Rapid and directed group motility in B. subtilis does not rely on individual motility or chemotaxis — Source link ↗

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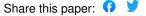
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1   **Physical factors contributing to regulation of bacterial surface motility**

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7   **Microbes routinely face the challenge of acquiring territory and resources on wet surfaces. Cells move**

8   **in large groups inside thin, surface-bound water layers, often achieving speeds of 30 μm/s within this**

9   **environment, where viscous forces dominate over inertial forces (low Reynolds number). The canonical**

10   **Gram-positive bacterium *Bacillus subtilis* is a model organism for the study of collective migration over**

11   **surfaces with groups exhibiting motility on length scales three orders of magnitude larger than**

12   **themselves within a few doubling times. Genetic and chemical studies clearly show that the secretion**

13   **of endogenous surfactants and availability of free surface water are required for this fast group motility.**

14   **Here we show that: (i) water availability is a sensitive control parameter modulating an abiotic**

15   **jamming-like transition that determines whether the group remains fluidized and therefore collectively**

16   **motile, (ii) groups self-organize into discrete layers as they travel, (iii) group motility does not require**

17   **proliferation, rather groups are pulled from the front, and (iv) flow within expanding groups is capable**

18   **of moving material from the parent colony into the expanding tip of a cellular dendrite with implications**

19   **for expansion into regions of varying nutrient content. Together, these findings illuminate the physical**

20   **structure of surface-motile groups and demonstrate that physical properties, like cellular packing**

21   **fraction and flow, regulate motion from the scale of individual cells up to length scales of centimeters.**

22   **Introduction**

23   In their search for resources microbes contend with physically distinct environments ranging from

24   soft surfaces to bulk Newtonian fluids and complex fluids like mucus. In bulk fluid environments, canonical

25   microbes like *Escherichia coli* (1) and *Bacillus subtilis* (2, 3) ascend favorable chemical gradients via run-

26   and-tumble chemotaxis (4). This mechanism of gradient ascent requires both flagellar-mediated motility

27   and a complex system of phosphorylation-memory and chemical sensors on the bacterial surface, that

28   together regulate the run-tumble transition frequency (5). In contrast, bacterial surface motility has

29   different requirements and inputs, and different species have evolved distinct modalities of surface

30   motion. For instance, in the predatory species *Myxococcus xanthus,* individual cells move in back-and-

31   forth motions that employ two distinct sets of protein machinery for 'twitching' and 'gliding' motility, and

32   cells assemble into larger motile groups that traverse surfaces as monolayers (6, 7). Many other species,

33   including the opportunistic pathogens *Serratia marcescens* (8, 9) and *Proteus mirabilis* (10, 11), also form

34   large groups of motile cells that are capable of rapidly expanding over surfaces, in some cases even against

35   bulk fluid flow (12). Similarly, when present in sufficient numbers *Paenibacillus dendritiformis* form

36   intricate fractal-like patterns on soft agar surfaces in response to lateral chemical gradients (13, 14). Even

37   baker's yeast have been observed to exhibit group movement over fluid surfaces (15). Other species

38   exhibit surface motility in response to non-chemical fields; for instance the cyanobacterium *Synechocystis*

39   is phototactic, responding to incident light by asymmetrically extending and retracting pili from its surface

40   to create a biased random walk toward a light source (16, 17). Crucial to its motion, *Synechocystis* modifies

41   the local surface environment by secreting exopolysaccharides, and only when enough cells have

42   participated in such surface modification can the group move toward the light source. These examples

43   demonstrate that in response to various gradients, microbes have evolved distinct sensing capabilities

44   and modalities of motion to acquire resources and respond to selective pressures on surfaces. Despite

45   their differences, surface motility in all of these species (and even in abiotic systems (18)) appears to be a

46   collective phenomenon, requiring the motion of and/or surface modification by large numbers of cells

47   (19, 20). Uncovering biochemical and genetic factors that regulate motion is crucial for understanding

48   how each species executes surface motility, but those factors are only part of the full picture. Physical

49   forces that produce, regulate, and guide microbial group motion on surfaces may be relevant across

50   species and contexts, and are thus integral to our understanding of microbial ecology in natural

51   environments and will expand the suite of design tools for engineering microbial systems.

52   Modeling plays an important role in these systems because it has the potential to connect

53   experimental observations with physical forces and regulators of motion (21–29). Different models posit

54   qualitatively distinct physical mechanisms for group motility, with basal assumptions motivated by

55   species-specific attributes. In *P. dendritiformis* growth and spreading of cells in dendritic patterns are

56   modeled as a diffusion-limited conversion of nutrients into biomass at the growing tips of the dendrite

57   (13, 30). Cellular motion is thought to rely on chemotaxis that follows nutrient gradients that are created

58   by metabolic consumption of the colonies themselves (22, 30, 31). Together these effects recapitulate

59   many of the classes of bacterial surface patterning observed in experiments (26, 32–36). However, within

60   a dense and active (e.g. swarming) group whose velocity correlations rapidly decay on the length scale of

61   a few cells (21), it is unclear how individuals could effectively modulate their tumble frequency and

62   accumulate sufficiently persistent runs to deliberately bias their random walks and hence execute run-

63   and-tumble chemotaxis. Indeed, previous work in *B. subtilis* (37–40) (and *Pseudomonas aeruginosa* (41),

64   *Salmonella enterica* (42)*, and *Escherichia coli* (43)) shows that neither chemotaxis nor motility of

65   individuals are necessary for rapid and outward-directed surface motility. Other models of surface motility

66   (e.g. swarming or sliding) in *B. subtilis* or *P. aeruginosa* focus on the role of surfactants (23, 44) and/or

67   osmotic potential (24), both of which appear to be crucial for rapid surface motility in those species (37,

68   45–47).

69   While cell density is clearly an important factor in motion, in so much as many cells are required

70   for collective movement, and genetics implicates endogenous surfactant production as a physical

71   requirement for collective motion, it remains unclear how density, material flow, and dendrite structure

72   contribute to regulation of this ubiquitous behavior. In this work we combined high resolution video and

73   time-lapse microscopy with computational image processing to clarify the relative contributions of

74   density, flow and structure by examining the surface motility of the extensively-studied Gram-positive

75   bacterium *B. subitilis* (5, 24, 48, 49). Within a few hours of deposition, a small central inoculum of wild-

76   type cells can rapidly colonize the entire surface of a wet 10 cm agar plate via apparent swarming motility

77   (50). Such group motility over soft surfaces has been shown to depend on the secretion of 'surfactin', a

78   bacterially produced surfactant and wetting agent (22, 23, 25, 37, 51–53). Functional knockouts for

79   surfactin production (Δ*srf*) result in a phenotype where individual cells are still motile and chemotactic in

80   bulk fluid, but bacterial groups cannot move across surfaces (37, 40, 54). Localized secretion of surfactin

81   is thought to generate a gradient in surface tension, and thus produce collective motion via the Marangoni

82   force (23, 55, 56).

83   Building on that biophysical picture, we found evidence that the movement of *B. subtilis* over soft

84   surfaces was regulated by cell density, whereby groups of cells are subject to a jamming-fluidization

85   transition (57) that correlates with packing fraction (and thus water availability). These results

86   complement findings that the viscosity of bacterial suspensions depends on cell concentration, shear rate,

87   and level of motility (58, 59). Further, we show that such groups operate in two distinct modalities of

88   motion: groups can move without growth as 'islands' that translocate independent of the parent colony,

89   or dendrites can extend with large-scale flow of material from the parent colony to the dendrite tip. We

90   used a chemotropic assay to show that when connected to a nutrient-rich parent colony, extending

91   dendrites can venture into nutrient-barren regions, while colonies that originate in nutrient-barren

92   regions are not collectively motile. These data suggest that, in addition to genetically regulated production

93   of surfactants, multiple physical and abiotic factors regulate motion independent of the chemotactic or

94   motile abilities of the constituent cells, and that groups move differently when presented with anisotropic

95   nutrient environments. When combined with previous work, these data suggest a model in which

96   individual motility and subsequent swarming are mechanisms that maintain a fluidized state on which

97   surface-tension gradient forces (Marangoni forces) and osmotically driven colony hydration can act to

98   precipitate group motion over a surface, and that a jamming-like transition, like those found in other

99   macroscopic granular systems (57, 60–65), may be a key regulator of whether bacterial groups are able

100 to move.

