed in Table S1. The pSac-CM-P*nasA-yfp* vector was a gift from Michael Elowitz (California Institute of Technology). To make overexpression strains, we used polymerase chain reaction (PCR) to amplify the desired region from the wild type strain. The PCR product is then put under the Hyperspank IPTG inducible promoter, using the HindIII/SalI and the NheI sites within the pDR111 vector (gift from David Rudner, Harvard Medical School). The Hyperspank promoter controls overexpression of the gene through isopropyl-β-D-thiogalactopyranoside (IPTG) induction. Vectors for deletion strains were made in a similar way using the pER449 plasmid (gift from Wade Winkler). All constructs were confirmed by direct sequencing and then integrated into the chromosome of the wild type strain by a standard one-step transformation procedure[31]. Finally, chromosomal integrations were confirmed by colony PCR using the corresponding primers.

Table S1. List of strains used in this study

Strain	Genotype	Source
Wild type	*B. subtilis* NCIB 3610	*30*
P_{hyp}-CFP, P_{nasA}-YFP	*amyE::* $P_{Hyperspank}$-*cfp*, *sacA::* P*nasA-yfp* (Sp^R, Cm^R)	This study
P_{hyp}-RocG	*amyE::* $P_{Hyperspank}$-*rocG* (Sp^R)	This study
P_{hyp}-tapA operon	*amyE::* $P_{Hyperspank}$-*tapA operon* (Sp^R)	This study
ΔtapA operon	*tapA-sipW-tasA::* cat	This study
ΔoppA-D	*oppABCD::* cat	This study
ΔcomX	*comX::* cat	*14*
Δhag	*hag::* cat	This study
ΔrocG	*rocG::* kan	This study

II. Mathematical model of metabolic codependence

Model description

We describe the dynamics of biofilm growth in terms of two distinct populations, corresponding to the interior and the periphery of the biofilm. The two populations are assumed to be located in a moving frame of reference as the biofilm grows, so that they are always located at the same distance from the physical edge of the biofilm (Extended Data Figure 6a).

The metabolic state of the biofilm is determined by the following quantities: 1) The concentrations of glutamate in the biofilm interior (G_i) and in the periphery (G_p); 2) the concentration of ammonium (A), which is assumed to be equal for the two populations due to its fast diffusion; 3) The concentration of active glutamate dehydrogenase (GDH) in the interior cells (H_i); and 4) the rate of biomass production, which is assumed to be given by the concentrations of housekeeping proteins (such as ribosomal proteins) in the interior (r_i) and in the periphery (r_p). The dynamics of these state variables are described by the following set of ordinary differential equations:

$$\frac{dA}{dt} = \alpha G_i H_i - \delta_A A(r_i + r_p)$$
$$\frac{dG_i}{dt} = D\left(G_p - G_i\right) - \alpha G_i H_i - \delta_G G_i r_i$$
$$\frac{dG_p}{dt} = D\left(G_i - G_p\right) + D_E\left(G_E - G_p\right) - \delta_G G_p r_p$$
$$\frac{dH_i}{dt} = \beta_H \frac{G_i^n}{K_H^n + G_i^n} - \gamma_H H_i$$
$$\frac{dr_i}{dt} = \beta_r A G_i - \gamma_r r_i$$
$$\frac{dr_p}{dt} = \beta_r A G_p - \gamma_r r_p$$

The terms in the equations are interpreted as follows:

- $\alpha G_i H_i$: ammonium production from glutamate, catalyzed by the enzyme GDH (Figure 2a)
- $\delta_A A(r_i + r_p)$: ammonium consumption by interior and peripheral cells
- $\delta_G G_i r_i$ and $\delta_G G_p r_p$: glutamate consumption by interior and peripheral cells, respectively
- $D(G_p - G_i)$: glutamate diffusion between peripheral and interior regions
- $D_E(G_E - G_p)$: glutamate diffusion between the environment and the periphery of the biofilm

