Supplementary Information for "A competitive trade-off limits the selective advantage of increased antibiotic production"

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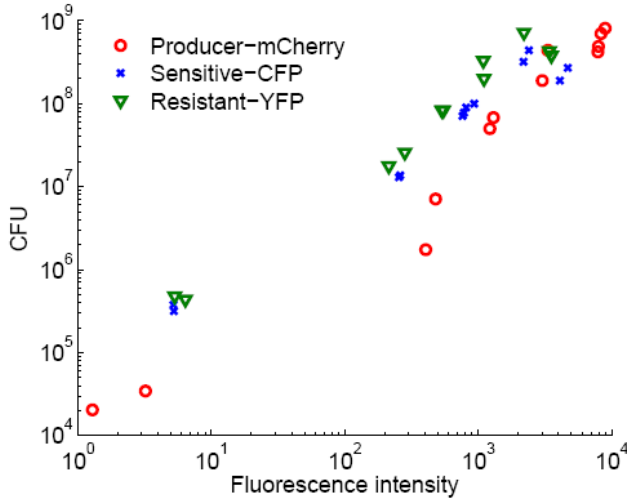
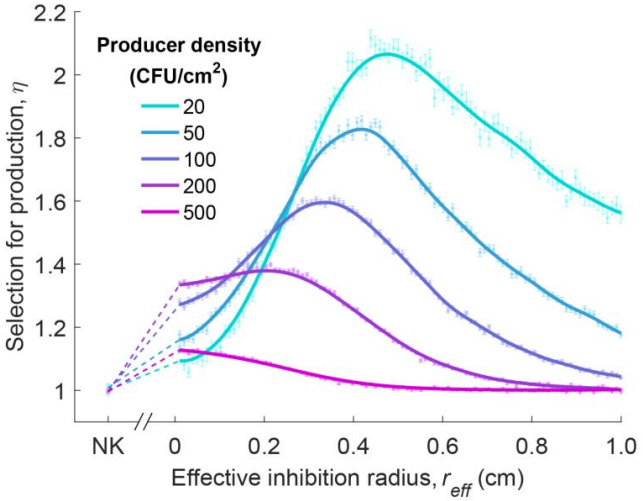
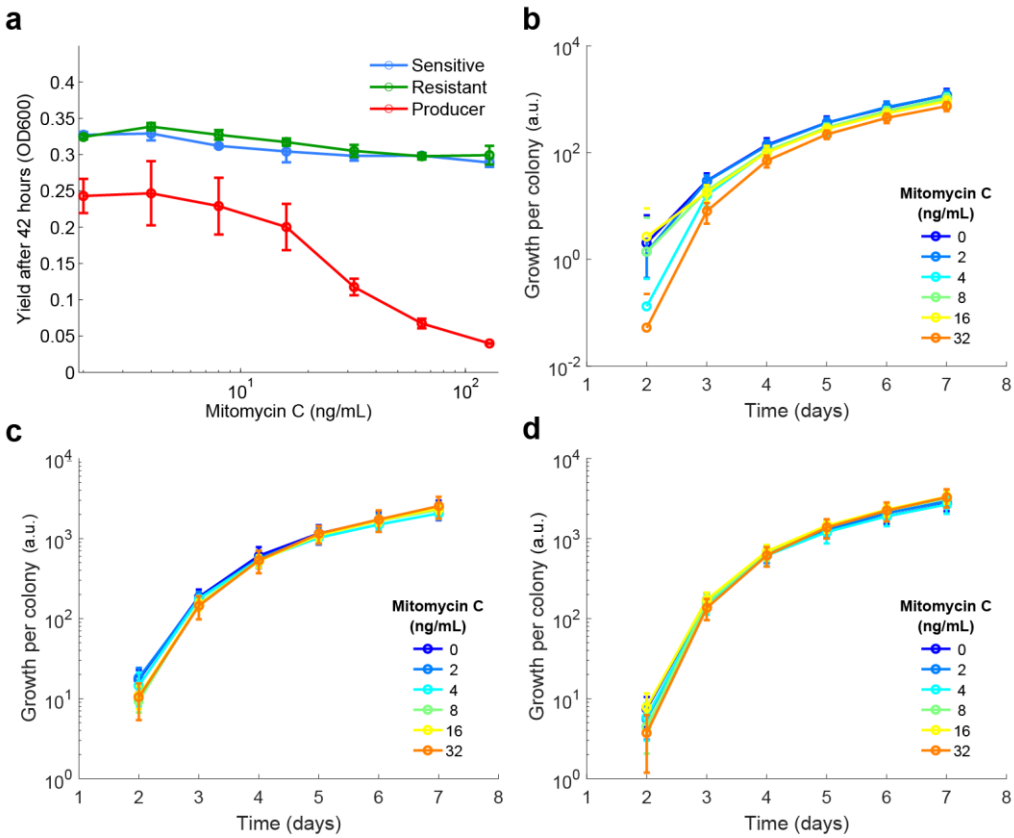
Supplementary Figures