101

102 **Results**

103 *Bacillus subtilis* is a model motile Gram-positive bacterium, capable of sensing and responding to

104 its chemical environment in bulk fluid via run-and-tumble chemotaxis (2, 5, 66). On wet surfaces wild-type

105 *B. subtilis* rapidly move out from a central inoculum (67), apparently via collective swarming motility (50)

106 that requires secretion of the endogenous bio-surfactant 'surfactin'. Mutants that lack the ability to

107 produce surfactin (Δ*srf*) do not exhibit collective motility from their central inoculum (37) (and Movie S1).

108 We inoculated small, dense (OD ~ 10 - 20) droplets of wild-type *B. subtilis* on soft (~0.5% w/v agarose)

109 nutrient-rich surfaces. After a brief quiescent phase, groups of cells rapidly expanded over the surface in

110 a dendritic pattern that reached the edge of the plate (~5 cm of travel) in less than 6 hours (Movie S2).

111 Dendrites robustly moved outward away from the original point of inoculation into fresh territory at an

112 average group motility rate of ~5 μm/s and up to ~15 μm/s (SI Fig. 1). Cells within the dendrites were

113 highly motile, exhibiting swarming motility (37) with individual cells moving at rates up to ~30 μm/s in

114 highly circuitous paths. Consistent with previous work (37, 38), we confirmed that mutants lacking the

115 ability to run (tumble-only, Δ*CheY*), lacking the ability to tumble (run-only, Δ*CheB*), and mutants lacking

116 flagella (Δ*hag*) were all able to exhibit rapid surface motility, albeit with some differences in dendrite

117 speed and spreading pattern (SI Fig. 2, Movies S3 - S5).

118   In our early observations we noted transitions in the motile state of groups, wherein cells within

119 a *contiguous region* appeared to be either highly motile and moving as a group or immobilized on both

120 the individual and group levels. These distinct states of motion were frequently observed at the same

121 time, and cellular groups were observed transitioning between these states on the time scale of a minute,

122 too short to be accounted for by phenotypic changes.

123

124 **Movement is regulated by a jamming-like transition**

125   While surface motility of *B. subtilis* is robust to genetic manipulations of chemotaxis and motility,

126 it was very sensitive to gel stiffness as measured by agarose concentration (37). Below ~0.4% agarose by

127 weight the gel is sufficiently porous that bacteria can penetrate and swim within it, akin to canonical swim

128 plates (50, 68). Above ~0.7% agarose, limited water availability hinders surface motility (50), leading to

129 expansion across plates through the replication of sessile cells at the leading edge of growth, as seen in *P.*

130 *dentritiformis* (dendrite-like) or *E. coli* (growing circular colonies), both of which expand at much slower

131 rates (31, 69, 70). Thus, across a relatively narrow range of agarose percentages, groups of *B. subtilis*

132 exhibit three qualitatively distinct behaviors: swimming through agarose (below ~0.4%), rapid surface

133 motility (between ~0.4% and ~0.7%), and slow growth by replication (above ~0.7%). Why does the

134 transition from rapid surface motility to slow proliferative growth occur over such a narrow range of

135 agarose stiffness, and correspondingly, water availability?

136   Many previous studies of *B. subtilis* characterized spreading and attendant morphological

137 behaviors at the length-scale of colonies (mm to cm) and on the timescale of bacterial replication, imaging

138 colony morphology and spreading with minutes or hours between frames (26, 32, 37, 50). These excellent

139 studies revealed much of what is known about spreading phenotypes, their genetic mechanisms, and

140 biochemical correlates. We performed high temporal and spatial resolution imaging of spreading colonies

141 to illuminate processes that potentiate spreading. We captured images at 30 or 60 frames per second

142 with spatial resolution of 5 µm/pixel or 15 µm/pixel, respectively, both of which enabled us to see intensity

143 variations produced by the movements of cells within the swarm. We wrote a custom image analysis script

144 that measured a scalar correlate of motion as a function of position through time. Briefly, the algorithm

145 measures the mean absolute value of local intensity fluctuations at a position across a set of N (usually 5

146 – 7) frames and thus reports on the level of motile activity at each position through time (see Methods).

147 With this 'activity' filter, we were able to visualize on the scale of 10s to 100s of microns which parts of

148 the colony were actively motile and in which parts cells were stationary (Fig. 1A).

149   In wild-type cells, the rapid movement of dendrites and cellular groups was correlated with

150   significant increases in the quantitative measure of activity from our image analysis, indicating that

151   movement of individuals positively correlates with movement of the group. Conversely, regions whose

152   constituent cells had a low measure of activity were stationary and did not move or flow over the surface

153   (Fig. 1 and Movie S6). Frequently, stark boundaries in activity level formed within contiguous regions of

154   cells, indicating that within a genotypically and phenotypically identical population, sub-populations could

155   adopt two qualitatively distinct states of motion at the same time. We interpreted the high activity state

156   as a fluidized (low viscosity) state in which cells were swarming, and the low activity state as a 'jammed'

157   state where the network of contacts and forces between cells led to local immobility. We observed that

158   jammed regions could be fluidized if they came into contact with a fluidized region. Further, the phase

159   boundary between these regions actively fluctuated in time, translating fractions of a micron per second

160   (Fig. 1B), meaning that individual cells transitioned between motile and immotile states faster than

161   (potential) phenotypic changes in the local environment. Physical theories of jamming in granular

162   materials (61, 63, 64, 71) predict that the transition between jammed and fluidized states result from

163   small (technically infinitesimal) differences in packing fraction.

164   We took two approaches to assess the connection between packing fraction and motion. Phase

165   contrast video microscopy allowed us to visualize the cells as dark objects, while open spaces – even a

166   fraction of a cell's area – are relatively bright (SI Fig. 3A, Movie S7A). First, we reasoned that at suitably

167   high magnification (60X), the average intensity in a region is directly correlated with the amount of open

168   space (assuming the cells are in a monolayer, see next section of Results), or in other words, brighter

169   regions have lower packing fractions. We hypothesized that if packing fraction was controlling the

170   transition, there should be a positive correlation between local intensity and the local level of activity. We

171   averaged both the intensity and the activity over regions ~0.8 μm^2, and then calculated (i) the histogram

172   of spatially correlated values between those two measurements, (ii) the mean local intensity vs. the local

173   activity, and (iii) the bivariate correlation coefficient (SI Fig. 3B). The mean intensity values increased

174   monotonically with activity and the correlation coefficient was 0.34 ($p < 10^{-6}$, relative to the null-

175   hypothesis of no correlation between the two signals, SI Fig. 3B). Second, we hypothesized that there

176   should be a negative correlation between packing fraction and flow speed – with the understanding that

177   the nature of the jamming transition means that the relationship between those observables is non-linear

178   (i.e. we would expect a significant, but weak negative correlation). After removing low frequency

179   background variations, we used a fixed threshold to define which pixels were cells (dark) and which were

180   open space (light), and averaged the resulting packing fraction over square regions 12.4 μm^2 in area. We

181   then used open-source particle image velocimetry software (72) to measure the local speed of cellular

182   flow through the same pixel groups. We calculated the histogram of spatially correlated values between

183   flow speed and packing fraction, with a bivariate correlation coefficient of − 0.11 (with $p < 10^{-6}$, relative to

184   the null-hypothesis of no correlation between the two signals) (SI Fig. 3C). Thus both methods provide

185   significant support for the hypothesis that packing fraction regulates collective movement.

186   Next, we wanted to determine if available water (and its anticorrelate: packing fraction) could

187   regulate the transition between the fluidized / collectively motile state and the jammed immotile state.

