**Supplementary Information**

**Substrate richness and toxicity govern the assembly and spatial organization of microbial community involving metabolic division of labor**

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**S1 MODEL DESCRIPTION**

Here, we describe our individual-based model framework in detail.The model is implemented on a well-built cell colony growth simulator, *gro* ((Gutierrez et al 2017, Jang et al 2012), https: <https://github.com/liaupm/GRO-LIA>). Supplementary Figure 2 provides the basic logic of the model. We programed this logic using *gro* language, resulted in a good workflow to simulate dynamics of a consortium involving metabolic division of labor (MDOL). Parameters and variables used in the model are listed in Supplementary Table 1 and Supplementary Table 2, respectively. The source *gro* code of all the simulations can be found in Github:

<https://github.com/RoyWang1991/MDOLcode/tree/master/MDOL-spatial>.

**Modeling kinetics of metabolic division of labor in a degradation pathway.** As mentioned before, we assumed a two-step pathway was implemented by sequential division of labor between two populations (Supplementary Figure 1A). The two enzymatic reactions occurred intracellularly and were modelled by basic Michaelis-Menten equations. S, I, and P were assumed to be transported across cells via passive diffusion at a rate proportional to the concentration gradient between two intra- and extracellular compartments. In addition, P would be consumed as the growth resource by cells following Monod equations. Thus, we modelled the material dynamics of the Detoxifier cells following three equations:

Similarly, another three equations were used to describe the dynamics within the Embezzler cells:

Since the model described cells as rod-shaped capsule, the volume of cells, andcan be calculated from the cell length and,as well as the width (diameter) of the capsular cells, , via function

In the individual-based simulations, all these mass concentrations were updated iteratively using the discretized calculations. Eventually, to describe the spatial heterogeneity of S, I, P, we modelled their diffusion using the finite element method built in *gro*.

**Cell growth, division and movement.** As most of the previous studies (Frost et al 2018, Lardon et al 2011, Li et al 2019, Smith et al 2017), the model described cells as rod-shaped capsule, which elongated at a rate of . In this regime, the volume of each cell just grows exponentially through elongation, from an initial length :

Following our hypothesis that P is the sole limited growth resource for both populations, iscalculated using the P-mediated Monod equations associated with a first-order death rate:

Where is the yield coefficient for biomass production; is the death rate. In addition, to involve substrate toxicity, a term representing toxic effects was introduced to these equations, which is related to the intracellular concentration of S and described by a hyperbolic toxic function (Quintas et al 2005), then

Therefore, higher toxic strength or intracellularly accumulating more S will lower the growth rate. During the simulations, cell volumes are updated iteratively as well. Cells divided via binary fission when its volume reach the division length , where is the random noise in the cell cycle, following a uniform distribution. Moreover, we did not consider active cell movement in the model, but passive cell shoving derived from cell-cell contact was included, modelled by the ‘collision detection and collision response’ strategy built-in *gro* (Gutierrez et al 2017, Jang et al 2012).

**Model parameterization.** Supplementary Table 2 lists all the parameters (24 parameters in total) and their definitions, units, values and sources used in our model. Mechanical and numerical parameters, related to cell shape and cell movement, were assigned with the default values of *gro* platform. Parameters deciding the enzymatic kinetics of the two reaction steps were taken from the previous publications, which reported the exact kinetic parameters of salicylate 1-hydroxylase (Camara et al 2007) and catechol 2,3-dioxygenase (Viggiani et al 2004), consistent with our synthetic system. Parameters governing the inherent growth of bacteria, that is, yield coefficient, maximum resource consuming rate , as well as death rate were estimated directly from experimental observations of the growth our *Pseudomonas* strains using one of the final products, pyruvate, as the sole carbon source. The permeability coefficients for mass transfer across the cell of S (), I (), and P (), were taken or evaluated from an estimation model reported previously (Ahn et al 2006).

**Model to simulate ‘competition’ scenarios.** As a control, we built an additional model to simulate the situation where the two populations just competed for the limited resource. We did not include S and I in this model, while supplied P at the beginning which was evenly distributed across the environments. P was still the sole limited resource, and the growth kinetics of the two cell types were totally same as before:

Values of the parameters used in this model were also totally same as before.

