Supplemental Information: Entropy-driven motility of
Sinorhizobium meliloti on a semi-solid surface

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1 Supplemental Movies

The experimental apparatus for time-lapse fluorescence imaging of spreading S. meliloti was described in [1]. The agar plate with the spreading S. meliloti was imaged through a $2\times$ achromatic lens (MAP1075150-A, ThorLabs) and (for fluorescence) a GFP emission filter (MF525-39, ThorLabs) onto a cooled, 1300 $\times$ 1030 CCD array (Princeton Instruments). Images were collected at a rate of 0.001 Hz for (typically) 70 h.

We used a white-light imaging system to measure the effect of agar concentration on the rate of (purified) EPSII spreading. Here the EPSII concentrate was deposited onto an agar slab and imaged under white LED illumination by an $f = 8$ mm lens onto a $1024 \times 768$, CCD array (Imaging Source) at 0.004 Hz for 12 h.

- Movie SM1 shows the complete time lapse series of fluorescence images for a colony of S. meliloti strain Rm8530 spreading on semi-solid agar (0.4 % (w/v) agar / 5% (w/v) TY) (Figure 1 of main text). The migration of actively-dividing clusters toward the outer boundary of the spreading mucoid layer is apparent at $t \approx 8h$. The slight off-center position of the spreading disk relative to the initial droplet is due to imperfect leveling of the agar surface. (In some movies below this same effect introduces a slight curvature or twist in the radial spreading patterns.)

- Movie SM2 consists of fluorescence images of the spreading and aggregation of gfp-labeled S. meliloti Rm1021 on 0.4 % (w/v) agar / 5% (w/v) TY, driven by addition of the washed, concentrated HMW EPS II fraction.

- Movie SM3 consists of fluorescence images of the spreading and aggregation of gfp-labeled MT102 E. coli on 0.4 % (w/v) agar / 5% (w/v) TY, driven by addition of the washed, concentrated HMW EPS II fraction. Movie SM7 shows the control experiment using the same strain with no added EPSII.

- Movies SM4 and SM5 are additional time lapse fluorescence images (like SM1) of S. meliloti Rm8530 spreading on 0.4 % (w/v) agar / 5% (w/v) TY.

- Movie SM6 consists of time lapse fluorescence images (like SM1) of S. meliloti Rm8530 +PwggR-gfp spreading on 0.4 % (w/v) agar / 5% (w/v) TY.
2 Osmotic equilibration model

Here we show that the equilibration of the osmotic gradient between the agar and the EPS II-rich bacterial colony can generate exponential relaxation kinetics (Eqns. 1-2, main text) in the colony area $A$, as seen in the experimental data of Figure 1(b) (main text). We consider a droplet of bacterial culture that is inoculated onto a horizontal agar surface. The droplet produces by time $t_0$ a mass $M$ of a high molecular weight osmolyte (i.e. EPS II) that cannot readily diffuse into the agar. We expect that as the osmolyte begins to absorb water from the agar a mucoid disk or puddle develops, grower thicker (deeper) until it reaches a certain depth $h$ (in the $z$-direction) where it can begin to spread outward. Subsequently it continues to grow by expanding outward in radius, while its depth does not change. (For simplicity we ignore the earliest stage in which the depth $h$ of the mucoid first begins to grow, before significant radial spreading has occured. The fluid dynamics of such an early phase in a spreading biofilm are studied by Seminara et al. [2].)

Therefore we model the mucoid puddle as a flattened cylindrical volume centered at $r = 0$, with a time-dependent radius $R(t)$ and constant, uniform depth $h \ll R$. We assume that the water activity is reasonably constant within the mucoid layer - i.e. that the EPS II concentration is independent of position $r$. Then the mucoid layer and agar are dilute solutions with molar concentrations $n$ and $n_{\text{agar}}$ and osmotic pressures $\Pi = nRT$ and $\Pi_0 = n_{\text{agar}}RT$ respectively [3]. By Darcy’s Law the flux of water across unit area of the agar/mucoid interface is proportional to the net osmotic pressure difference:

$$v_z = \frac{\kappa}{\mu L} (\Pi - \Pi_0) = \frac{\kappa RT}{\mu L} (n - n_{\text{agar}}).$$

(1)