188   We imaged expanding tips that were suddenly subjected to evaporation, and hence reduced water

189   availability. As water evaporated, the expanding tips exhibited a dynamic transition from a fluidized and

190   rapidly swarming state to a state where both individuals and the group were immotile (Fig. 2A/B and

191   Movie S7B). The motile-to-immotile transition did not occur at the same time across the group, and local

192   variations in density allowed some cells to 'rattle' in place, even after the group as a whole had become

193   immotile – consistent with the appearance of 'rattlers' in jammed granular systems (73, 74). These

194   observations further support the hypothesis that the transition is not a phenotypic change and that local

195   drag and frictional forces are not preventing motion. Then, to examine the reversibility of this transition,

196   we took the same plates and expanding tips, resealed them to halt evaporation, and continued imaging

197   while water from the gel rehydrated the cells. Over a few minutes, the colony re-fluidized into domains

198   of high activity sub-groups, which then coalesced until the entire tip region regained fluidity and continued

199   to expand (Fig. 2C/D and Movie S7C). This strongly indicates that re-wetting and the corresponding

200   reduction in packing fraction 'reverses' the jamming-like transition back to a fluidized and collectively

201   motile state (61, 71, 75).

202

Motile groups are pulled by the front in discrete layers

204   In our bright-field imaging it appeared that motile dendrites frequently traversed surfaces in

205   discrete layers of cells, first traversing the surface as dense monolayers, then double layers, and so forth,

206   up to 4 discrete layers (see Movies S6, S8 and S9). To confirm that groups migrated in discrete layers we

207   measured the surface height of expanding dendrites with high Z-resolution using an interferometric

208   profilometer (see Methods). In Fig. 3A/B we show the height field and a 1D profile for an expanding mono-

209   layer dendrite – its height across the expansion region was almost exactly 1 μm, the thickness of a single

210   *B. subtilis* cell. As dendrites slowed and/or encountered space constraints, the layer thickness jumped to

211   higher integer values, as shown in Fig. 3C/D whose dendrites transition between 0 and 3 layers of cells (4

212   layers visible in Movies S6 and S10). Looking back to the jamming data in Figs. 1 and 2, multiple discrete

213   layers are visible in the interior of the colony. Frequently, the fastest dendrites were advancing as

214   monolayers (the fastest moving groups in Movies S6, S8, and S9, notably tips in S10 appeared multi-

215   layered). In addition to being valuable *in situ* data of motile-group structure, this also meant that our

216   imaging of rapid motile groups (and attendant activity filtering) was characterizing the motion of all cells

217   in *Z*, as opposed to there being motile and immotile cells at different *Z* positions (e.g. a biofilm blocking

218   view of motile cells). This discrete structuring is noteworthy because relevant models of surfactant-driven

219   spreading take cell-layer thickness as a continuous variable, whereas these data demonstrate that

220   (frequently, though not strictly) motile groups move in monolayers with possible transitions to other

221   discrete heights, especially when near the jamming transition.

222   The boundary shape between bare agarose and dense cellular monolayers also contained

223   information. While the shape of the meniscal boundary can be influenced by surface pinning and

224   depinning (76), this suite of imaging data showed that such boundaries have different shape statistics near

225   and far from the advancing front. At the advancing front, the boundary curvature is smooth (low variation)

226   with an overall positive curvature whereas behind the front retraction of material causes curvature to

227   vary widely in a so-called 'lacunar' structure (SI Fig. 4A). Further, within the regions whose boundaries

228   were lacunar, voids appeared with similar distributions of boundary curvature to the external boundary

229   (SI Fig. 4B). Consistent with previous work on 'viscous fingering' (77) and models of surfactant-driven flow

230   (23, 24), these data support the hypothesis that the advancing front has positive pressure (pushes

231   outward, covers new territory) while the boundary behind the front experiences negative pressure (pulls

232   inward, retracts and reduces coverage), both consistent with cellular groups primarily being front-pulled

233   by gradients in surface tension, not pushed from the back by proliferation. Consequently, on one occasion

234   we observed a dendrite tip contacting its own surfactant field (78), resulting in an almost immediate

235   (within ~ 15 s) cessation of motion (SI Fig. 5 and Movie S11) despite the number of cells in the tip increasing

236   even after self-contact. Also consistent with a front-pull mechanism, much of the same imaging data

237   showed independent islands of cellular monolayers, completely detached from their parent colony,

238   traversing the surface independently, with characteristic positive curvature at the front and negative

239   curvature regions in the back (Movies S6, S8, S12 and S13).

240   While high numbers of cells are required for group motility, these data indicate that proliferation

241   alone is not generating outward pressure to drive group motion. To definitely determine this, we

242   measured the area of dense monolayer islands of cells as they moved over time. In Fig. 4, we show one

243   such island traversing an agarose surface over a distance many times its length. Rather than increasing

244   due to cell division and growth, the island area decreased over time as such islands tend to leave a trail of

245   cells immobilized on the surface by the meniscus. Therefore, during surfactin-driven surface motility

246   groups of cells are capable of large-scale motion without growth in the population and without connection

247   to their parent colony. Rather, boundary curvature analysis and the existence of independent islands

248   provide evidence that cells are pulled by the front, frequently in monolayers at a density near the

249   threshold for jamming.

250

251   **Material flows from parent colony to dendrite tip**

252   While our data were consistent with outward front-pressure pulling the group away from the

253   parent colony, multiple time-lapse experiments showed apparent rapid flow of discrete cellular layers

254   from parent colony to the expanding dendrite tip (e.g. see Movie S10). To confirm that these observations

255   were indeed flow of material over the scale of millimeters to centimeters, we performed our typical wild-

256   type expansion assay, but doped the initial cellular deposition with 1 µm fluorescent polystyrene beads,

257   at a ratio of ~ 1 bead per 100 cells (see Methods). We imaged the motion of the tracer beads at 30 fps,

258   which allowed us to see the trajectories of individual beads on the time scale of minutes (Movie S14, SI

259   Fig. 6A). The swarming motility of wild-type cells caused the motion of individual beads to be erratic, akin

260   to a non-thermal random walk with drift (79) especially when flow speed was slow in comparison to

261   cellular swim speeds (Movie S15, SI Fig. 6B). This erratic motion prohibited flow tracking in wild-type

262   groups, however, as we had confirmed earlier (38), mutants of *B. subtilis* unable to perform flagellar self-

263   propulsion (Δ*hag*) exhibit rapid surface motility with speeds and morphologies similar to wild-type cells

264   (see SI Fig. 2). This strain did not cause severe bead agitation and thus we were able to perform *in situ*

265   flow measurements using those mutants (Fig. 5 and Movie S16).

266   Using open-source cross-correlation software, we measured bead flow via particle image

267   velocimetry (72). Similar to the flow characteristics of a typical incompressible fluid, the flow speed of

268   dense cellular monolayers decreased in regions with wide meniscal boundaries and significantly sped up

269   at points of constriction (see inset Fig. 5). The similarity between incompressible 2D flow and flow of dense

270   cellular monolayers is consistent with the fact that cells are effectively solid steric objects densely packed

271   in (incompressible) water. Average flow speeds, and following the motion of individual beads, showed

272   that material originating in the parent colony frequently made its way to the expanding tip (Fig. 5 and

273   Movie S16). Thus, while our island data showed that motile groups need not be connected to the parent

274   colony and that cellular proliferation was not required for group motility, these data indicate that when

275   connected back to the parent colony, cells and material (e.g. nutrients) can be supplied to the expanding

276   tip of a dendrite.

277   Having confirmed that our bright-field data was indeed revealing large scale flow, we examined

278   how the speed of tip expansion into fresh territory compared with the speed of cellular flow along

279   established dendrites connecting the tip to the parent colony (SI Fig. 7). Consistent with the bead-flow

280   data, kymograph analysis showed that when expanding dendrites are connected to their parent colony,

281   cells (and surrounding fluid) flow many centimeters from the colony into the expansion zone. Such flows

282   along an established dendrite are significantly faster (~ 10 µm/s) than the speed of tip movement (~ 3

283   µm/s) into fresh territory, as they must be for material to reach the tip from the colony. At this point it is

284   unclear how groups create these differentials in speed along a dendrite, but we offer a potential

285   mechanism in the Discussion.

