Supporting Information: A Growing Microcolony can Survive and Support Persistent Propagation of Virulent Phages

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May 30, 2017

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1 Simulation method

1.1 Detailed protocol of the numerical simulation

Cell growth and cell-cell interaction

We constructed a 3-dimensional model of cells and surrounding phage particles. The cell are labeled by an index i and modeled as spheres with radius r_i, a position $\vec{x}_i$, and a state S_i. The cell feels a repulsive potential V_{ij} from every other cell j of the form $V_{ij}(d_{ij}) = \frac{\tilde{k}}{2}\left(\frac{d_{ij}-r_i-r_j}{r_i+r_j}\right)^2$ if $d_{ij} \leq r_i + r_j$ [1]. Here $\vec{d_{ij}} = \vec{x_i} - \vec{x_j}$ and $d_{ij} = ||\vec{d}_{ij}||$ is the euclidean distance between the cells. $\tilde{k}$ parametrizes the repulsion strength. The potential is set to be zero when $d_{ij} > r_i + r_j$. The motion of cells is computed in the over-damped limit, $\dot{\vec{x}}_i = -\frac{1}{\mu}\sum_{j\neq i}\frac{\mathrm{d}}{\mathrm{d}d_{ij}}V_{ij}\vec{\hat{d}}_{ij}$, where $\vec{\hat{d}}_{ij}$ is the unit vector parallel to $\vec{d}_{ij}$ and μ is a viscosity parameter. This viscosity and the repulsion strength $\tilde{k}$ can be absorbed together to a single variable k.

Cell growth follows a Monod growth, $\dot{r_i} = \frac{g(n)}{3}r_i$, where the nutrient dependent growth rate $g(n) = g_{\max}\frac{n}{n+K}$. The maximal growth rate $g_{\max}$ is taken to be $\frac{1}{30\ \mathrm{min}}$ which corresponds to a doubling time of approximately 20 minutes. The Michelis-Menten constant was chosen to be $K = n(t=0)/5$.

Nutrient depletion

For simplicity we only allow uninfected cells to grow and consume nutrients. We further assume that nutrients diffuse infinitely fast and not to be hindered by the occupation of the cells. Hence there is no spatial dependency in the growth rate. We confirmed that the relevant range of colony sizes were small enough that the simulation with finite diffusion rate of nutrient with realistic parameters did not deviate noticeably from our infinite diffusion simulation. The depletion of nutrients takes the form $\dot{n} = -\frac{g(n)}{V}\sum_i \delta_{S_i,0}$, where state variable $S_i = 0$ denotes that the cell i is uninfected by phage, δ is the Kronecker delta function, and V is the volume of the system.

Cell Division

Each cell grows until it reaches the volume 2 μm^3 (or $R_d = 0.782$ μm) where it divides. The choice for R_d is such that the median cell volume in the simulation is 1.3 μm^3. During division, the parent cell p is removed and the two daughter cells are placed close to the center of the parent cell with some randomness $x_i^{(j)} = x_p^{(j)} + \zeta$, where $i = (1,2)$ refer to the daughter cells, (j) denotes the j'th component of the position $\vec{x}$ and ζ is a uniformly distributed number in $[-\frac{R_d}{4}, \frac{R_d}{4}]$. One daughter cell receives the fraction $\alpha \sim 0.5$ of the parents volume, while the other receives the remaining fraction: $r_1 = r_p\alpha^{\frac{1}{3}}$ and $r_2 = r_p(1-\alpha)^{\frac{1}{3}}$. Here noise is introduced through the random number α that is drawn from the Normal distribution with average 0.5 and standard deviation 0.1.

Phages

The phages are treated as individual point particles described with positions $\vec{y}$. The phages diffuse in the simulation volume, following the over-damped Langevin equation: $\dot{y}_k^{(j)} = \vec{F}_k(y_k^{(j)}(t)) + \xi(t)$, where $y_k^{(j)}$ is the jth-component of the position of the k'th phage; $\xi(t)$ is a noise term with a Gaussian probability distribution: $\langle\xi(t)\rangle = 0$ and $\langle\xi(t), \xi(t')\rangle = 2D_p\delta(t-t')$. There is no phage-phage interaction and when there is no cell-phage collisions the force term vanishes $\vec{F}_k = 0$. However, when a phage collides with a cell it is exposed to a repulsive force $V_k(d_{ki}) = \frac{\tilde{k}}{2}\left(\frac{d_{ki}-r_i}{r_i}\right)^2$ if $d_{ki} \leq r_i$, where d_{ki} is the euclidean distance between the k'th phage and the i'th cell with radius r_i. Furthermore, we include phage decay at a rate δ. The simulation was done in a cube of volume V with a reflective boundary condition for phages.