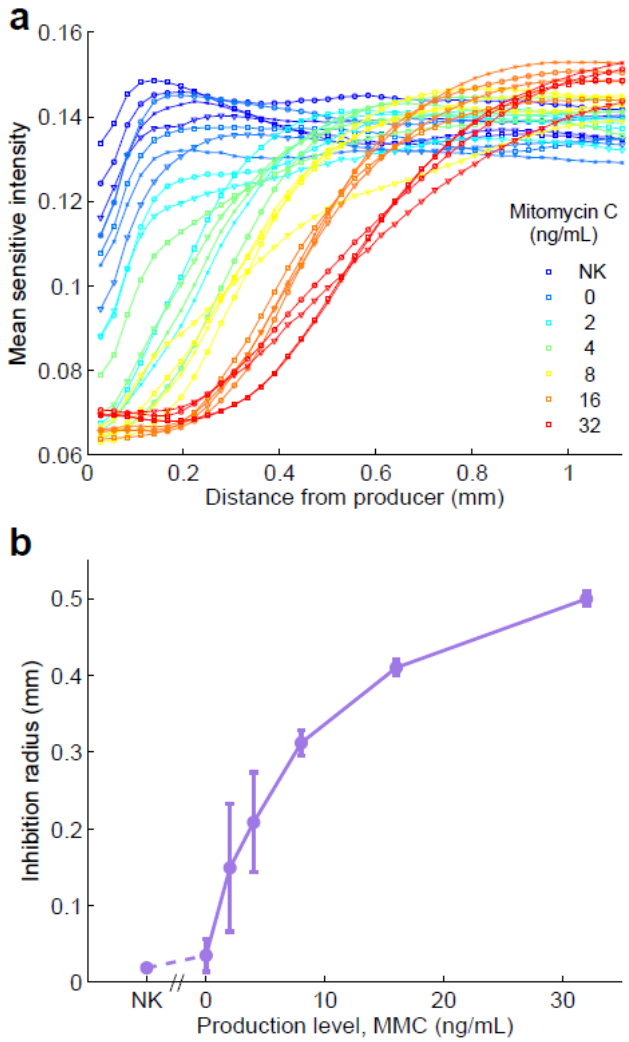
Supplementary Figure 1. Total fluorescence intensity is a good proxy for CFU for measuring colony growth. Individual colonies of each strain were assayed by imaging (calculating fluorescence intensity using the image processing pipeline, see Methods) and by counting colony-forming units (CFU).



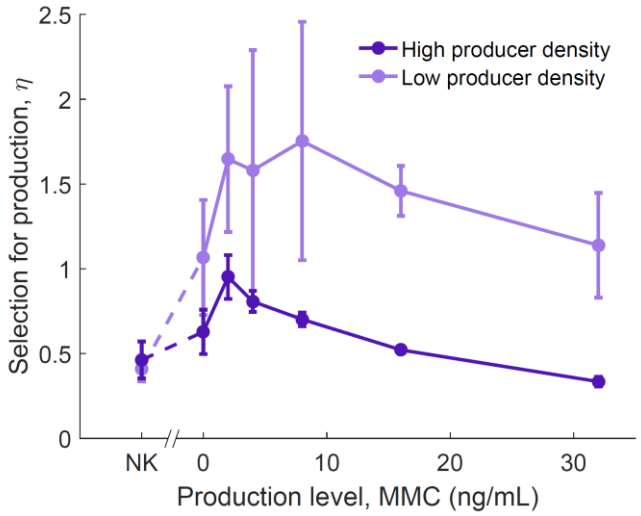
Supplementary Figure 2. Simulations accounting for the cooperative effect of toxicity from nearby producer colonies acting as instantaneous point sources of diffusing antibiotic. Selection for antibiotic production as a function of the effective inhibition radius r_{eff}, the distance at which the antibiotic concentration equals *MIC* for an isolated producer colony. NK corresponds to no production, c=0. Parameters are the same as in Figure 3c with the addition of σ=0.25, *MIC*=1, and $c=2\pi\sigma^2 MIC * \exp(r_{eff}^2/2\sigma^2)$. Mean of n=50 simulation runs per parameter set, error bars show s.e.m.).