Here $v_z$ is the speed of water flowing upward into the mucoid disk, $\kappa$ is the Darcy permeability of the agar, $\mu \simeq 10^{-3}$ Pa s is the dynamic viscosity of water, $RT \simeq 2480$ J/mol, $L$ is a characteristic length scale for the osmotic pressure gradient (such as the thickness of the agar base). When the disk has area $A = \pi R^2$ the molar concentrations of osmolyte in the mucoid layer ($n$) and agar ($n_{\text{agar}}$) are

$$n = \frac{M}{mw A h}$$

(2)

$$n_{\text{agar}} = \frac{C_a}{mw_a}$$

(3)

where $mw$ and $mw_a$ are the molecular weight of the EPS II and agar respectively and $C_a$ is the concentration (mass/volume) of the agar. The final, equilibrated area $A_\infty$ of the mucoid is characterized by equal values of these two concentrations:

$$\frac{C_a}{mw_a} = \frac{M}{mw A_\infty h}.$$  

(4)

Eqn. 1 can then be written

$$v_z = \frac{\kappa RT}{\mu L} \frac{M}{mw A h} \left(1 - \frac{A}{A_\infty}\right).$$

(5)

Because of the upward fluid flux into the mucoid layer, the conservation of flow

$$\nabla \cdot \mathbf{v} = 0$$

(6)

requires a divergence in the radial component $v_r$ of the fluid velocity $\mathbf{v}$ in the mucoid layer, sufficient to match the influx of water from the agar:

$$\frac{1}{r} \frac{d(v_r r)}{dr} h = v_z.$$  

(7)

Integrating this expression, subject to the condition that $v_r$ is finite at $r = 0$, gives

$$v_r = \frac{v_z r}{2h}.$$  

(8)
We use Eqn. 8 for the advective fluid velocity in the diffusive simulations of the spreading colony.) Then the disk radius expands at speed $\frac{dR}{dt} = v_r(R)$, or
\[
\frac{dR}{dt} = \frac{v_r R}{2h}
\] (9)
and the disk area $A = \pi R^2$ expands as
\[
\frac{dA}{dt} = \frac{v_r A}{h}
\] (10)
or, using Eqn. 5,
\[
\frac{dA}{dt} = \frac{\kappa RT}{\mu L h^2 m w} \left( 1 - \frac{A}{A_\infty} \right)
\] (11)
We can define the constant $k$ as
\[
k = \frac{\kappa RT}{\mu L h m w_a}
\] (12)
and obtain Eqn. 1 of the main text,
\[
\frac{dA}{dt} = k(A_\infty - A).
\] (13)
Its solution is an exponential relaxation $A \to A_\infty$ in which $k$ is the relaxation rate, with units of $h^{-1}$.

Note that $k$ depends linearly on the agar concentration $C_a$, while the equilibrated area $A_\infty$
\[
A_\infty = \frac{M m w_a}{C_a h m w}
\] (14)
varying inversely with $C_a$. However $k$ is independent of the mass $M$ of EPS II present in the mucoid layer. These predictions are consistent with the data of Figure 3 in the main text.

Estimating the magnitude of $k$ requires some assumptions about the parameters $\kappa$ and $L$ appearing in Darcy’s Law. A literature value $\kappa \simeq 600$ nm$^2$ is available for 2% agarose, although $\kappa$ was found to be very sensitive to agar concentration [4]. If we assume that $\kappa$ scales roughly as the inverse of the agar concentration, we can estimate $\kappa \sim 3000$ nm$^2$ for 0.4% agar. Using that $\kappa$ with $L \sim 3$ mm and $h \sim 1$ mm, and $m w_a \sim 10^5$ g/mol for the agar molecular weight, we have $k \sim 1 \times 10^{-4}$s$^{-1} \simeq 0.3$ h$^{-1}$. This is roughly 3 – 6 times larger than the $k$ we measure for sinorhizobial colonies on agar, which is satisfactory numerical agreement considering that $L$, $m w_a$ and $\kappa$ are very crudely estimated here.

### 3 Simulations of colony spreading

To test our understanding of spreading and pattern formation in $S. meliloti$ colonies we used MATLAB (Mathworks Inc.) to simulate patterns of cell dispersion in a suspension of bacteria subjected to the physical forces of a spreading radial flow (advection), where clusters of cells are located on the perimeter of the colony. We model the fluid as a flattened disk lying atop the $xy$ plane (the agar surface), with outer radius $R(t)$ and uniform depth $h$, and centered at $r = 0$. The disk expands radially outward due to the osmotically-driven radial fluid velocity $v_r(R)$, leading to exponential relaxation kinetics in the growth of the disk area $A(t)$, as described in Eqns. 8 and 13 above.