Cell-Phage interaction

Collision between phage k and cell i is defined as $d_{ki} \leq r_i$. The infection of the i'th cell by the k'th phage occur at a rate γ as long as the phage is within the cell radius. When the cell is infected by the phage, the state S_i is set to $S_i = 1$ if the cell was previously in the uninfected state ($S_i = 0$), otherwise the state is unchanged. To take into account the time delay before cell lysis, we use a ten-step Poisson process. It start when a cell is first infected and we increase the cell state S_i at a rate $10 \cdot r$ until the state is $S_i = 11$, where lysis occurs and β new phages are spawned uniformly around the cell's center and the cell is removed. $\gamma = \infty$ describes the diffusion limited adsorption of phage. Various phage adsorption rate η in Fig. 3D in main text was simulated by changing γ, where η for a given γ was measured by simulating adsorption to a single cell in a limited volume.

Simulation initialization

The simulation starts with a single cell of volume 1.3 μm^3, which is allowed to establish a colony for T_i time, after which the phages are added. At time T_i, the geometric center of the colony is computed $x_{gc}^{(j)} = \frac{1}{N}\sum_i x_i^{(j)}$, where N is the number of cells at time T_i. The maximal distance of any cell to the geometric center $r_{\max} = \max_i ||\vec{x_i} - \vec{x_{gc}}||$ is computed. Then $P_0 \cdot V$ phages as then spawned uniformly in the simulation space outside the sphere defined by position $\vec{x_{gc}}$ and radius $r_{\max}$.

Time Integration

The model was implemented using the explicit Euler method with a time-step of Δt.

1.2 Simulation parameters

Table S 1: Summary of the default parameters. These values are used unless otherwise mentioned. The values are chosen to be applicable for phage P1 parameters when available.

Name	Value	Units	Description	Comments / References
ΔT	10^{-6}	h	Size of the time step	
L_{box}	65	μm	Side length of simulation volume	
n_0	0.5	$1/\mu$m^3	Initial concentration of nutrient	
g_{max}	2	1/h	Maximal growth rate for the cells	[2]
K	$n_0/5$	$1/\mu$m^3	Michaels-Menten constant for Monod growth	[2]
R_d	0.782	μm	The length scale for division	
k	10^3	μm^2/h	Parameter for cell-cell interaction potential	
ν	$R_d/4$	μm	Size of displacement noise	
P_0	0.01	$1/\mu$m^3	Density of invading phage	
γ	∞ [1]	1/h	Phage infection rate for diffusion limited case	
	10^5 to 10^6	1/h	to reduce the phage adsorption rate	
β	400		Burst size	[3]
δ	0.003	1/h	Rate of phage decay	[3]
$\tilde{r}$	0.6		Constant for lysis latency time	[3]
D_P	$1.3 \cdot 10^4$	μm^2/h	Diffusion constant for the phage	[2]
D_n	$4 \cdot 10^5$	μm^2/h	Diffusion constant for the nutrient	[2]
				Used in supplement

2 Comparison with explicit modeling of nutrient diffusion and local consumption in a microcolony growth

We investigate the accuracy of using the meanfield approximation for nutrient depletion:

$$\dot{n} = -\frac{g(n)}{V}\sum_i \delta_{S_i,0}, \tag{S1}$$

and compare it to the complete field computation. For the complete field computation, we construct a cubic grid for the space: $x_i = i\Delta_{\text{grid}}$, $y_j = j\Delta_{\text{grid}}$ and $z_k = k\Delta_{\text{grid}}$, and divide the time into the discrete points: $t_l = l\Delta T$. For this grid, we define the density of susceptible cells $S(i,j,k,t_l)$, by assigning each cell to the closest grid point. The complete field computation has the continuous form:

$$\dot{n} = -g(n(\vec{x},t)) \cdot S(\vec{x},t) + D_n \nabla^2 n(\vec{x},t). \tag{S2}$$

In our discretized scheme the field equation becomes:

$$\frac{n_{i,j,k}^{l+1} - n_{i,j,k}^{l}}{\Delta T} = -g(n_{i,j,k}^{l}) \cdot S_{i,j,k}^{l} + D_n \hat{\tilde{L}} n_{i,j,k}^{l}, \tag{S3}$$