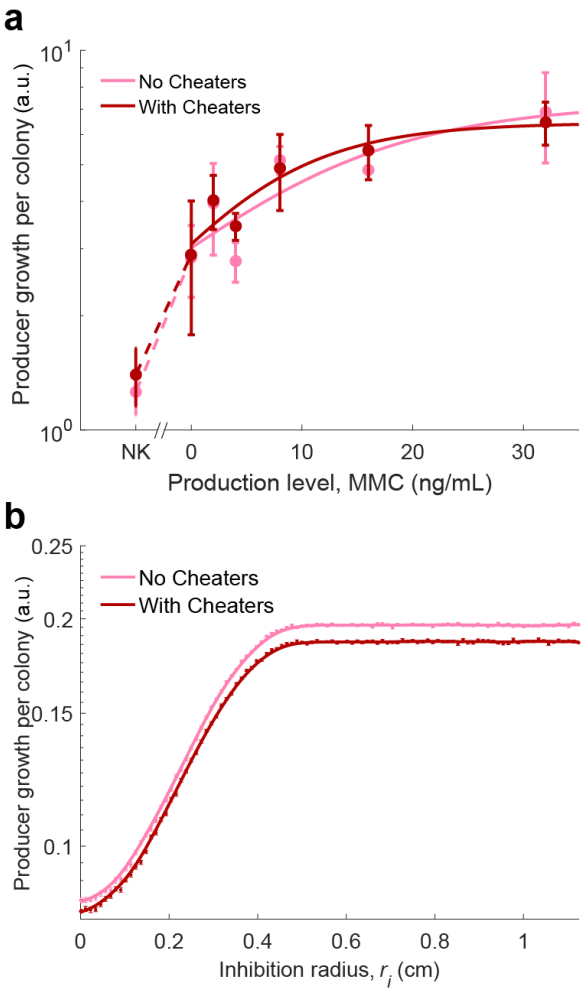
Supplementary Figure 3. Growth of single strains with varying concentrations of mitomycin C. a, Mean growth in liquid culture, showing that mitomycin C reduces producer yield at concentrations where non-producing strains are not significantly affected (n=4 wells per condition, error bars show standard deviation). **b-d,** Mean growth (arbitrary units) of colonies on solid media (n=30-50 colonies per condition, error bars show s.d.). **b,** Producer-mCherry. **c,** Resistant-YFP. **d,** Sensitive-CFP.



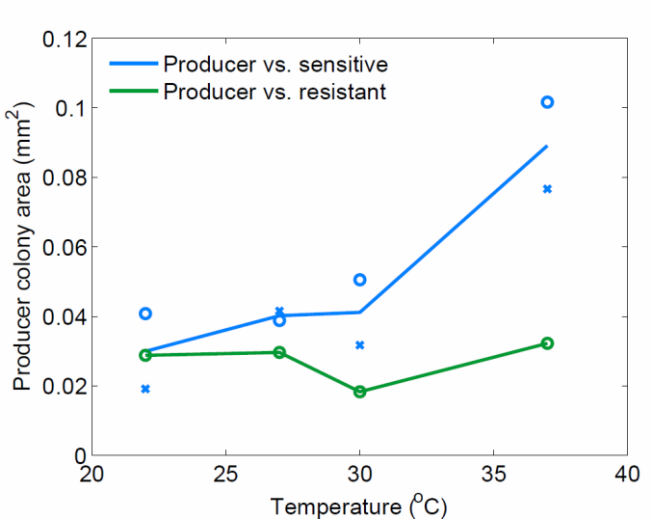
Supplementary Figure 4. Varying mitomycin C concentration tunes the size of inhibition zones. a, Mean fluorescence intensity in the sensitive channel (CFP) decreases in proximity to producer colonies (each trace is an individual plate image with [P] = 2 CFU/cm^2, [S] = 2,000 CFU/cm^2, 4 plates per condition). **b,** Mean inhibition radius increases with the amount of colicin induction via mitomycin C (n=4 plates per condition, error bars show s.d.).