**Modifications of the model to predict the pattern formation assays.** To predict the spatial organization of our synthetic consortium, we modified the basic model by introducing several additional assumptions. Firstly, since one-unit salicylate would be converted to two-unit final product (one pyruvate and one acetyl-CoA) via one-unit catechol, we modified the kinetics of the second enzymatic reaction, resulted in a novel dynamic equation of the intracellular final product of the Embezzler cells:

Secondly, we introduced the measured the exact toxic strength of salicylate, 0.62 (Figure 3C), to the model. Thirdly, we also considered the fitness different between AN0010 and AN0001 because they expressed different enzymes. We performed competitive fitness assays between AN0010 and AN0001 using the standard protocol reported in (Travisano and Lenski 1996) and (Pande et al 2016) using pyruvate (0.34 mol/L) as the sole carbon source as well as supplying IPTG (2 mM) to induce the expression of the functional operon (Figure 3B), which showed that AN0001 generally grew a little bit slower than AN0010, with a relative fitness of 0.982±0.016. The liquid medium and culture conditions used in these measurements was described in the Methods section. The related statistical analysis was performed on *Wolfram Mathematica* (version 12.0). We then added this fitness cost in the function characterizing the growth of Embezzler by set a new growth function:

Eventually, to simulate the small ‘sectors’, we introduced another limited resource, L, to the model, which is equally essential to both populations. We modelled its consumption also by Monod equation, thus the growth kinetics of both populations were modified,

where andare the maximum consuming rate and half-saturation constant for cell to consume limited resource L.

**Simulation protocols.** Individual-based model simulations were run on a ThinkPad laptop computer (X1 Carbon 2019) running windows 10.1 using *gro* platform available online (https: https://github.com/liaupm/GRO-LIA). Cell behaviors were simulated in a 2D plane, and would finally develop to a monolayer colony. For the model simulate MDOL, S were evenly initialized across the plane with the designed concentration, while the initial concentration of I and P, were set to zero. For the model with another limited resource L, in addition to S, L were also evenly initialized across the plane. For the model simulating ‘competition’ scenarios, instead, only P was included and evenly initialized across the plane. To start the colony growth, we used 200-cell inocula consisting of a 1:1 mix of Detoxifier and Embezzler cells, randomly scattered and oriented within a 200-μm circle. Simulations were set to terminate once the cell population exceeded 8,100 individuals.

**Simulation data processing.** Images of simulated colony pattern at each time point were simply generated from *gro* using the built-in function ‘*Snapshot*’. Videos were created from the time-series image sequences exported from *gro*, and assembled in software *Image J* (version 1.53c). Custom functions were included in the *gro* codes to record the position coordinates of every cell, intra- and extra- concentrations of the masses, as well as the cell number of each population at each time point. These simulation data were analyzed and visualized using custom *Wolfram* *Mathematica* scripts (version 12.0). Specifically, to fit simulation with the proposed function, we used NonlinearModelFit function of Wolfram Mathematica (version 12.0) with the default method. To calculate the spatial assortment, we used the similar method (Estrela and Brown 2013, Yanni et al 2019) used for quantifying the colony images described in the Methods section. Two differences are that the cell position data of the final simulation time point was used as the input, and the distance of 5 μm was used as the neighborhood distance, which makes the absolute assortment values incomparable between experiment and model, but the variation trend should be the same, as tested in the previous study (van Gestel et al 2014). The source code used for all these analyses are available on Github:

<https://github.com/RoyWang1991/MDOLcode/tree/master/MDOL-spatial>.