Where the Laplace-operator $\hat{\tilde{L}}$ computes the diffusion on the grid. Using the central difference the explicit computation becomes:

$$D_n \hat{\tilde{L}} n_{i,j,k}^{l} = \frac{D_n}{\Delta_{\text{grid}}^2}(n_{i-1,j,k}^{l} + n_{i+1,j,k}^{l} + n_{i,j-1,k}^{l} + n_{i,j+1,k}^{l} + n_{i,j,k-1}^{l} + n_{i,j,k+1}^{l} - 6n_{i,j,k}^{l}). \tag{S4}$$

We test the difference between the full field computation and the meanfield computation using the parameters listed in table 1 with two exceptions: $L_{\text{box}} = 50$ μm and $n_0 = 0.1$ μm^{-3}. We observe only minute differences

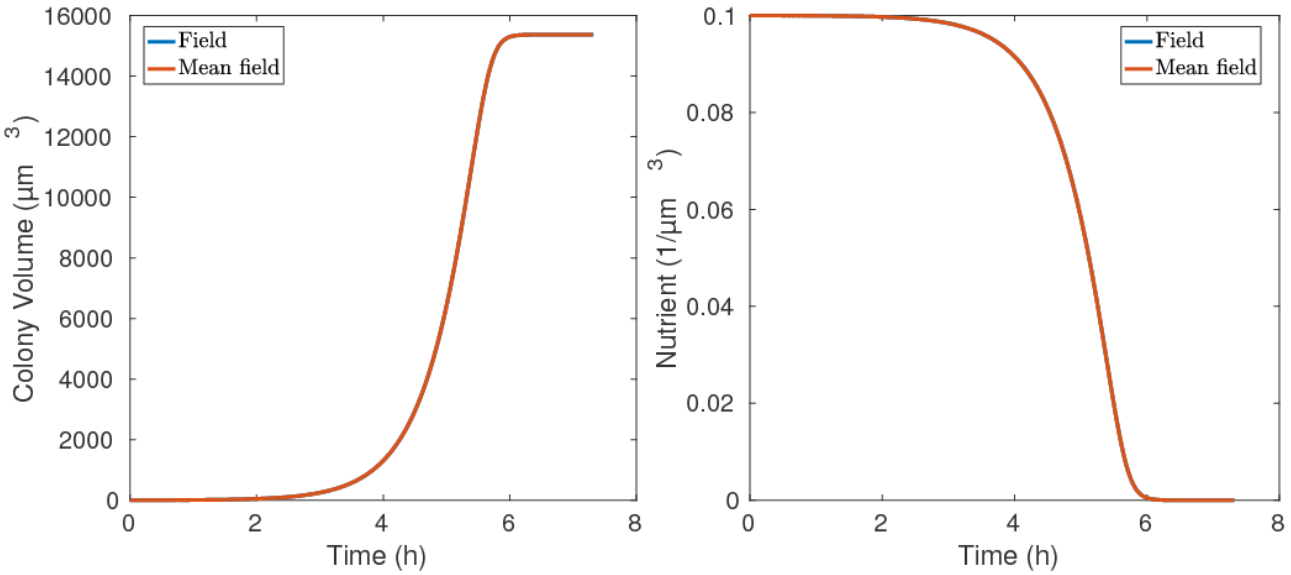

Figure S 1: Difference between the full field computation (blue) and the meanfield approximation (red). **Left**. The volume of the colony as a function of time. **Right**. The density of nutrient in the simulation.

between the methods on this scale, but we expect the impact to increase as the total colony volume increases.

3 Modeling colony growth under phage attack

3.1 Model equation and solution

We model the bacterial microcolony as a solid sphere consisting of an inner core of susceptible cells and a thick outer shell of depth ΔR of infected cells. The model system is illustrated in Fig. S2.

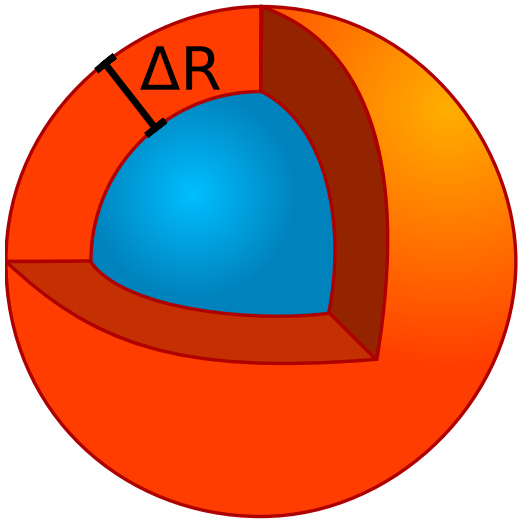

Figure S 2: The model system: The cell colony is considered a solid sphere, where the susceptible cells are located in the core (blue), and the infected cells are located as a thick shell (orange) of depth ΔR around the core.