- $\beta_H \frac{G_i^n}{K_H^n + G_i^n}$: GDH activation in the interior cells
- $\gamma_H H_i$: GDH deactivation in the interior cells
- $\beta_r AG_i$ and $\beta_r AG_p$: production of housekeeping proteins in the interior and peripheral cells, respectively
- $\gamma_r r_i$ and $\gamma_r r_p$: degradation of housekeeping proteins in interior and peripheral cells, respectively

We make the following assumptions:

- Peripheral cells rely on ammonium synthesized by interior cells. As a simplification, we assume that only the interior cells have active GDH.
- Activation of GDH depends on the glutamate availability. Specifically, H_i is reduced when the concentration of available glutamate (G_i) is below a given threshold. This can be due to explicit regulatory interactions or simply as a consequence of the slowdown of cellular processes in the absence of nutrients.
- Consumption of ammonium and glutamate depends on the metabolic activity of the cell. The higher the concentration of housekeeping proteins – a proxy for the metabolic state of the cell – the faster the consumption of nutrients.
- The production of housekeeping proteins increases with the concentrations of glutamate and ammonium.

In order to extract from the model the population expansion, which can be measured experimentally, we consider that the dynamics of the cell density $\boldsymbol{\rho}$ of the two populations are given by:

$$\frac{d\rho_{i,p}}{dt} = \eta r_{i,p} \rho_{i,p} \left(1 - \frac{\rho_{i,p}}{K(G_{i,p})}\right) - \lambda_{i,p} \rho_{i,p}$$

The first term in the right-hand side is a logistic-growth term, where the maximal growth rate is considered to be proportional to the concentrations of housekeeping proteins r_i and r_p. Additionally, we assume that the carrying capacity K depends on the concentration of glutamate:

$$K(G) = \frac{G^m}{K_k^m + G^m}$$

Thus $K(G)$ varies between 0 and 1 depending on whether glutamate concentration is below or above a given threshold, denoted as K_k. Note that the cell density $\rho_{i,p}$ defined here is relative to the carrying capacity, therefore, both K and ρ are dimensionless.

The logistic-growth term in the density equation shown above describes the standard birth/death processes that occur in an unmoving bacterial population. In our system, however, the peripheral cells are always expanding into the open area outside of the biofilm. We represent this fact by adding an effective decay term, $-\lambda_{i,p}\rho_{i,p}$ in the density equation of the expanding population (i.e. the peripheral population for all situations considered, except in the case of chemical attack, where the peripheral population is eradicated and consequently the interior cells can expand). This decay term accounts for the effective loss of cells undergone locally by the biofilm front as it expands (in our moving reference frame) into the cell-free area surrounding it.

Given the above-described dynamics for the cell densities, the growth rate (measured experimentally as the area of non-zero local motion within the biofilm) is given by the logistic term, since this is the only term related to actual growth of the population:

$$\mu_{i,p} = \eta r_{i,p}\rho_{i,p}\left(1 - \frac{\rho_{i,p}}{K(G_{i,p})}\right)$$

Addition of glutamine to the media

Glutamine is synthesized by glutamine synthase (GS) in the cell, and it also regulates the activity of GS through negative feedback [27]. Therefore, external addition of glutamine reduces GS activity, and consequently lowers its consumption of ammonium and glutamate (used to synthesize glutamine). Additionally, we assumed that glutamine inhibits either directly or indirectly GDH activity, affecting the production of ammonium from glutamate. This is implemented in the model as non-competitive inhibition on the parameters α and δ. Specifically, the effective $\bar{\alpha}$ and $\bar{\delta}$ are given by:

$$\bar{\alpha} = \frac{\alpha}{\frac{[Gln]}{K_\alpha} + 1}, \qquad \overline{\delta_{A,G}} = \frac{\delta_{A,G}}{\frac{[Gln]}{K_\delta} + 1}$$

Figure 3f in the main text shows the model prediction: in agreement with the experimental observations, external addition of glutamine leads to the quenching of oscillation. A systematic analysis of the effect of glutamine addition is shown in Extended Data Fig. 5b, where a bifurcation diagram of the peripheral glutamate concentration with respect to the added glutamine concentration is shown.