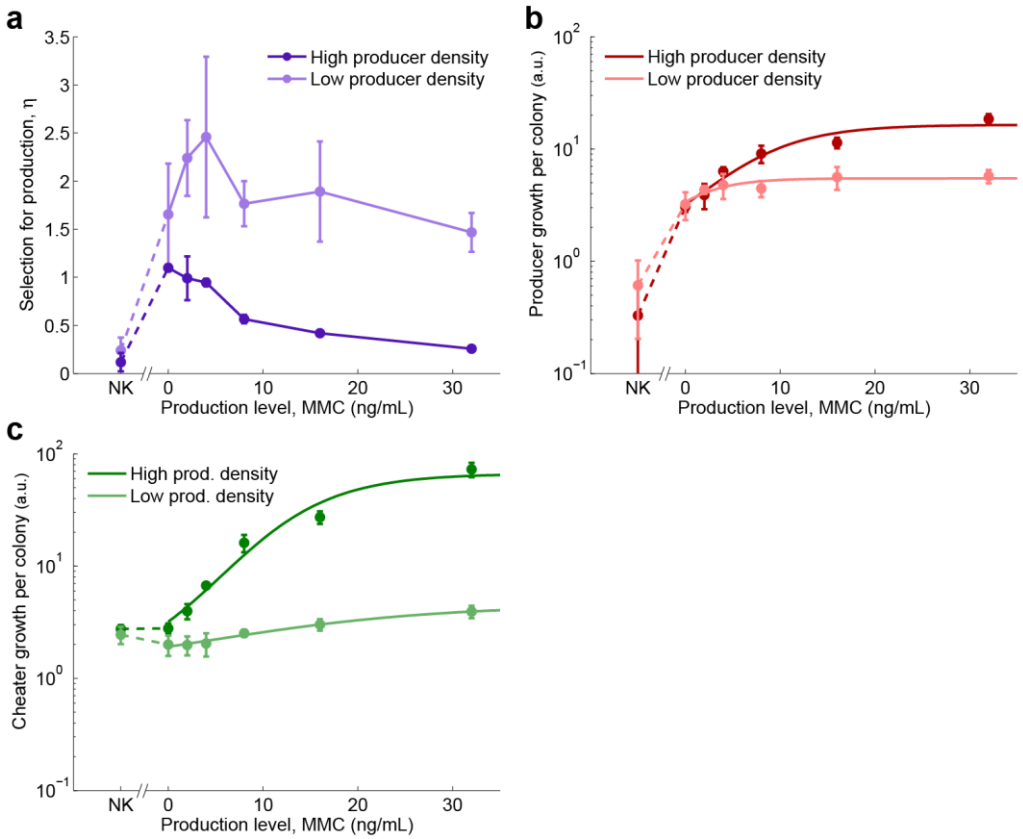
Supplementary Figure 5. Confirmation of optimal production level in dye-swapped experiment. The experiment in Figure 4a was replicated using YFP-marked producers and mCherry-marked cheater strains. All other experimental conditions are the same (n=4 plates per condition, error bars show s.d.).



Supplementary Figure 6. Producer growth plateaus with and without cheaters. a, Mean growth (arbitrary units) of producer colonies in experimental competitions ([S]=2,000 CFU/cm^2, [P]=20 CFU/cm^2, [C]=0 or 20 CFU/cm^2; mean of 4 replicate plates for each point, error bars show s.d.). **b,** Mean growth (arbitrary units) in model simulations ([P] = 0.5 CFU/cm^2, [C]=0 or 0.5 CFU/cm^2, all other parameters as in Figure 3c; mean of 50 simulation runs, error bars show s.e.m.). Growth units are arbitrary.



Supplementary Figure 7. Colicin expression is temperature-dependent. Mean growth of producer colonies in competition with sensitive or resistant competitors at varying temperatures. [P]=8 CFU/cm^2, [S] or [R] =2000 CFU/cm^2, [mitomycin C]=0, n=2 replicate plates for producer versus sensitive and n=1 for producer versus resistant.



Supplementary Figure 8. The advantage of producers over cheaters is maximized at an intermediate level of antibiotic production. Data are as in Figure 4, but growth data was normalized by average seeding density instead of number of observed colonies per plate. **a**, Selection for production in three-way competitions as a function of varying levels of colicin induction via mitomycin C ([S]=2,000 CFU/cm^2; high density: [P]=[C]=20 CFU/cm^2, low density: [P]=[C]=2 CFU/cm^2; mean of 4 replicate plates for each point, error bars show s.d.). Left-most points represent no-killing (NK) controls, where the sensitive competitor was replaced with resistant. **b**, Mean growth (arbitrary units) of producer colonies increases monotonically with production level (n=4, error bars show s.d.). **c**, Mean growth (arbitrary units) of resistant colonies at low and high producer density for varying production levels (n=4, error bars show s.d.).