The dynamics of the microcolony can be described by the volume of the core V_O and of the shell $V_{\Delta R}$.

$$\frac{\mathrm{d}V}{\mathrm{d}t} = \frac{\mathrm{d}V_O}{\mathrm{d}t} + \frac{\mathrm{d}V_{\Delta R}}{\mathrm{d}t}$$

The cells are assumed to be in the exponential growth phase and thus the core of uninfected grows at a rate g and the infected shell is diminished at a rate $rg = 1/\tau_l$ with τ_l being the latency time of phage burst, and thus

assumes that the phage lysis is dependent on cell growth, which is the case for phage P1.

$$\frac{\mathrm{d}V}{\mathrm{d}t} = gV_O - rgV_{\Delta R} \tag{S5}$$

This expression can be rewritten in terms of the colony radius R.

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \frac{g}{3}\left[(r+1)\frac{(R-\Delta R)^3}{R^2} - rR\right]$$

This equation allows for stable solutions as long as the growth term is larger than the decay term. There is as such a critical radius R_C, where the derivative is zero:

$$(r+1)(R_C - \Delta R)^3 - rR_C^3 = 0 \tag{S6}$$

The solution of this equation linearized with ΔR is presented in the main text eq. (2). The full solution of eq. (S6) is given by:

$$R_C = \Delta R\left(r^{\frac{1}{3}}(r+1)^{\frac{2}{3}} + r^{\frac{2}{3}}(r+1)^{\frac{1}{3}} + r + 1\right). \tag{S7}$$

Other than predicting the threshold value of the colony size, (S5) can also be solved by using a few approximations, and then be compared to the volumetric growth curves of the colonies. We expand the first term in equation (S5) :

$$\frac{(R-\Delta R)^3}{R^2} = R - 3\Delta R + \frac{3\Delta R^2}{R} - \frac{\Delta R^3}{R^2}$$

and consider solutions of order ΔR, where the equations simplifies to:

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \frac{g}{3}\left[(r+1)\left(R - 3\Delta R\right) - rR\right]$$

The differential equation is a simple first order linear differential equation and can be solved:

$$R(t) = R_0 \cdot \exp\left(\frac{gt}{3}\right) + 3\Delta R \cdot (r+1) \tag{S8}$$

With R_0 as an integration constant, where the radius at time zero is given (S8) by $R(0) = R_0 + 3\Delta R \cdot (r+1)$.

3.2 Growth rate fitting

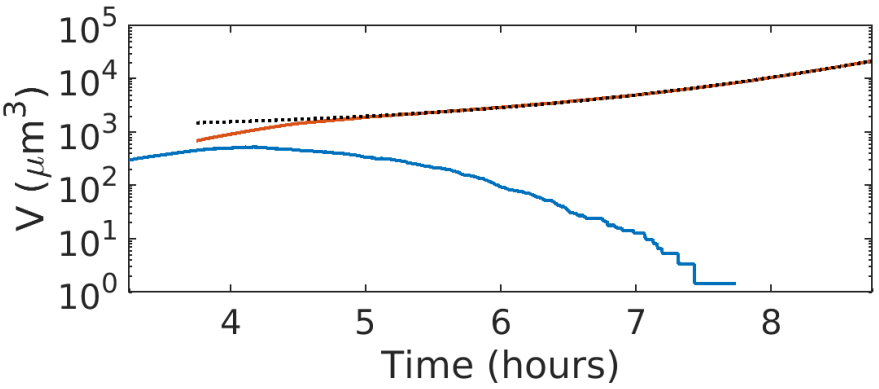

Figure S 3: Example of time traces of the colony volume. The blue line is a time-trace from a colony which was just below the survival threshold when the phage attack began. Since the colony is exterminated, a growth rate of zero is assigned. The red line is an example of colony which was just above the survival threshold. The growth rate is determined by fitting of (S8) (black line)

From simulation time-traces, the growth-rate of the colony is fitted to (S8), with R_0 and g being the fitting parameters (Fig. S3 red). When colonies are completely eradicated and those are assigned a growth rate of 0 (Fig. S3 blue).