Addition of glutamate to the media

The concentration of glutamate in the external medium is explicitly defined in the model by the parameter G_E. Thus, supplementation with additional glutamate is represented by simply increasing the value of G_E. Figure 3g in the main text shows the model prediction: consistent with the experimental observations, a moderate increase in external glutamate does not eliminate the

oscillations. A systematic study also shows that further increasing glutamate leads to quenching of oscillations (Extended Data Fig. 5c).

Addition of ammonium to the media

The concentration of ammonium is explicitly represented in the model with the variable A, and addition of ammonium to the media can be represented as an additional creation term (α_0) in the ammonium equation:

$$\frac{dA}{dt} = \alpha G_i H_i - \delta_A A(r_i + r_p) + \boldsymbol{\alpha_0}$$

Figure 3h in the main text shows the model prediction: in agreement with the experiments, externally adding ammonium quenches oscillation. We also systematically explored the effect of different ammonium concentrations through a bifurcation diagram of the system with respect to α_0 (Extended Data Fig. 5d).

Overexpression of GDH in cells

We also investigated the effects of overexpressing GDH in the biofilm. The overexpression is implemented in the model by an additional creation term β_0 into the equation for GDH (H_i). Furthermore, since the overexpression is applied throughout the entire biofilm, we include active GDH for the peripheral cells (H_p), and consequently the production of ammonium from those cells. To that end, the differential equations for A, G_p and H_i are modified as shown below, and an equation for GDH in the peripheral cell population (H_p) is also added:

$$\frac{dA}{dt} = \alpha G_i H_i + \boldsymbol{\alpha G_p H_p} - \delta_A A\left(r_i + r_p\right)$$

$$\frac{dG_p}{dt} = D\left(G_i - G_p\right) + D_E\left(G_E - G_p\right) - \boldsymbol{\alpha G_p H_p} - \delta_G G_p r_p$$

$$\frac{dH_i}{dt} = \boldsymbol{\beta_0} + \beta_H \frac{G_i^n}{K_H^n + G_i^n} - \gamma_H H_i$$

$$\boldsymbol{\frac{dH_p}{dt} = \beta_0 - \gamma_H H_p}$$

Figure 4e in the main text shows the model prediction: in agreement with the experiments, overexpressing GDH leads to quenching of oscillation. A systematic analysis on different levels of overexpression is shown in the bifurcation diagram in the Extended Data Fig. 5e.

Addition of hydrogen peroxide to the media

Hydrogen peroxide is a strong oxidizer that can kill the cells on the periphery of the biofilm. Dead cells in the biofilm will still affect glutamate diffusion, but will be metabolically inactive. Thus, the killing is implemented in the model by removing the production term of housekeeping proteins in the peripheral cell population. Additionally, a new negative term in the cellular density equation is introduced to account for cell death. To that end, the differential equations for r_p and ρ_p are modified as shown below:

$$\frac{dr_p}{dt} = -\gamma_r r_p$$

$$\frac{d\rho_p}{dt} = \eta r_p \rho_p \left(1 - \frac{\rho_p}{K(G_p)}\right) - \boldsymbol{\lambda_{H_2O_2} \rho_p} - \lambda_p \rho_p$$

The new term is also added to the equation for the rate population expansion:

$$\mu_p = \eta r_p \rho_p \left(1 - \frac{\rho_p}{K(G_p)}\right) - \boldsymbol{\lambda_{H_2O_2} \rho_p}$$

Finally, in the case of GDH overexpression, hydrogen peroxide entirely eliminates GDH production in the peripheral cell population, and the differential equation for H_p becomes:

$$\frac{dH_p}{dt} = -\gamma_H H_p$$

Figure 4h in the main text shows the model prediction on the average growth rate and death in interior and peripheral populations after the addition of hydrogen peroxide, for both wild type and GDH overexpressing biofilms.