**S2 TABLE OF SIMBOLS**

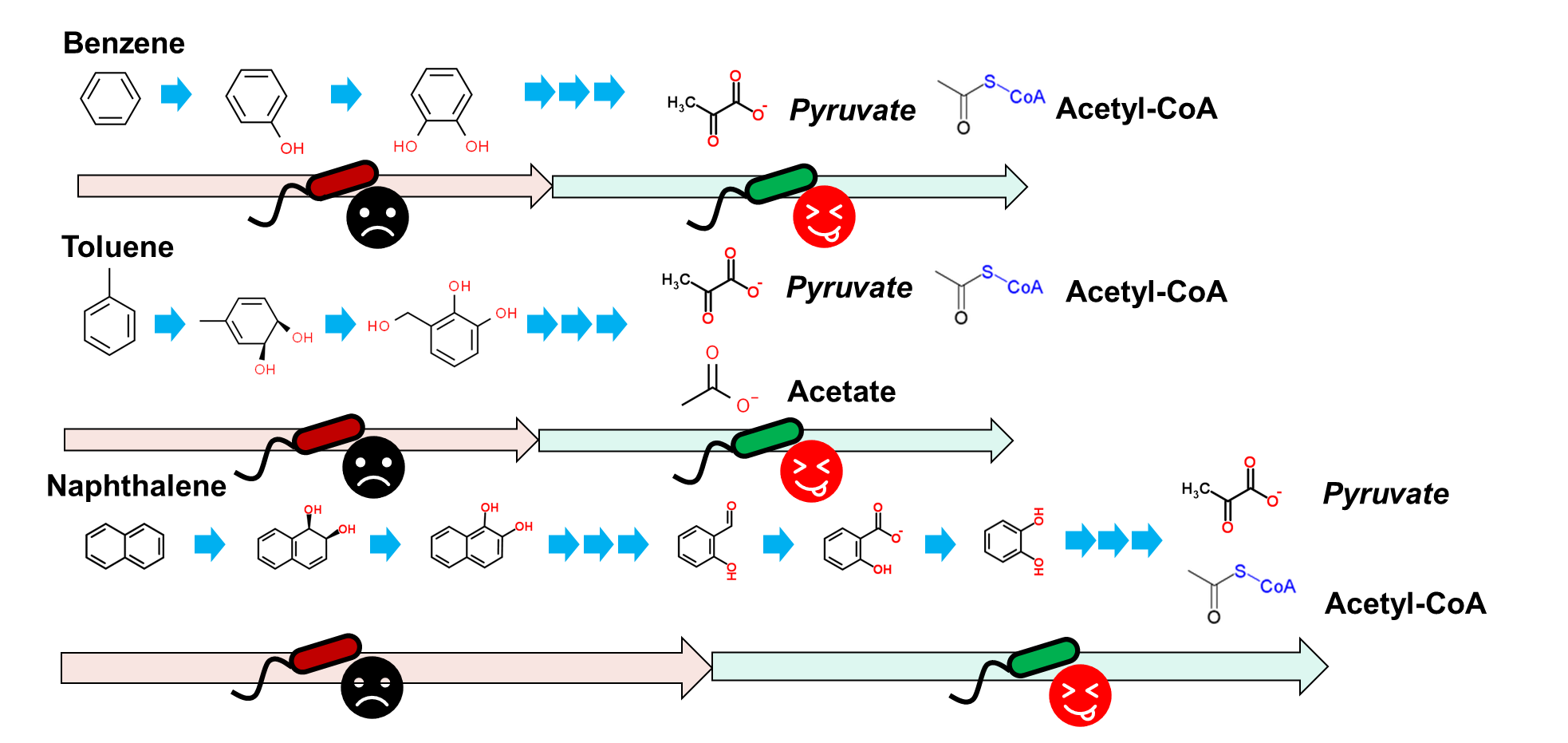
**Supplementary Table 1** Summary of model variables

|  |  |  |
| --- | --- | --- |
| **Variable** | **Description** | **Units** |
|  | Concentration of Intracellular substrate in the cells from Detoxifier population. | C-mmol/L |
|  | Concentration of Intracellular substrate in the cells from Embezzler population. | C-mmol/L |
|  | Concentration of extracellular substrate. | C-mmol/L |
|  | Concentration of Intracellular intermediate in the cells from Detoxifier population. | C-mmol/L |
|  | Concentration of Intracellular intermediate in the cells from Embezzler population. | C-mmol/L |
|  | Concentration of extracellular intermediate. | C-mmol/L |
|  | Concentration of Intracellular final product in the cells from Detoxifier population. | C-mmol/L |
|  | Concentration of Intracellular final product in the cells from Embezzler population. | C-mmol/L |
|  | Concentration of extracellularfinal product. | C-mmol/L |
|  | Concentration of Intracellular limited resource in the cells from Detoxifier population. | C-mmol/L |
|  | Concentration of Intracellular limited resource in the cells from Embezzler population. | C-mmol/L |
|  | Concentration of extracellularlimited resource. | C-mmol/L |
|  | Volume of the Detoxifier cells | fL |
|  | Volume of the Embezzler cells | fL |
|  | Length of the Detoxifier cells | μm |
|  | Length of the Embezzler cells | μm |
|  | Growth rate of the Detoxifier cells | min-1 |
|  | Growth rate of the Embezzler cells | min-1 |