4 Summary of experiments with phage infection of microcolonies at different soft-agar depths

In this section, we summarize the result of two additional repeats of the experiment shown in Fig. 5 in main text (we call them the repeat experiment 1 and the repeat experiment 2). The general protocol is given in the method section in the main text, with the exception that 3ml of top agar per plate was used instead of 2.5ml, and that 250 μl of phage lysate was sprayed onto the plates instead of 500 μl. The phage lysates used in the repeat experiment 1 and 2 had concentrations of 4×10^{10} PFU/ml and 3.5×10^{10} PFU/ml, respectively. The bacterial culture was diluted to $\sim$ 600 CFU/ml and 150μl was added per plate in the repeat experiment 1, while in the repeat experiment 2, the cell culture was diluted to $\sim$ 300 CFU/ml and 250μl was added per plate. In addition, in repeat experiment 1, we only prepared one plate for phage spray and one plate for the no-phage control per time point, and the control plates were also used for microscope imaging during the first 8 hours, which means that they were moved from 37C to room temperature several times during the incubation time.

In both of the repeat experiments, the colony size development was consistent with exponential growth in the first 9 hours, with the fitted doubling time being 29 min and 26 min in the test experiment 1 and the test experiment 2, respectively (Fig. S4).

The fraction of visible colonies as a function of pre-incubation time before phage spray is shown in Fig. S5. We see the clear increase of the survival fraction at 6-7 hours, consistent with the result presented in the main text. One notable difference is that in the test experiment 2, a small number of surviving colonies were observed at all time points, even for the plate sprayed with phage at the 0-hour time point. We however noticed that the colonies in the early time-points (especially up to 4 hours) were clustered at one side of the plate, and that the top-agar layer in some cases was noticably thicker at that side of the plate. This suggests that the thicker layer of top-agar provided protection by substantially delaying phage arrival.

Finally Fig. S6 shows the estimate of the radius of final colonies, with and without exposure to phage. The sizable reduction in size as a consequence of phage exposure is seen in both repeat experiments. In repeat experiment 1, the smaller radius from the fluorescence image than from the dark-field image of phage-exposed colonies is evident, suggesting that the surface layer is dead (not producing GFP). In repeat experiment 2, the difference is less pronounced, likely due to additional protection by the thicker top agar.

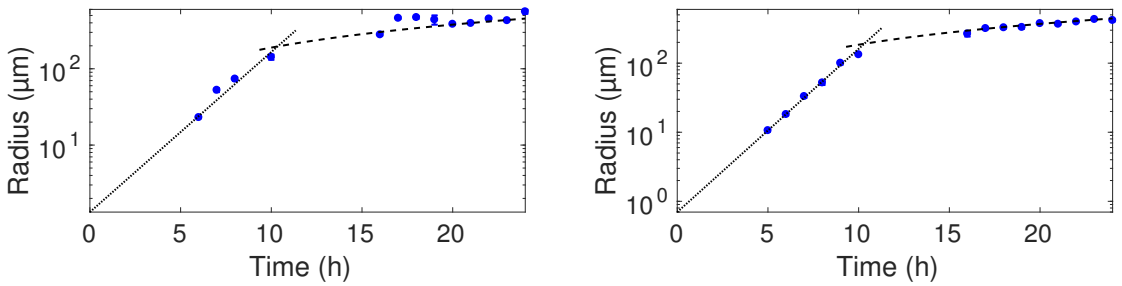

Figure S 4: Growth curves for repeat experiment 1 (left) and repeat experiment 2 (right). The fitted doubling time is 28.7 $\pm$ 1.2 min and 25.5 $\pm$ 0.3 min for the repeat experiment 1 and 2, respectively.

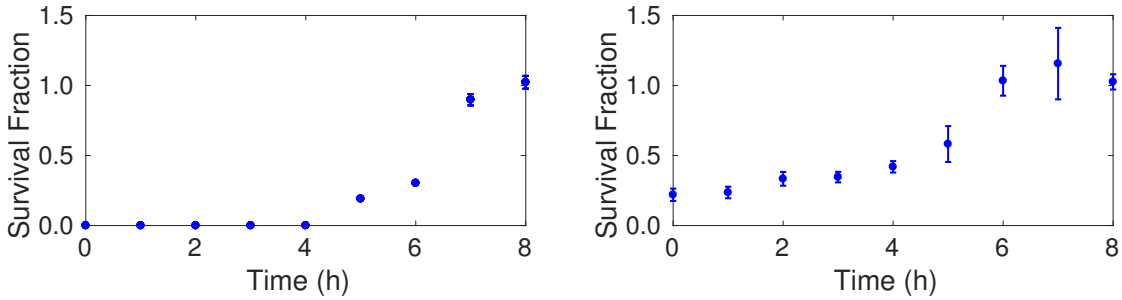

Figure S 5: Survival fractions from repeat experiment 1 (left) and repeat experiment 2 (right). The average number of colonies per control plate was 89 $\pm$ 4 and 82 $\pm$ 4 for repeat experiments 1 and 2, respectively.