286

287   **Motile groups use material flow to traverse nutrient poor regions**

288   We knew from previous work (37, 38) that neither chemotaxis nor flagellar-mediated motility is

289   required for group surface motility, but that surface motility was potently modulated by (e.g.) the

290   presence of monovalent ions (K+ (25)). This suggested that groups might respond to their chemical

291   environment indirectly via changes in metabolism and/or gene expression. For instance, differences in

292   nutrient concentration might affect the rate of synthesis and/or secretion of surfactin, and thereby affect

293   surfactin-mediated motility. Further, our large-scale flow data suggested that differences across the

294   chemical landscape that affect group surface motility could be ameliorated by flow of material from other

295   regions.

296   We wanted to test whether motile groups had differential responses to exogenously presented

297   nutrient gradients, and specifically whether groups would avoid negative nutrient gradients. We created

298   agarose plates with two distinct halves – one half containing the same rich defined medium (RDM) used

299   earlier, the other half containing (potassium-free) NaCl buffer osmotically matched with the RDM to

300   maintain the same osmotic potential (see Methods). A thin impermeable barrier separated the two

301   halves, initially keeping the nutrients on one side. Once the agarose set, we poured a thin (~ 1 mm) layer

302   of osmotically matched NaCl buffer with agarose, and thus linked the two regions into a contiguous

303   surface with uniform mechanical properties that allowed cells to move freely between them. The nutrient-

304   rich half also contained a red fluorescent tracer dye (rhodamine) that served as an approximate reporter

305   of nutrient diffusion over the barrier. With or without cells, these plates showed diffusion of the dye from

306   the nutrient rich side into the side devoid of nutrients, meaning that there was a negative concentration

307   gradient on the surface from the nutrient rich to the nutrient poor side. We reasoned that if wild-type

308   cells were responding (potentially indirectly) to the outward-facing chemical gradients on a standard plate

309 (i.e. with initially isotropic nutrients) or to the absence of required potassium ions on the split-plate, they

310 would react to the reverse nutrient gradient on these split plates and either avoid the nutrient poor region

311 or show reduced motility toward it.

312   To test this, we simultaneously inoculated such a split-plate in two positions from the same

313 isogenic population of wild-type cells, one colony on the nutrient rich side, one on the nutrient poor side

314 (Fig. 6A/B). The colony inoculated on the nutrient poor side did not exhibit collective motility and was

315 effectively stationary, while the colony on the nutrient rich side rapidly covered the nutrient rich zone and

316 simultaneously ventured into the nutrient poor zone. Initially, dendrites that expanded into the nutrient

317 poor zone covered the plate less densely than dendrites that never left the nutrient rich zone (Fig. 6B),

318 but tip speeds were similar in both zones. Ultimately, cells originating from the nutrient-rich zone

319 colonized the entire plate (both zones, see Movie S17). The difference in initial coverage between the

320 zones supports the hypothesis that groups do exhibit a (mild) chemotropic response to gradients in

321 nutrients, ions, or potentially their own secreted waste products. Interestingly, these data show that

322 connection to a parent colony in a nutrient rich zone allows cells to explore nutrient-poor regions, even if

323 those regions lack critical chemical species ($K+$ ions in wild-type (25)). This also complements findings that

324 bacteria can transport other bacteria as cargo during surface motility (80). We did not observe any

325 independently moving islands of cells in the nutrient-poor region. Our flow data offer a qualitative

326 mechanism for this 'scouting' ability, that large-scale flow of material from parent colony to the extending

327 tip brings metabolic resources, presumably to fuel, in part, surfactin production, thus allowing Marangoni

328 forces to continue to pull the group outward via positive tip pressure.

329

330 **Discussion**

331   Our data support a model in which water availability – a proxy for bacterial packing fraction – is a

332 sensitive control parameter for an abiotic jamming transition in the granular material that is densely

333 packed, expanding bacterial populations. We hypothesize that motility plays a contributory role,

334 encouraging a fluidized state via motility-dependent viscosity (59, 81). When sufficient fluid is extracted

335 from the substrate by endogenous osmolyte secretion and cells are in a fluidized state, groups flow over

336 the surface driven and guided by surface-tension gradients (Marangoni forces). Conversely, when water

337 availability decreases, either by (e.g.) evaporation or decreasing osmotic potential, packing fraction

338 increases and cells transition to a jammed and immotile state. It is worth noting that while our data are

339 consistent with a jamming-fluidization transition, we cannot use our current data to *prove* that we are

340 meeting the technical requirements of such a transition. Jamming is defined by a non-crystalline network

341   of steric contacts and forces between particles that are 'isostatic', that is, they do not have free modes of

342   motion (other than rattlers (73)), but we are unaware of a modality of microscopy that permits live, *in situ*

343   visualization of the force and contact network in a dense bacterial group (stationary or collectively motile).

344   Nonetheless, the potential existence of an abiotic jamming transition that regulates group motility

345   through the dense packing of nematic actors offers a mechanism for why small increases in agarose gel

346   stiffness (~0.05%) switch groups from rapid surface migration to slow steric growth. It also clarifies why

347   the secretion of both surfactants and osmolytes is required for collective motility (37, 50); the former

348   being crucial for force generation and the latter for maintaining fluidization. Thus we hypothesize that

349   colonies that are not able to achieve the fluidized state – for any reason – are constrained to expand on

350   the order of 100 times slower over surfaces using the forces generated by cell wall growth and cell division

351   (i.e. the mode of sessile growth one typically associates with single colonies growing on a petri dish).

352   Our examination of boundary structure (SI Fig. 4) was consistent with outward-facing pressure at

353   the expanding front and inward-facing pressure behind the front. However, many data sets showed

354   persistent flow from parent colony, or other regions behind the front, toward the advancing front, without

355   the appearance of lacunar structure (e.g. Movies S2 and S10). We speculate that these differences in

356   boundary structure, and correspondingly flow, might reflect differences in the magnitude of force being

357   generated at the front and/or changes to the effective viscosity of the cellular suspension. Differences in

358   force magnitude might arise from varying rates of surfactin secretion and/or processes that affect the

359   spreading or degradation of surfactin. Differences in effective viscosity of the cellular suspension could

360   arise due to changes in cellular density (including but not limited to jamming), cellular aspect ratio (9, 82),

361   internal shear arising from individual motility (59, 81), or processes that affect the rate of fluid extraction

362   (e.g. via osmolytes) or loss (e.g. via evaporation) from the substrate. Similarly, we hypothesize that the

363   difference in tip speed vs. flow speed along an established dendrite (SI Fig. 7) – which allows material to

364   be brought from parent colony to dendrite tip – might reflect different energetic costs for motion in those

365   two scenarios. A tip advancing into fresh territory uses surface tension differences to generate force, but,

366   given our layering data, it must also pay a line-energy cost along the meniscus on each side of the dendrite,

367   which to first order would appear like a constant cost in energy per unit length of movement (equivalent

368   to a constant reduction in overall force). Conversely, assuming that cells flowing within an established

369   dendrite are using the same mechanisms for force generation, they move within the same meniscal

370   boundaries established by the tip, thus do not pay this energetic cost, and thus have a larger effective

371   force pulling them outward.

372   The existence of large scale flow from parent colony to dendrite tip presents a number of

373 biophysical questions and possibilities. How far into nutrient poor zones can expanding dendrites

374 venture? How do those distances depend on the available nutrients or the position of the parent colony?

375 How do surface properties like local water availability and topography (83, 84) affect flow and

376 colonization? How do structures on the surface guide and/or impeded collective motility? The fact that

377 we observed islands of cells moving independently on nutrient-rich surfaces, whereas flow from a parent

378 colony was required for exploration of nutrient-poor zones, suggests that those modalities of motion

379 (island vs. flowing dendrite) depend on environmental conditions. For instance, an island that ventures

380 into a nutrient poor region might quickly deplete its resources, and thus decrease secretion of osmolytes

381 and surfactants required for motion (54). Likewise, the transport of materials and nutrients from a

382 common pool (parent colony) into discrete tendrils far from the common pool (80) spur game-theoretic

383 questions of how those resources are divided, whether phenotypic changes are playing a role, and – like

384 other collectively motile and reproductive systems (e.g. *Dictyostelium* (85, 86)) – how the specific

385 genomes of individuals propagate in space and time relative to their nearly isogenic kin.