**Supplementary Table 2** Summary of model parameters

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Description** | **Default value and units** | **Source** |
|  | Michaelis-Menten constant first reaction. | 0.096 C-mmol⸱L-1 | (Camara et al 2007) |
|  | Enzyme concentration of the first reaction. | 0.002 C-mmol | (Zimmerman and Trach 1991) |
|  | Specific rate of thefirst reaction. | 460 min-1 | (Camara et al 2007) |
|  | Michaelis-Menten constant second reaction. | 0.0015 C-mmol⸱L-1 | (Viggiani et al 2004) |
|  | Enzyme concentration of the second reaction. | 0.002 C-mmol | (Zimmerman and Trach 1991) |
|  | Specific rate of thesecond reaction. | 2000 min-1 | (Viggiani et al 2004) |
|  | Permeability coefficient for the substrate transfer across cell membrane. | 2.63 min-1 | (Ahn et al 2006) |
|  | Permeability coefficient for the intermediate transfer across cell membrane. | 0.427 min -1 | (Ahn et al 2006) |
|  | Permeability coefficient for the final product transfer across cell membrane. | 2.51 min -1 | (Ahn et al 2006) |
|  | Permeability coefficient for the listed resource transfer across cell membrane. | 2.62 min -1 | (Ahn et al 2006) |
|  | Diffusivity coefficient for the substrate | 2.0 | Maximum value in *gro* |
|  | Diffusivity coefficient for the intermediate | 2.0 | Maximum value in *gro* |
|  | Diffusivity coefficient for the final product | 2.0 | Maximum value in *gro* |
|  | Diffusivity coefficient for the limited | 2.0 | Maximum value in *gro* |
|  | Initial length of the cells | 1.0 μm | Default value in *gro* |
|  | The mean division length of the cells | 3.75 μm | Default value in *gro* |
|  | Random noise in the cell cycle | 0.15 μm | Default value in *gro* |
|  | Width (diameter) of the capsular cells | 1.0 μm | Default value in *gro* |
|  | Half-saturation constant of Monod growth. | 0.02C-mmol⸱L-1 | Experimental measurement |
|  | Maximum consumption rate of final product for cell growth. | 0.32 C-mmol⸱min-1 | Experimental measurement |
|  | Half-saturation constant of the limited resource L for cell growth. | 1.0 C-mmol⸱L-1 | Experimental measurement |
|  | Maximum consuming rate of the limited resource L for cell growth. | 0.04 C-mmol⸱min-1 | Experimental measurement |
|  | Apparent maintenance rate | 0.0003 min-1 | Experimental measurement |
|  | Yield coefficient for biomass production | 0.1 C-mmol-1 | Experimental measurement |
|  | Toxic strength of the substrate | 0 ~ 2 L⸱C-mmol -1 |  |
|  | Initial concentration of the substrate | 0 ~ 40 C-mmol⸱L-1 |  |
|  | Initial concentration of the limited resource | 76 C-mmol⸱L-1 | Experimental measurement |

**S3 SUPPLEMENTARY FIGURES AND VIDEOS**

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**Supplementary Figure 1** Three typical cases of organic compound degradation suggest that carbon source allocation is asymmetric between different populations when implementing these pathways by MDOL. Since direct carbon sources, such as small organic acids or coenzyme A, are normally present as the final product of a pathway, the population performing the last steps can get more benefit.