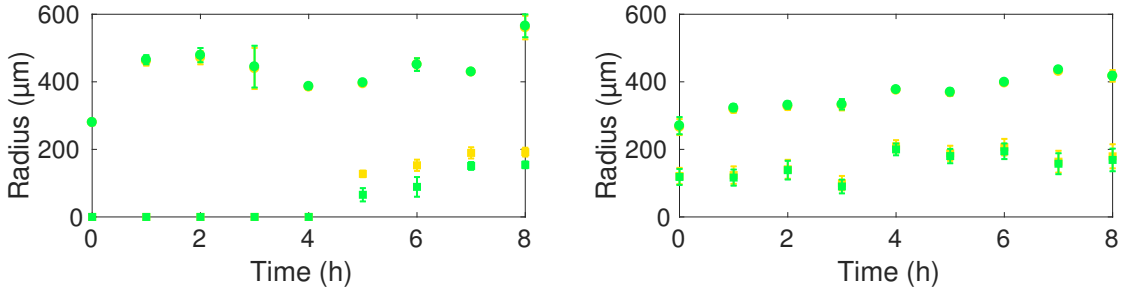

Figure S 6: Radii of the final colonies from repeat experiment 1 (left) and repeat experiment 2 (right). The horizontal axis shows the total incubation time. The circles show the final colony size in the absence of phage-exposure, while the squares show the radii of colonies exposed to phages. The time at which phages were introduced is given on the horizontal axis. Yellow symbols show the radii estimated from dark-field images, while green symbols show the radii estimated from green fluorescence images.

5 Detailed result of the $P1_{vir}$ resistance test

The table S2 summarizes the detailed results of the $P1_{vir}$ resistance test. As described in the main text, we picked 10 colonies from each phage-sprayed plate that had been pre-incubated for at least 4 hours (shown in the first column), and streaked the colonies on fresh plates containing sodium citrate to inactivate free phage and thereby permit the growth of both sensitive and resistant cells from the original colony. The 10 colonies from each plate are numbered in the second column (Parent colony id #). The third column (# colonies tested) lists the number of new colonies formed from each parental colony that were tested for phage resistance. When the number is less than 10, it equals the number of colonies that grew on the fresh plate. When more than 10 colonies grew, we randomly picked 10 for cross-streaking. The fourth column (Resistant), lists the number of colonies that tested $P1_{vir}$ resistant by cross-streaking, while the fifth column (Susceptible) lists the number of colonies that were found to be susceptible to $P1_{vir}$. If any colonies were found to be resistant then the original colony is listed as resistan in Table 1 in the main text. The sum of the fourth and fifth columns should equal the number in the third column.

Table S 2: Full results of the $P1_{vir}$ resistance test.

Time	Parent colony id #	# colonies tested (When less than 10, equal to number of recovered colonies)	Resistant	Susceptible
4h	1	0	-	-
	2	10	3	7
	3	10	0	10
	4	10	0	10
	5	10	0	10
	6	0	-	-
	7	0	-	-
	8	0	-	-
	9	10	10	0
	10	10	0	10
5h	1	0	-	-
	2	1	0	1
	3	10	10	0
	4	0	-	-
	5	0	-	-
	6	0	-	-
	7	0	-	-
	8	10	0	10
	9	1	0	1
	10	10	0	10

6h	1	0	-	-
	2	10	0	10
	3	10	10	0
	4	0	-	-
	5	10	10	0
	6	10	0	10
	7	10	10	0
	8	10	10	0
	9	10	10	0
	10	0	-	-
7h	1	10	10	0
	2	6	5	1
	3	0	-	-
	4	10	10	0
	5	10	0	10
	6	10	0	10
	7	10	0	10
	8	10	10	0
	9	10	10	0
	10	10	0	10
8h	1	10	0	10
	2	10	0	10
	3	10	10	0
	4	10	10	0
	5	10	10	0
	6	10	0	10
	7	10	9	1
	8	10	0	10
	9	10	10	0
	10	10	0	10

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