386   Whereas individual cells in bulk fluids propel themselves and direct their motion according to the

387 algorithms of chemotaxis, these data and previous work strongly suggest a fundamentally different set of

388 rules govern group surface motility. Specifically, that collective secretion of surfactants and osmolytes

389 drives and directs motion, that large-scale flow plays a critical role in exploration of territories with

390 differing nutrient concentrations, and that bacterial packing fraction – modulated through multiple

391 mechanisms – is a sensitive control parameter that regulates transitions between fluidized (collectively

392 motile) and jammed (immotile) states of the group. Thus continued understanding and modeling of

393 microbial communities on surfaces must consider how these physical factors – not fully described by

394 genetics and biochemistry – contribute to population dynamics.

395

396 **Methods**

397 *B. subtilis culture storage and preparation*

398   Isogenic cultures of *B. subtilis* strains were grown to OD 0.5, mixed 50% in glycerol aliquots,

399 individually snap frozen and stored at - 80 C. For each experiment, 200 μL aliquots were removed and

400 thawed, then diluted in 10 ml LB medium and grown in a shaking incubator at 37 C for 4 hours until mid-

401 log phase, then pelleted by centrifugation at 4000 g for 10 mins. The supernatant was removed, and the

402 bacterial pellets were then resuspended in 200 μL LB, yielding a culture with an approximate density OD

403 10, then 1.5 μL of that culture was deposited on 0.5% agarose plates with Teknova EZ-RDM media (a rich

404   defined medium), then dried for 10 mins in air and sealed with parafilm. For bead flow experiments, 1 μm

405   fluorescent polystyrene beads (Bangs Laboratories, Dragon Green) were diluted into the inoculation

406   culture and vortexed before deposition.

407   Depending on the type of time lapse experiment, plates were then either incubated for 1 hr then

408   imaged or imaged immediately at 37 C. For culture profilometry (height measurements), cultures were

409   incubated for 2 - 3 hrs at 37 C, then unsealed within 10 minutes of imaging to minimize evaporation and

410   jamming. For the multilayer height measurements (Fig. 3B), cultures were unsealed, transitioned to

411   immotility, and were then imaged between 60 to 80 minutes later.

412

413   *Plate preparation*

414   Plates were created by mixing 100 mL Teknova EZ-RDM and 0.5% agarose by weight and

415   autoclaving for 15 minutes at 121 C. Media was cooled to 50 C in an incubator before being poured into

416   4 plates (25 ml each). Each plate was cooled and solidified under flame in open air for 10, 15, or 30 minutes

417   before immediate inoculation, creating a range of initial gel hydrations to examine the effects of water

418   availability on colony morphology. For the split-plate experiment (Fig. 6) zero-nutrient buffer was made

419   from de-ionized water, 0.5% agarose w/v and NaCl concentration matched to the calculated osmotic

420   potential of the rich defined medium.

421

422   *Microscopy and Imaging*

423   Transmitted-light and oblique-illumination microscopy were performed using a Nikon SMZ-25

424   stereo zoom microscope, with a P2-SHR Plan Apo 1x objective, and a Prior ES111 OptiScan stage. High

425   speed images were taken using an Andor Zyla 5.5 CMOS camera. Phase contrast microscopy was

426   performed using a Nikon Eclipse Ti-E inverted microscope with an CFI S Plan Fluor ELWD 20x Ph1 ADM

427   objective (Fig. 2) or CFI S Plan Fluor ELWD 40X Ph2 ADM objective (SI Fig. 3). High speed images were

428   taken using an Andor iXon Ultra 888 EMCCD camera. Culture height images were captured using a Zygo

429   NewView 7300 optical 3D profilometer in the University of Oregon's CAMCOR facility. Low-frequency

430   variations in plate height were computationally removed by subtraction of a fitted a polynomial surface

431   to the background.

432   Activity overlays were generated using custom Matlab© scripts which calculated the per-pixel

433   sum over the absolute differences between 5 or 7 consecutive frames. Kymographs were generated using

434   custom Matlab scripts to identify and interpolate contours, then sum over cross-sectional slices of

435   intensities along the contours over time. All scripts and Matlab code are available on request.

436

441

442 **References**

443 1. R. Mesibov, J. Adler, Chemotaxis Toward Amino Acids in Escherichia coli. *J. Bacteriol.* **112**, 315–326
444   (1972).

445 2. L. F. Garrity, G. W. Ordal, Chemotaxis in Bacillus subtilis: how bacteria monitor environmental
446   signals. *Pharmacol. Ther.* **68**, 87–104 (1995).

447 3. D. S. Bischoff, G. W. Ordal, Bacillus subtilis chemotaxis: a deviation from the Escherichia coli
448   paradigm. *Mol. Microbiol.* **6**, 23–28 (1992).

449 4. H. C. Berg, D. A. Brown, Chemotaxis in Escherichia coli analysed by Three-dimensional Tracking.
450   *Nature* **239**, 500 (1972).

451 5. C. V. Rao, G. D. Glekas, G. W. Ordal, The three adaptation systems of Bacillus subtilis chemotaxis.
452   *Trends Microbiol.* **16**, 480–487 (2008).

453 6. R. Balagam, O. A. Igoshin, Mechanism for Collective Cell Alignment in Myxococcus xanthus Bacteria.
454   *PLOS Comput. Biol.* **11**, e1004474 (2015).

455 7. D. B. Kearns, L. J. Shimkets, Chemotaxis in a gliding bacterium. *Proc. Natl. Acad. Sci.* **95**, 11957–
456   11962 (1998).

457 8. A. E. Patteson, P. E. Arratia, A. Gopinath, Quenching a swarm: Effect of light exposure on
458   suppression of collective motility in swarming Serratia marcescens. *bioRxiv*, 331801 (2018).

459 9. A. Rabani, G. Ariel, A. Be'er, Collective Motion of Spherical Bacteria. *PLOS ONE* **8**, e83760 (2013).

460 10. T. Matsuyama, *et al.*, Dynamic Aspects of the Structured Cell Population in a Swarming Colony of
461   Proteus mirabilis. *J. Bacteriol.* **182**, 385–393 (2000).

462 11. H. H. Tuson, M. F. Copeland, S. Carey, R. Sacotte, D. B. Weibel, Flagellum Density Regulates Proteus
463   mirabilis Swarmer Cell Motility in Viscous Environments. *J. Bacteriol.* **195**, 368–377 (2013).

464 12. B. V. Jones, Role of swarming in the formation of crystalline Proteus mirabilis biofilms on urinary
465   catheters. *J. Med. Microbiol.* **54**, 807–813 (2005).

466 13. E. Ben-Jacob, I. Cohen, H. Levine, Cooperative self-organization of microorganisms. *Adv. Phys.* **49**,
467   395–554 (2000).

468 14. E. Ben-Jacob, From snowflake formation to growth of bacterial colonies II: Cooperative formation of
469   complex colonial patterns. *Contemp. Phys.* **38**, 205–241 (1997).

470 15. S. Atis, B. T. Weinstein, A. W. Murray, D. R. Nelson, Microbial Range Expansions on Liquid
471   Substrates. *Phys. Rev. X* **9**, 021058 (2019).

472 16. T. Ursell, R. M. W. Chau, S. Wisen, D. Bhaya, K. C. Huang, Motility Enhancement through Surface
473   Modification Is Sufficient for Cyanobacterial Community Organization during Phototaxis. *PLoS*
474   *Comput. Biol.* **9** (2013).

475 17. N. Schuergers, *et al.*, Cyanobacteria use micro-optics to sense light direction. *eLife* **5**, e12620 (2016).

476 18. M. Driscoll, *et al.*, Unstable fronts and motile structures formed by microrollers. *Nat. Phys.* **13**, 375–
477   379 (2017).

478 19. M. F. Copeland, D. B. Weibel, Bacterial Swarming: A Model System for Studying Dynamic Self-
479   assembly. *Soft Matter* **5**, 1174–1187 (2009).

480 20. R. M. Harshey, Bacterial Motility on a Surface: Many Ways to a Common Goal. *Annu. Rev. Microbiol.*
481   **57**, 249–273 (2003).

482 21. A. Be'er, G. Ariel, A statistical physics view of swarming bacteria. *Mov. Ecol.* **7**, 9 (2019).