**Supplementary Figure 2** The logic of the individual-based model. (A) We assumed a conceptualized organic substrate (S) could be degraded an intermediate metabolite (I) by one enzyme (E1), then to a final product (P) by a second enzyme (E2). An Detoxifier population was assumed to perform the first step, while an Embezzler population performed the second. All the reactions occurred intracellularly, while S, I, and P were passive diffused across the cell membrane. Importantly, the growth of both populations was dependent on the intracellular concentration of P, the sole limited resource of this system, following the Monod equation with a one-order death rate *d*. When applicable, a hyperbolic toxic term was added to the growth equation, with a toxic strength *t*, to represent substrate toxicity. (B) Individual-based simulations were performed on a 2D plane. Bacterial cells were characterized as rigid capsules of variable length and fixed radius. Cells of the two populations were initialized in an inoculating ring with a radius of 200 μm, and 100 cells for each population were randomly distributed. They grew and divided in this habitat by division of labor, driving the range expansion of the colony. By sequentially updating the cell configuration according to the rule proposed in (A), we simulated the development of colony structure until the total number of cells reaches 8100. We focused on the assembly of this community, as well as its spatial patterning.



**Supplementary Figure 3** Dynamics of extra- and intracellular final product concentration of both populations suggest that increasing initial substrate concentration can weaken final product privatization by the Embezzler population. (A) Diagram shows the definition of ,, and (See Supplementary Table 1 section for detailed description). (B) Dynamics of average ratio of the extracellular (grid right outside the cell, ) to intracellular product concentration () among all the Embezzler cells across different conditions of substrate richness and toxicity. The ratio lower than 1 means majority of final product was privatized by the Embezzler cells, while higher ratio indicates more final product was released outside of cells. The plot indicates that higher substrate input leads to higher **/** ratio, i.e., more release of final product. (C) Dynamics of ratio of the average intracellular product concentration of Detoxifier cells () to this of the Embezzler cells () across different conditions of substrate richness and toxicity. The ratio lower than 1 means Embezzler accumulate more product inside the cell than Detoxifier, while increased ratio indicates this benefit is reduced. The plot indicates that higher substrate input leads to higher **/** ratio, suggesting benefit from product privatization was decreased.



**Supplementary Figure 4** Dynamics of intracellular substrate concentration of both populations suggest that Detoxifier population can benefit from the detoxification of the toxic substrate. (A) Diagram shows the definition of , and(See Supplementary Table 1 section for detailed description). (B) Dynamics of ratio of the average intracellular substrate concentration of Detoxifier cells () to this of the Embezzler cells () across different conditions of substrate richness and toxicity. The ratio lower than 1 means Detoxifier accumulate lower amount of substrate inside the cell than Embezzler. The plot indicates that Detoxifier cells always possess lower intracellular substrate concentration than Embezzler cells, showing potential benefit when the substrate is toxic.



**Supplementary Figure 5** Fitting the initial substrate concentration (*S*) and substrate toxic strength (*t*) to relative fraction of Detoxifier (*DF*) suggests simple functions to predict community assembly of consortium involving MDOL. The fit results from the basic model (A), the modified model for experiment results prediction (B), as well as the updated model considering another limiting resource, L, are shown, which gives three fitted prediction function PF1, PF2, and PF3, respectively. The hyperbolic surfaces in these plots show the predicted value from the corresponding function (indicated at the bottom of each graph), while the green points indicate the average value from eight independent simulations in the related conditions of *S* and *t*.



**Supplementary Figure 6** Spatial assortment increased positively with toxic strength of the substrate. Data used here are same as Figure 2C. Correlation coefficients (R2) and *P*-value were labeled in plot. These correlation analyses were performed on Wolfram Mathematica (version 12.0) using the function LinearModelFit with the default set-up.



**Supplementary Figure 7** Verification of the Superman Detoxifier, Embezzler, and Cheater strain. (A) Enzymic activity assays of salicylate 1-hydroxylase and catechol 2,3-dioxygenase were performed to verify the metabolism of the substrate, salicylate, and the intermediate, catechol, of the four strains. Three replicates were performed for each assay. (B) Growth dynamics from the mono-culture of four strains using salicylate (solid line) or catechol (dash line) as the sole carbon source. Five replicates were performed for each treatment. 10 C-mmol/L salicylate or catechol were used in the culture experiments.



**Supplementary Figure 8** Liquid co-culturing dynamics of the Detoxifier and Embezzler supplying salicylate as the sole carbon source. Biomass dynamics (A) and relative fraction of Detoxifier strain (B) are shown. Five replicates were performed for each treatment.