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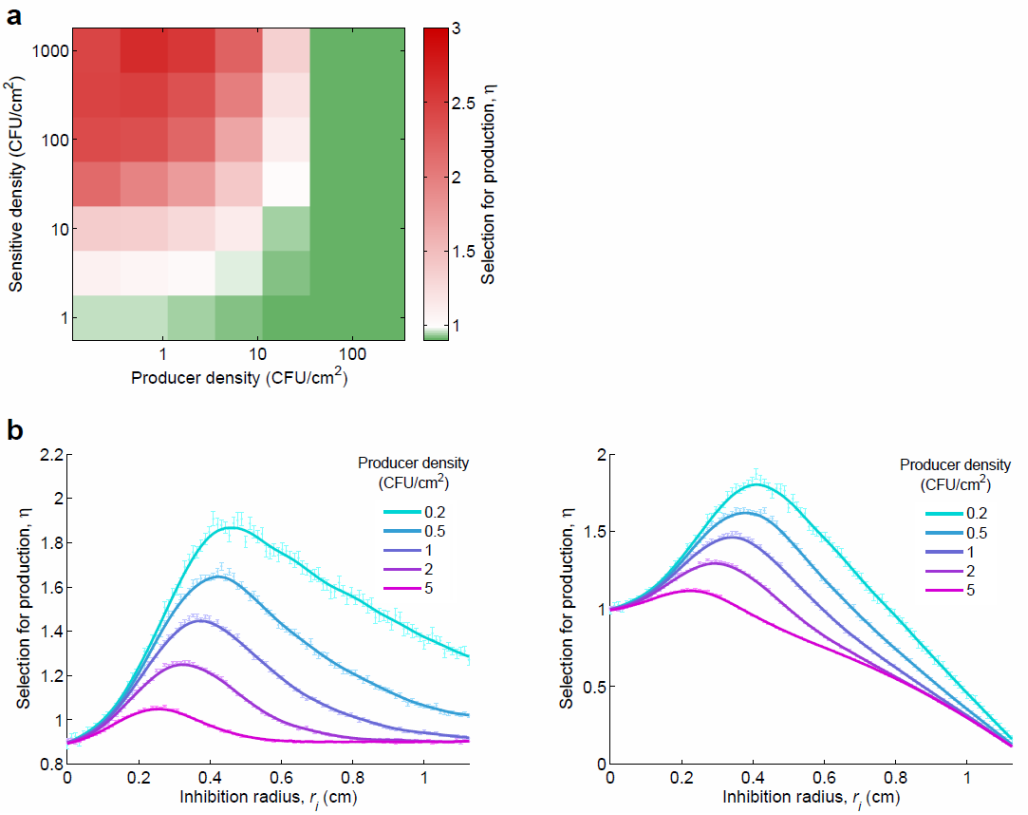
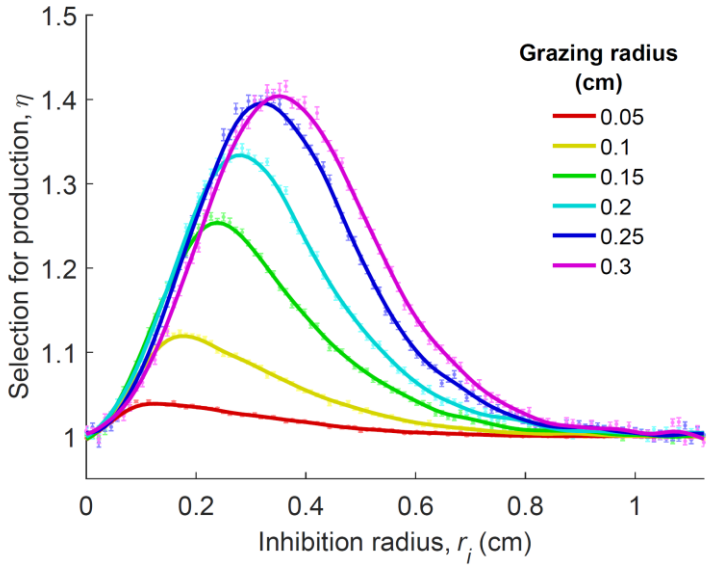
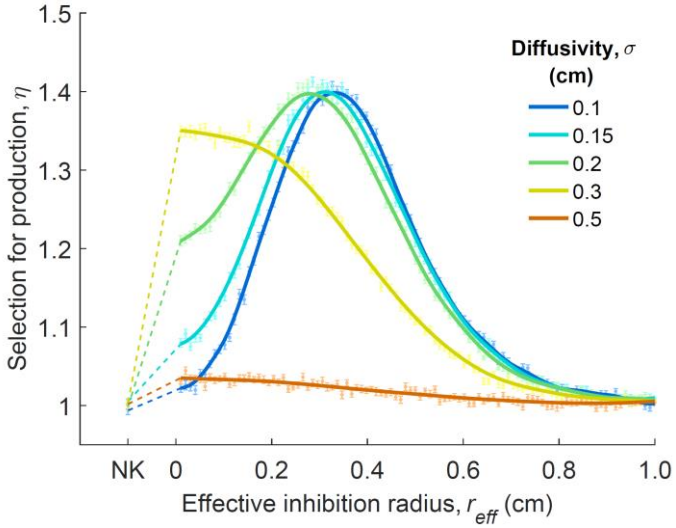