Effect of varying the ratio of interior to peripheral cells

As a consequence of biofilm expansion the relative size of interior and peripheral cell populations changes over time. Since the variables of the mathematical model represent intensive quantities (their value does not depend on the total volume) most of the equations would not be affected by changes in the relative size of both cell populations. The only exception is the equation for ammonium, as it describes the concentration of this species in the whole biofilm, taking into account reactions that occur exclusively in one or the other population region. In this case the relative size of each one of these two regions will modulate the relative effect of these reactions.

To explore the effects of changes in the relative sizes of the two populations, we define f_i as the fraction of the size of the interior population over the whole biofilm population. This parameter allows us to distinguish the contributions of the interior and peripheral regions to both the production and the consumption of ammonium:

$$\frac{dA}{dt} = f_i \alpha G_i H_i - \delta_A A\left(f_i r_i + (1 - f_i)\, r_p\right)$$

This equation allows us to determine the effect of an increase in f_i (such as the one that occurs in the biofilm as it expands) on the growth oscillations reported above. Extended Data Figure 6f shows that the oscillations persist for a wide range of f_i values, with a period that increases only slightly with f_i, in agreement with the experimental observations.

Sensitivity analysis

Extended Data Figure 6g and h show how changes in each one of the intrinsic parameters of the model affect the period and the modulation depth of the oscillations. The values of the parameters were scanned from 50% to 150% of its original value. Whenever a modulation depth lower than 2% was measured the system was considered to be non-oscillating, and labeled in gray in the color plot.

Table S2: Parameter values used in the paper.

	Description	*Value*	*Units*
α	Glutamate dehydrogenation coefficient	50	$\mu M^{-1}\ h^{-1}$
δ_A	Ammonium consumption coefficient	4	$\mu M^{-1}\ h^{-1}$
δ_G	Glutamate consumption rate	4	$\mu M^{-1}\ h^{-1}$
D	Glutamate diffusion constant within the biofilm.	0.4	h^{-1}
D_E	Glutamate diffusion constant between biofilm and exterior	0.6	h^{-1}
G_E	Glutamate concentration in the external medium	30	mM
β_H	Maximal activation rate of GDH	50	$\mu M\ h^{-1}$
γ_H	Deactivation rate of GDH	7.5	h^{-1}
K_H	GDH activation threshold	7.2	mM
n	Hill coefficient for GDH activation	7	
β_r	Expression coefficient of ribosomal/housekeeping proteins.	0.14	$mM^{-1}\ h^{-1}$
γ_r	Degradation rate of ribosomal/housekeeping proteins	2	h^{-1}
η	Population growth rate coefficient	100	mM^{-1}
K_K	Glutamate threshold for carrying capacity	0.85	mM
m	Hill coefficient for carrying capacity	12	
λ_i	Expansion rate of interior cells	0	h^{-1}
λ_p	Expansion rate of peripheral cells	0.032	h^{-1}
$[Gln]$	Concentration of glutamine in the medium	1	mM
K_α	Glutamine inhibition threshold on GDH activity	$5{\cdot}10^{-8}$	mM
K_δ	Glutamine inhibition threshold on GS activity	$5{\cdot}10^{-2}$	mM
α_0	Rate of ammonium entering the biofilm from the external medium	0.03	$mM\ h^{-1}$
β_0	Expression rate of GDH from the additional copy of the gene	$1.5{\cdot}10^{-6}$	$mM\ h^{-1}$
$\lambda_{H_2O_2}$	Death rate due to hydrogen peroxide	5	h^{-1}

Reference

[31] Jarmer, H., Berka, R., Knudsen, S., and Saxild, H. H. (2002) Transcriptome analysis documents induced competence of Bacillus subtilis during nitrogen limiting conditions, *FEMS Microbiol Lett 206*, 197-200.