As we can envision several possible mechanisms that may cause cells to move within the fluid and/or relative to the agar, our simulations are aimed at understanding which mechanisms can generate patterns of cells and aggregates similar to those of Figure 1 of the main text. We explored through simulation the patterns generated by the following mechanisms: (a) Clusters of cells are forced to the perimeter (i.e. to $r = R$) of the mucoid disk (e.g. by the depletion force, Discussion) and are therefore swept outward at speed $v_r(R)$ with the expanding mucoid fluid; (b) The clusters shed daughter cells into the disk area (i.e. $r \leq R$). In one set of simulations these daughter cells diffuse through the fluid with a diffusion coefficient $D$ while also being transported by the fluid at speed $v_r$ (advection). In another set of simulations the daughter cells within the disk area move by actively swimming in random directions at constant speed $v_{swim}$. 

3
To capture the behavior observed in numerous experimental trials, a simulation begins with 20 - 30 clusters of cells, each located on the perimeter of the mucoid disk, and each containing a very large number (e.g. $\geq 10^2 - 10^3$) of individual, replicating cells. During spreading each cluster sheds daughter cells at a rapid rate ($\simeq 10^2 - 10^3$ daughter cells per hour). Our experimental data indicate that few of these daughter cells subsequently divide, and that although they may remain mobile in the fluid for a few hours they eventually settle out of the fluid layer. Therefore in our simulations the clusters at the perimeter (at $r = R$) shed daughter cells into the interior of the mucoid layer (i.e. to $r < R$). These daughter cells remain mobile (either by swimming or diffusion) for a period of $\sim 2 - 4$ h and then become immobilized. The simulations do not model the aggregation kinetics of the daughter cells.

In simulations where the daughter cells diffuse (rather than swim) through the mucoid, we estimate their diffusion coefficient from the Einstein-Stokes relation $D = \frac{6\pi k_B T}{\eta a}$, where $k_B$ is Boltzmann’s constant, $T$ is absolute temperature, $\eta$ is the dynamic viscosity of the mucoid fluid, and $a \sim 1$ $\mu$m is the radius of a single cell. In the case where daughter cells swim, the simulation assigns each new daughter cell a velocity $v_{\text{swim}}$ (typically $0.01$ $\mu$m/sec) in a random direction.

The simulations in Figure 3(b)-(c) of the main text use parameters obtained from the experimental data shown in panel (a) of that figure: $\sim 32$ actively dividing cell clusters located around the mucoid perimeter, disk radius $R$ spreads from 2.8 to 4.6 mm, spreading rate $k = 0.046$ h$^{-1}$, each cluster sheds $\simeq 360$ cells/s, and daughter cells remain mobile for 11 h before settling.

Although both of the simulations shown in Figure 3(b)-(c) of the main text capture the dispersal of the cells in a radial pattern, the swimming model (main text Figure 3(b)) more closely resembles the experimental data. Diffusive motion of the daughter cells allows the spreading fluid to carry the cells radially outward until the cells settle to the agar surface. Therefore the daughter cells are not found far from the fluid perimeter. Furthermore, as diffusion is inefficient on long length scales, the daughter cells do not travel far in the azimuthal direction (transverse to the radial expansion). Consequently the clusters in the diffusive simulation (main text Figure 3(c)) deposit short, narrow trails of cells on the agar. By contrast, the swimming motility simulated in main text Figure 3(b) captures the key features of the data: Near the disk perimeter the daughter cells swim outward from the source clusters, streaming away from each cluster in a conical pattern. Active swimming allows the cells to explore a larger portion of the disk area before they settle onto the agar surface. This conical streaming pattern, which is especially apparent in the supplemental movies such as SM6, is characteristic of swimming (vs. diffusive) motion of daughter cells shed by a moving source: as with a wave front generated by a source traveling at constant speed, the half-angle $\alpha$ of the conical vertex is determined by the ratio of the swimming speed ($v_{\text{swim}}$) to the speed of the source clusters that move with the boundary. The observed angles suggest that the swimming speed is comparable to the speed of the mucoid boundary motion, $v_{\text{swim}} \sim kR \sim 10^{-5}$ mm/s, a slow velocity consistent with bacterial swimming in a highly viscous medium.