Supplementary Figure 9. Fixed and variable-cost model simulations. a, Varying-density simulations (as in Fig. 3b) with an additional 10% cost imposed on the final producer growth. **b,** Varying-inhibition simulations (as in Fig. 3c), showing mean selection for production as a function of inhibition radius (n=50, error bars are s.e.m.). Left, simulations with a 10% fixed cost on producer growth. Right, simulations with cost as a function of inhibition radius r_i (cost = $0.7r_i^2$).

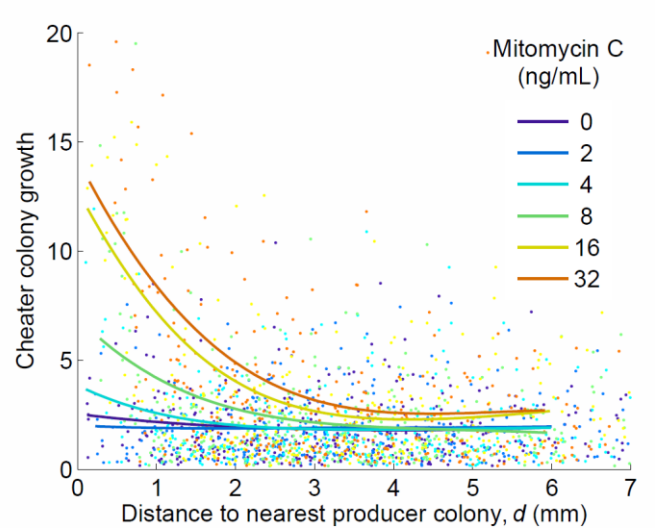


Supplementary Figure 10. Location of selection peak depends on the grazing zone radius. Simulations were run as in Figure 3c ([P] = [C] = 2 CFU/cm^2, n=50 simulation runs per parameter set, error bars show s.e.m.).



Supplementary Figure 11. Shape and location of selection peak depends on antibiotic diffusivity in the cooperative model. Selection as a function of the effective inhibition radius r_{eff}, the distance at which the antibiotic concentration equals *MIC* for an isolated producer colony. NK corresponds to no

production, *c*=0. Simulations were run as in Supplementary Figure S2 ([P] = [C] = 2 CFU/cm^2, n=50 simulation runs per parameter set, error bars show s.e.m.).



Supplementary Figure 12. Growth of individual cheater colonies close to producer colonies increased with colicin induction. Each series is combined data from 4 replicates where [P]=[C]=2 CFU/cm^2; solid lines are smoothened averages calculated by local linear regression.

Supplementary Tables

Supplementary Table 1. Parameters used for the simulations shown in Figure 3. Simulations were performed as described in Methods.

Parameter	Description	Value (Fig. 3a)	Value (Fig. 3b)	Value (Fig. 3c)
r_i	Inhibition zone radius	0.25 cm	0.25 cm	0-1.125 cm
r_g	Grazing zone radius	0.25 cm	0.25 cm	0.25 cm
A	Environment area	2 cm^2	100 cm^2	100 cm^2
dx	Grid-cell length	0.002 cm	0.02 cm	0.02 cm
P	Number of producer colonies	4	10-10,000	20-500
C	Number of cheater colonies	4	10-10,000	20-500
S	Number of sensitive colonies seeded	1,000	100-100,000	1,000
k	Colony area scale factor	1.5	N/A	1.5