483 22. C. Giverso, M. Verani, P. Ciarletta, Branching instability in expanding bacterial colonies. *J. R. Soc.*
484   *Interface* **12**, 20141290 (2015).

485 23. S. Trinschek, K. John, U. Thiele, Modelling of surfactant-driven front instabilities in spreading
486   bacterial colonies. *Soft Matter* **14**, 4464–4476 (2018).

487 24. S. Srinivasan, C. N. Kaplan, L. Mahadevan, A multiphase theory for spreading microbial swarms and
488   films. *eLife* **8**, e42697 (2019).

489 25. R. F. Kinsinger, M. C. Shirk, R. Fall, Rapid Surface Motility in Bacillus subtilis Is Dependent on
490   Extracellular Surfactin and Potassium Ion. *J. Bacteriol.* **185**, 5627–5631 (2003).

491 26. D. Kaiser, Bacterial Swarming: A Re-examination of Cell-Movement Patterns. *Curr. Biol.* **17**, R561–
492   R570 (2007).

493 27. J. D. Partridge, R. M. Harshey, Swarming: Flexible Roaming Plans. *J. Bacteriol.* **195**, 909–918 (2013).

494 28. T. A. Witten, L. M. Sander, Diffusion-Limited Aggregation, a Kinetic Critical Phenomenon. *Phys. Rev.*
495   *Lett.* **47**, 1400–1403 (1981).

496 29. J. Y. Wakano, S. Maenosono, A. Komoto, N. Eiha, Y. Yamaguchi, Self-Organized Pattern Formation of
497   a Bacteria Colony Modeled by a Reaction Diffusion System and Nucleation Theory. *Phys. Rev. Lett.*
498   **90**, 258102 (2003).

499 30. I. Golding, Y. Kozlovsky, I. Cohen, E. Ben-Jacob, Studies of bacterial branching growth using reaction–
500   diffusion models for colonial development. *Phys. Stat. Mech. Its Appl.* **260**, 510–554 (1998).

501 31. E. Tamar, M. Koler, A. Vaknin, The role of motility and chemotaxis in the bacterial colonization of
502   protected surfaces. *Sci. Rep.* **6**, 19616 (2016).

503 32. N. C. Caiazza, R. M. Q. Shanks, G. A. O'Toole, Rhamnolipids Modulate Swarming Motility Patterns of
504   Pseudomonas aeruginosa. *J. Bacteriol.* **187**, 7351–7361 (2005).

505 33. C. J. Ingham, E. B. Jacob, Swarming and complex pattern formation in Paenibacillus vortex studied
506   by imaging and tracking cells. *BMC Microbiol.* **8**, 36 (2008).

507 34. J. K. Parrish, L. Edelstein-Keshet, Complexity, Pattern, and Evolutionary Trade-Offs in Animal
508   Aggregation. *Science* **284**, 99–101 (1999).

509 35. E. B. Steager, C.-B. Kim, M. J. Kim, Dynamics of pattern formation in bacterial swarms. *Phys. Fluids*
510   **20**, 073601 (2008).

511 36. A. Marrocco, *et al.*, Models of Self-Organizing Bacterial Communities and Comparisons with
512   Experimental Observations. *Math. Model. Nat. Phenom.* **5**, 148–162 (2010).

513 37. D. B. Kearns, R. Losick, Swarming motility in undomesticated Bacillus subtilis. *Mol. Microbiol.* **49**,
514   581–590 (2003).

515 38. R. Fall, D. B. Kearns, T. Nguyen, A defined medium to investigate sliding motility in a Bacillus subtilis
516   flagella-less mutant. *BMC Microbiol.* **6**, 31 (2006).

517 39. S. Mukherjee, D. B. Kearns, The structure and regulation of flagella in Bacillus subtilis. *Annu. Rev.*
518   *Genet.* **48**, 319–340 (2014).

519 40. R. A. Calvo, D. B. Kearns, FlgM Is Secreted by the Flagellar Export Apparatus in Bacillus subtilis. *J.*
520   *Bacteriol.* **197**, 81–91 (2015).

521 41. T. S. Murray, B. I. Kazmierczak, Pseudomonas aeruginosa Exhibits Sliding Motility in the Absence of
522   Type IV Pili and Flagella. *J. Bacteriol.* **190**, 2700–2708 (2008).

523 42. A. Toguchi, M. Siano, M. Burkart, R. M. Harshey, Genetics of Swarming Motility in Salmonella
524   enterica Serovar Typhimurium: Critical Role for Lipopolysaccharide. *J. Bacteriol.* **182**, 6308–6321
525   (2000).

526 43. M. Burkart, A. Toguchi, R. M. Harshey, The chemotaxis system, but not chemotaxis, is essential for
527   swarming motility in Escherichia coli. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 2568–2573 (1998).

528 44. H. Du, *et al.*, High Density Waves of the Bacterium Pseudomonas aeruginosa in Propagating Swarms
529   Result in Efficient Colonization of Surfaces. *Biophys. J.* **103**, 601–609 (2012).

530 45. A. Yang, W. S. Tang, T. Si, J. X. Tang, Influence of Physical Effects on the Swarming Motility of
531   Pseudomonas aeruginosa. *Biophys. J.* **112**, 1462–1471 (2017).

532 46. R. Chen, S. B. Guttenplan, K. M. Blair, D. B. Kearns, Role of the σD-Dependent Autolysins in Bacillus
533   subtilis Population Heterogeneity. *J. Bacteriol.* **191**, 5775–5784 (2009).

534 47. A. Be'er, *et al.*, Paenibacillus dendritiformis Bacterial Colony Growth Depends on Surfactant but Not
535   on Bacterial Motion. *J. Bacteriol.* **191**, 5758–5764 (2009).

536 48. E. Ward, *et al.*, Organization of the flagellar switch complex of Bacillus subtilis. *J. Bacteriol.*,
537   JB.00626-18 (2018).

538 49. J. R. Kirby, T. B. Niewold, S. Maloy, G. W. Ordal, CheB is required for behavioural responses to
539   negative stimuli during chemotaxis in Bacillus subtilis. *Mol. Microbiol.* **35**, 44–57 (2000).

540 50. D. B. Kearns, A field guide to bacterial swarming motility. *Nat. Rev. Microbiol.* **8**, 634–644 (2010).

541 51. L. W. Schwartz, R. V. Roy, Some results concerning the potential energy of interfaces with
542   nonuniformly distributed surfactant. *Phys. Fluids* **13**, 3089–3092 (2001).

543 52. Á. T. Kovács, Bacillus subtilis. *Trends Microbiol.* **27**, 724–725 (2019).

544 53. T. E. Angelini, M. Roper, R. Kolter, D. A. Weitz, M. P. Brenner, Bacillus subtilis spreads by
545   surfing on waves of surfactant. *Proc. Natl. Acad. Sci.* **106**, 18109 (2009).

546 54. W.-J. Ke, Y.-H. Hsueh, Y.-C. Cheng, C.-C. Wu, S.-T. Liu, Water surface tension modulates the
547   swarming mechanics of Bacillus subtilis. *Front. Microbiol.* **6** (2015).

548 55. S. Srinivasan, N. C. Kaplan, L. Mahadevan, Dynamics of spreading microbial swarms and films.
549   *bioRxiv* (2018) https:/doi.org/10.1101/344267 (February 5, 2019).

550 56. M. Fauvart, *et al.*, Surface tension gradient control of bacterial swarming in colonies of
551   Pseudomonas aeruginosa. *Soft Matter* **8**, 70–76 (2012).

552 57. H. H. Wensink, *et al.*, Meso-scale turbulence in living fluids. *Proc. Natl. Acad. Sci.* **109**, 14308–14313
553   (2012).

554 58. H. M. López, J. Gachelin, C. Douarche, H. Auradou, E. Clément, Turning Bacteria Suspensions into
555   Superfluids. *Phys. Rev. Lett.* **115**, 028301 (2015).

556 59. A. Sokolov, I. S. Aranson, Reduction of Viscosity in Suspension of Swimming Bacteria. *Phys. Rev. Lett.*
557   **103**, 148101 (2009).