4 Rescue Experiments

We performed experiments to verify that adding washed, concentrated EPS II to non-spreading Rm1021 S. meliloti and MT102 E. coli could cause them to spread on semi-solid agar. We combined 10 $\mu$L of washed, concentrated EPS II with 3.5 $\mu$L of Rm1021 strain (expR- non-mucoid, non-spreading) or with MT102 E. coli, immediately prior to depositing the culture onto a 0.4% agar surface. Supplemental Figure 1(a) and movie SM2 show that not only did spreading occur but the Rm1021 colony exhibited dynamic streaming patterns at its outer perimeter, very similar to those seen in the Rm8530 (expR+) strain. Supplemental Figure 1(b) and movie SM3 show that the EPS II also activated similar spreading and aggregation behavior in E. coli.
Supplemental Figure 1: EPS II drives aggregation of cells and migration of clusters to the colony perimeter, in addition to colony spreading: (a) Fluorescence images showing a colony of Rm1021 (expR-) growing on semi-solid agar (0.4 % (w/v) agar / 5% (w/v) TY). The EPS II-deficient mutant does not produce aggregate or spread on soft agar; (b) Addition of concentrated EPS II induces both spreading and aggregation in strain Rm1021 (expR-) on semi-solid agar. Thirty hours after the addition of washed, concentrated HMW EPS II solution, clusters of actively dividing cells have formed and migrated to the perimeter of the spreading mucoid layer. See also Supplemental movie SM2; (c) MT102 E. coli does not produce EPS II and does not spread or aggregate on soft agar; (d) Spreading and aggregation can be induced in MT102 E. coli on semi-solid agar by addition of washed, concentrated EPS II. See also Supplemental movie SM3.

5 Flagellar Mutant

Previous authors have noted that motility-deficient S. meliloti do form a spreading mucoid layer, although lacking the complex patterns [5]. The white light images of Supplemental Figure 2 show that the mucoid layer produced by the immotile fliP mutant spreads with the same exponential kinetics that are seen in the wild type strain. However, while the fliP cells disperse to some extent within the fluid, the perimeter of the fluid layer spreads more rapidly than the cells. The fliP colony lacks the actively dividing clusters of cells on the perimeter of the mucoid layer; rather the cells remain closer to the site of the starting droplet and eventually settle onto the agar surface. As in the depletion effect study by Schwarz-Linek et al. [6], we observe the same behavior with heat-killed cells as with the flagellum-deficient mutant: dead cells mixed with EPSII settle to the surface within a few hours. They do not associate into clusters within a mucoid droplet (data not shown). Therefore although flagella
are not absolutely required for spreading of the mucoid layer, cell motility is important to maintaining the actively dividing cell clusters at the moving perimeter of the mucoid layer.

Supplemental Figure 2: Spreading of fliP-deficient S. meliloti strain MG320 on 0.4 % (w/v) agar / 5% (w/v) TY. (a) Time lapse images collected in white light reveal that the cells do not spread to the same extent as the surrounding mucoid layer. Cells settle to the agar surface early during spreading, while the mucoid continues to expand. (b) The mucoid area $A(t)$ (blue, data) exhibits an exponential relaxation (red, fit) with $k = 0.021 \, h^{-1}$ following the lag period.

6 Image analysis

Study of the time lapse images suggests that cell division appears mostly limited to a few discrete source locations on the perimeter of the expanding mucoid disk. These outwardly moving sources shed daughter cells that settle onto the agar surface, generating the feathered pattern of the spread colony. To test whether cell growth occurs primarily on the perimeter of the colony we examined the time dependence of the GFP fluorescence per unit area during the spreading of the Rm8530 constitutive gfp-reporting strain. As shown in Supplemental Figure 3(a) we express the GFP fluorescence intensity (fluorescence counts per pixel) of an image of the colony in polar coordinates as $g(r, \theta, t)$, where $r$ is the distance from the disk center at $r = 0$, $\theta$ is the polar angle, and $t$ is time. Supplemental Figure 3(c) shows $g(r, \theta, t)$ vs $r$, with $g$ averaged over a small angular interval of about 10 degrees: A residual bright ring near $r \simeq 2.5$ mm is seen marking the initial radius of the colony, while an outward-moving discontinuity corresponds to the expanding boundary at $r = R(t)$. Otherwise however the fluorescence profile of the colony is relatively featureless and shows little time dependence indicative of active growth.