**Supplementary Figure 11** Updated model successfully reproduces the ‘sector’ structure formed during the colony range expansion. (A) Diagram shows that we introducing an additional limited resource, L, to the basic model, which is equally available to the two populations. (B) A representative pattern generated from this modified model. In this case, the initial substrate concentration is *S* = 10 C-mmol/L, while toxic strength *t* = 0.62. The values of other parameters, including the initial concentration of L, are assigned with the default value defined in Supplementary Table 2**.**



**Supplementary Figure 10** Characterization of the spatial pattern formed by SMC-mdol. (A) Images show the colony growth dynamics of the SMC-mdol when performing division of labor in salicylate degradation. Alternative fluorescence labelling was used to eliminate the effect of the expression of different fluorescent proteins. The growth of typical ‘bubble’ areas is zoomed in. (B) Confocal imaging shows the three-dimensional structure of a typical ‘bubble’ area. (C) Analysis of the relative fluorescence intensity of the image showed in (B), suggesting the distribution of the two populations in this area.



**Supplementary Figure 11** Twitching motility of pilus-deficient mutants in agarose-based ‘stab’ assays. (A) The twitching speed of different strains after 3-day incubation in 6-well plate following the standard protocol (Filloux and Ramos 2014). Minimum medium containing 1.5% agarose and 57 C-mmol/L pyruvate was used in these cases. The speed was determined from the analysis of 6 replicated experiments per strain and was calculated by dividing the radius of expansion area with the incubation time. (B) Typical images of the ‘twitching rings’ (faint rings that form around colonies) of different strains formed at the bottom of plates, between the plastic and the agarose.



**Supplementary Figure 12** Swimming motility of flagellum-deficient mutants in agarose-based ‘swimming’ assays. (A) The swimming speed of different strains after 2-day incubation in 6-well plate following the standard protocol (Filloux and Ramos 2014). Minimum medium containing 0.3% agarose and 57 C-mmol/L pyruvate was used in these cases. The speed was determined from the analysis of 6 replicated experiments per strain and was calculated by dividing the radius of swimming area with the incubation time. (B) Typical images of the ‘swimming rings’ of different strains formed inside the agarose plates.

**Supplementary video 1** A representative simulated dynamics with no substrate toxicity and low substrate input. Note that the labels in the top left corner indicate the simulation time and number of total cells. Red cells are the from Detoxifier population, while green cells are the from Embezzler population. In this case, the initial substrate concentration is *S* = 5 C-mmol/L, while toxic strength *t* = 0. Embezzler cells (Green) largely dominated the space in this case with a final fraction of 0.978 (7918 of 8100 cells). The values of other parameters are assigned with the default value defined in Supplementary Table 2**.**

**Supplementary video 2** A representative simulated dynamics with no substrate toxicity and high substrate input. Note that the labels in the top left corner indicate the simulation time and number of total cells. Red cells are the from Detoxifier population, while green cells are the from Embezzler population. In this case, the initial substrate concentration is S = 40 C-mmol/L, while toxic strength *t* = 0. In this case, Detoxifier population (Red) possess a higher frequency than the case in Supplementary video 1, with a final fraction of 0.363 (2943 of 8100 cells). The values of other parameters are assigned with the default value defined in Supplementary Table 2**.**

**Supplementary video 3** A representative simulated dynamics with high substrate toxicity and high substrate input. Note that the labels in the top left corner indicate the simulation time and number of total cells. Red cells are the from Detoxifier population, while green cells are the from Embezzler population. In this case, the initial substrate concentration is *S* = 40 C-mmol/L, while toxic strength *t* = 2. Detoxifier cells (Red) dominated the space in this case with a final fraction of 0.640 (5188 of 8100 cells). The values of other parameters are assigned with the default value defined in Supplementary Table 2**.**

**Supplementary video 4** A representative simulated dynamics of the modified model, where an additional limited resource, L, was assumed. Note that the labels in the top left corner indicate the simulation time and number of total cells. Red cells are the from Detoxifier population, while green cells are the from Embezzler population. In this case, the initial substrate concentration is *S* = 10 C-mmol/L, while toxic strength *t* = 0.62. The final fraction of Detoxifier cells (Red) is 0.506 (4102 of 8100 cells). The values of other parameters, including the initial concentration of L, are assigned with the default value defined in Supplementary Table 2**.**

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