558 60. M. Burel, S. Martin, O. Bonnefoy, Jamming/flowing transition of non-Brownian particles suspended
559   in a iso-density fluid flowing in a 2D rectangular duct. *EPJ Web Conf.* **140**, 03086 (2017).

560 61. E. Woldhuis, V. Chikkadi, M. S. van Deen, P. Schall, M. van Hecke, Fluctuations in flows near
561   jamming. *Soft Matter* **11**, 7024–7031 (2015).

562 62. K. A. Dahmen, Y. Ben-Zion, J. T. Uhl, A simple analytic theory for the statistics of avalanches in
563   sheared granular materials. *Nat. Phys.* **7**, 554–557 (2011).

564 63. M. E. Cates, J. P. Wittmer, J.-P. Bouchaud, P. Claudin, Jamming, Force Chains, and Fragile Matter.
565   *Phys. Rev. Lett.* **81**, 1841–1844 (1998).

566 64. A. J. Liu, S. R. Nagel, Jamming is not just cool any more. *Nature* **396**, 21–22 (1998).

567   65. I. R. Peters, *et al.*, Dynamic jamming of iceberg-choked fjords. *Geophys. Res. Lett.* **42**, 1122–1129
568   (2015).

569   66. J. Henrichsen, Bacterial surface translocation: a survey and a classification. *Bacteriol. Rev.* **36**, 478–
570   503 (1972).

571   67. N. Morales-Soto, *et al.*, Preparation, Imaging, and Quantification of Bacterial Surface Motility
572   Assays. *J. Vis. Exp. JoVE* (2015) https:/doi.org/10.3791/52338 (October 7, 2019).

573   68. A. J. Wolfe, H. C. Berg, Migration of bacteria in semisolid agar. *Proc. Natl. Acad. Sci.* **86**, 6973–6977
574   (1989).

575   69. L. Jauffred, R. Munk Vejborg, K. S. Korolev, S. Brown, L. B. Oddershede, Chirality in microbial biofilms
576   is mediated by close interactions between the cell surface and the substratum. *ISME J.* **11**, 1688–
577   1701 (2017).

578   70. C. Beloin, A. Roux, J.-M. Ghigo, Escherichia coli biofilms. *Curr. Top. Microbiol. Immunol.* **322**, 249–
579   289 (2008).

580   71. C. Reichhardt, C. J. O. Reichhardt, Aspects of jamming in two-dimensional athermal frictionless
581   systems. *Soft Matter* **10**, 2932–2944 (2014).

582   72. W. Thielicke, E. Stamhuis, PIVlab – Towards User-friendly, Affordable and Accurate Digital Particle
583   Image Velocimetry in MATLAB. *J. Open Res. Softw.* **2**, e30 (2014).

584   73. M. Skoge, A. Donev, F. H. Stillinger, S. Torquato, Packing hyperspheres in high-dimensional
585   Euclidean spaces. *Phys. Rev. E* **74**, 041127 (2006).

586   74. S. Atkinson, F. H. Stillinger, S. Torquato, Detailed characterization of rattlers in exactly isostatic,
587   strictly jammed sphere packings. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **88**, 062208 (2013).

588   75. K. Roeller, J. Blaschke, S. Herminghaus, J. Vollmer, Arrest of the flow of wet granular matter. *J. Fluid
589   Mech.* **738**, 407–422 (2014).

590   76. M. Hennes, J. Tailleur, G. Charron, A. Daerr, Active depinning of bacterial droplets: The collective
591   surfing of Bacillus subtilis. *Proc. Natl. Acad. Sci.*, 201703997 (2017).

592   77. O. Praud, H. L. Swinney, Fractal dimension and unscreened angles measured for radial viscous
593   fingering. *Phys. Rev. E* **72**, 011406 (2005).

594   78. B. L. James, J. Kret, J. E. Patrick, D. B. Kearns, R. Fall, Growing Bacillus subtilis tendrils sense and
595   avoid each other. *FEMS Microbiol. Lett.* **298**, 12–19 (2009).

596   79. Y. Wu, H. C. Berg, Water reservoir maintained by cell growth fuels the spreading of a bacterial
597   swarm. *Proc. Natl. Acad. Sci.* **109**, 4128–4133 (2012).

598   80. A. Finkelshtein, D. Roth, E. Ben Jacob, C. J. Ingham, Bacterial Swarms Recruit Cargo Bacteria To Pave
599   the Way in Toxic Environments. *mBio* **6** (2015).

600 81. D. Lopez, E. Lauga, Dynamics of swimming bacteria at complex interfaces. *Phys. Fluids* **26**, 071902
601   (2014).

602 82. B. Ilkanaiv, D. B. Kearns, G. Ariel, A. Be'er, Effect of Cell Aspect Ratio on Swarming Bacteria. *Phys.*
603   *Rev. Lett.* **118**, 158002 (2017).

604 83. E. S. Gloag, *et al.*, Micro-Patterned Surfaces That Exploit Stigmergy to Inhibit Biofilm Expansion.
605   *Front. Microbiol.* **7** (2017).

606 84. M. Werb, *et al.*, Surface topology affects wetting behavior of Bacillus subtilis biofilms. *Npj Biofilms*
607   *Microbiomes* **3**, 1–10 (2017).

608 85. D. A. Brock, T. E. Douglas, D. C. Queller, J. E. Strassmann, Primitive agriculture in a social amoeba.
609   *Nature* **469**, 393–396 (2011).

610 86. G. D. Palo, D. Yi, R. G. Endres, A critical-like collective state leads to long-range cell communication
611   in Dictyostelium discoideum aggregation. *PLOS Biol.* **15**, e1002602 (2017).

612

Figure 1. Isogenic cells exist in motile and immotile phases within the same colony. (A) Time-lapse imaging of wild-type *B. subtilis* spreading dendritically on a nutrient-rich soft-agarose surface. We applied a computational image filter that uses local intensity fluctuations over time to report on the degree of movement. Green regions are high motile activity and stationary regions are left gray. Transient motile regions and fluctuating boundaries between motile and stationary regions emerged and dissipated on timescales shorter than (potential) phenotypic changes (see Movie S1). **(B)** Kymographs along the colored dashed lines in (A) (time is downward). Transitions from large-scale motility (green) to immobilized (jammed) states are denoted by the red arrows. Regions could transition from jammed back to fluidized if they came in contact with a fluidized region (see boundary movements in yellow). At later times, once the entire population had become immotile, cells grew 'upward' into bilayers that frequently coalesced. See Movie S6.

12

Figure 2. Water availability controls a jamming-like transition. **(A)** Time-lapse imaging of the tip of a wild- type *B. subtilis* dendrite on a nutrient-rich low-agarose surface. At *t* = 0 the plate was unsealed and its surface was exposed to air. Subsequent evaporation reduced water availability at the surface, causing both collective motion (green color) and the computational measure of mean motile activity **(B)** to drop as the cellular group entered the jammed state. Across the entire dendrite tip collective motility halted. **(C - D)** The same plate was then immediately resealed and imaged over 20 minutes. The punctuated drop in evaporation rate allowed cells to retain more water from the gel. Fluidized domains of motile cells subsequently emerged and coalesced until the entire dendrite was, once again, collectively motile. In both sets of images, the rectangular structure toward the top is a cellular bilayer (see Fig. 3). Noise in the activity measurement (B) was (in part) due to manual refocusing required by the retraction of the surface as water evaporated. Scale bar is 200 μm. See Movies S7A and S7B.