Cell growth and gfp-production can be mapped more specifically by examining the time derivative $\partial g/\partial t$, as shown in Supplemental Figure 3(d). There $\partial g/\partial t$ is much weaker in the interior of the colony than in a narrow zone located at the moving boundary, where an intense pulse of GFP-production occurs. The amplitude of this pulse fades at long time, indicating a loss of growth vigor as the mucoid disk reaches its final, equilibrated size.

These fluorescence profiles confirm that cell growth is most active in the narrow zone on the boundary of the spreading colony. Similarly the total, area-integrated fluorescence (Supplemental Figure 3(b)) of the spreading colony grows linearly with time (at $t > t_0$), consistent with linear - rather than exponential - growth in the number of live cells. Overall this image analysis indicates that only the small fraction of cells located at the air / agar / mucoid interface at the perimeter of the disk have sufficient access to nutrient to sustain active division. These form clusters or aggregates that each contain many individual cells, shedding daughter cells into the interior at a near constant rate, whereas these daughter cells generally do not further grow or divide.
Supplemental Figure 3: Cell growth is most active on the boundary of the spreading mucoid: (a) The GFP fluorescence per pixel $g(r, \theta, t)$ is obtained in polar coordinates. The image shows a spreading colony of the constitutive gfp-reporting Rm8530 strain with the polar coordinate system overlayed; (b) The total fluorescence intensity over the colony, summed over all area, approaches a straight line (red line), indicating linear growth in the number of cells in the colony; (c) The sum (denoted $F(r, t)$) of $g(r, \theta, t)$ over a small angular interval of about 10 degrees (dashed lines in (a)) is shown at several different times. Curves are offset vertically for clarity; (d) New GFP expression activity vs. $r$ and $t$, as revealed by $dF/dt$, is shown for several different times. Curves are offset vertically. The time derivative is small in the interior of the colony - indicating little if any GFP synthesis or growth - while an intense pulse of GFP-production is seen within a narrow region at the boundary of the spreading colony. This pulse corresponds to the actively growing clusters at the colony edge.
7 P\textit{wggR} expression during spreading

\textit{wggR} is a transcriptional activator for genes in the EPSII gene cluster of \textit{S. meliloti}. It is under the control of the SinI/SinR/ExpR quorum sensing system. To test whether EPSII may be synthesized during colony spreading, we imaged the spreading of cells containing a \textit{gfp}-reporter for the promoter P\textit{wggR}. A plasmid-borne P\textit{wggR}-\textit{gfp} reporter fusion was constructed by PCR amplification of the \textit{wggR} promoter from genomic DNA. The PCR fragments were cloned into the \textit{SpeI} site in front of a promoterless \textit{gfp} in the plasmid pRU1097. The resulting plasmid was sequenced to confirm the final construct (pGD3). The final construct was introduced into \textit{S. meliloti} by tri-parental conjugations as described in [7].

Supplemental Movie SM6 shows the time lapse images of the spreading and equilibration. Figure 4 shows that the equilibration closely follows the exponential relaxation behavior (Eqn. (2) - main text) for a period of \( \simeq 45 \) h, with a rate \( k \simeq 0.044 \) /h. Integrating the total fluorescence in a radial (wedge-shaped) region of the disk (as in Figure 3) gives the time dependence of the GFP fluorescence \( F \) of that region, while the time derivative \( dF/dt \) gives a crude measure of the activity of promoter P\textit{wggR}. Figure 4B indicates that P\textit{wggR} activity rises sharply during the first hours of colony growth, prior to the start of spreading at about 14 h. It reaches a peak at about 22 h, and then begins to decline as the colony spreads. The data imply that a burst of EPSII synthesis occurs in the early stages of colony spreading.
Supplemental Figure 4: A colony of Rm8530 strain carrying a P\textit{wggR-gfp} reporter spreads to near-equilibrium: (A) The spreading follows an exponential relaxation behavior until \( \sim 60 \) h, by which time the area growth and its accurate measurement are affected by drying of the agar, accumulation of minor asymmetries in the spreading disk, and other effects. (B) The total fluorescence \( F \) integrated over a small angular wedge of the colony of about 30 degrees shows total GFP synthesized, while (C) the time derivative \( dF/dt \) is a crude measure of the activity of P\textit{wggR}. The peak in activity (blue curve) near \( \sim 22 \) h suggests that P\textit{wggR} activity – and therefore EPSII synthesis - is strongest during a burst early in the spreading of the colony. By contrast, a constitutive reporter (green curve showing derivative \( dF/dt \) measured for the pDG71 \textit{S. meliloti} shown in movie SM5) remains active throughout the spreading phase.

References