23
Figure 3. Dendrites expand in discrete layers. The left-hand column shows height images of wild-type *B. subtilis* dendrites on soft agarose taken using an interferometric profilometer (see Methods); the image backgrounds have been computationally flattened. **(A)** Advancing dendrites flowed downward (white arrow) and the fronts of those dendrites exhibited overall positive curvature (red dashed lines, inset) indicating outward pressure. Behind the advancing front, boundaries between agarose and cells exhibited a 'lacunar' structure (e.g. inset, cyan dashed ellipse), where the mode of the curvature distribution was negative (SI Fig. 3). Voids appeared behind the front, consistent with negative pressure in those regions overcoming meniscal forces. Both voids and lacunar structure are consistent with viscous fingering and cells de-wetting and being pulled to the front. **(B)** A one-dimensional profile of the surface height along the black line in (A). Here the advancing front is a densely packed cellular monolayer, whose height is one cell thickness as shown by the horizontal grey dashed lines (1 μm apart). **(C)** Same imaging modality as in (A), now after dendrites have jammed on the surface and then grown for ~ 1 hr. The original flow direction is indicated by the white arrow. **(D)** A one dimensional profile of the surface height along the black line in (C). Within contiguous cellular regions, bacterial film height is discretized into layers one cell thick; here the profile shows transitions from monolayer, to bilayer, to trilayer. Discretization ceased above four layers, when presumably cells move in any orientation, rotating about their long axis within the entire vertical structure (see also Movies S6, S8 and S9).
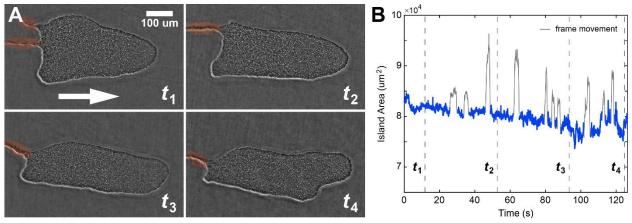
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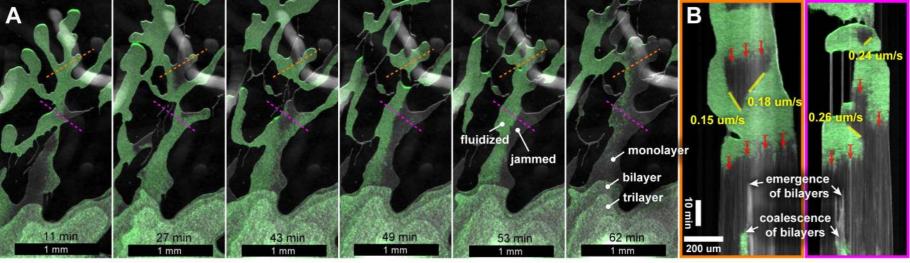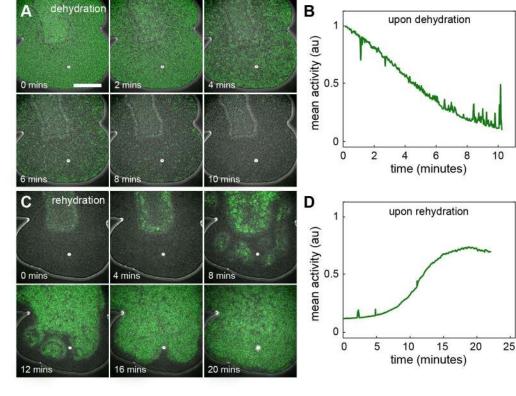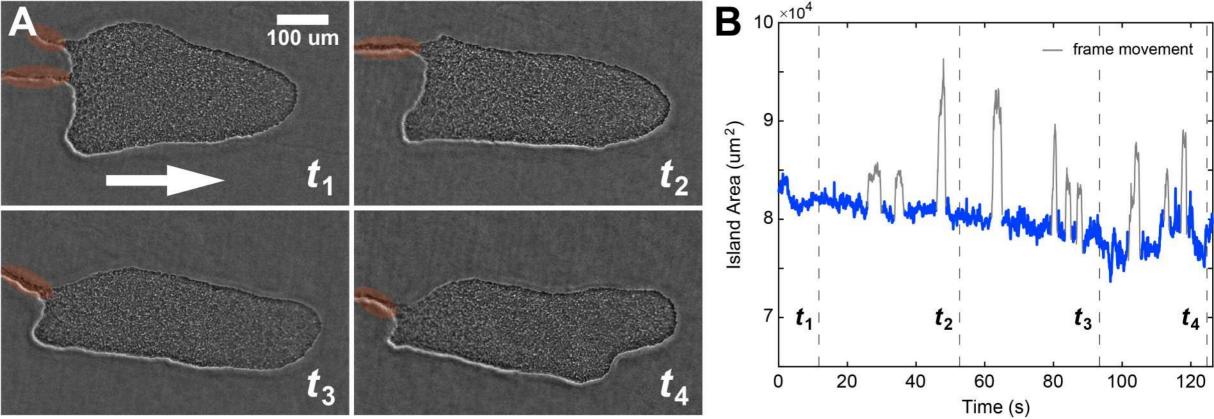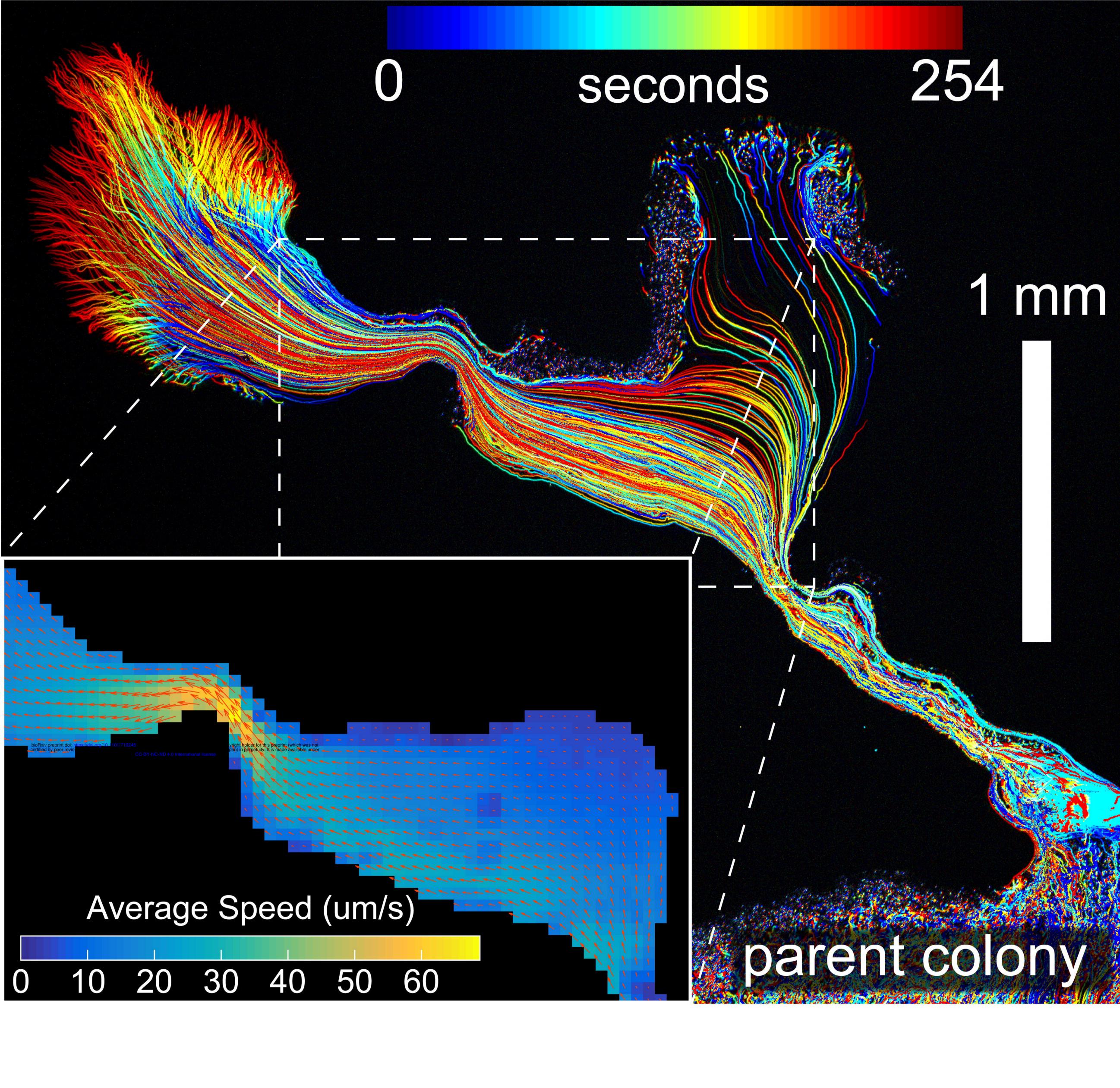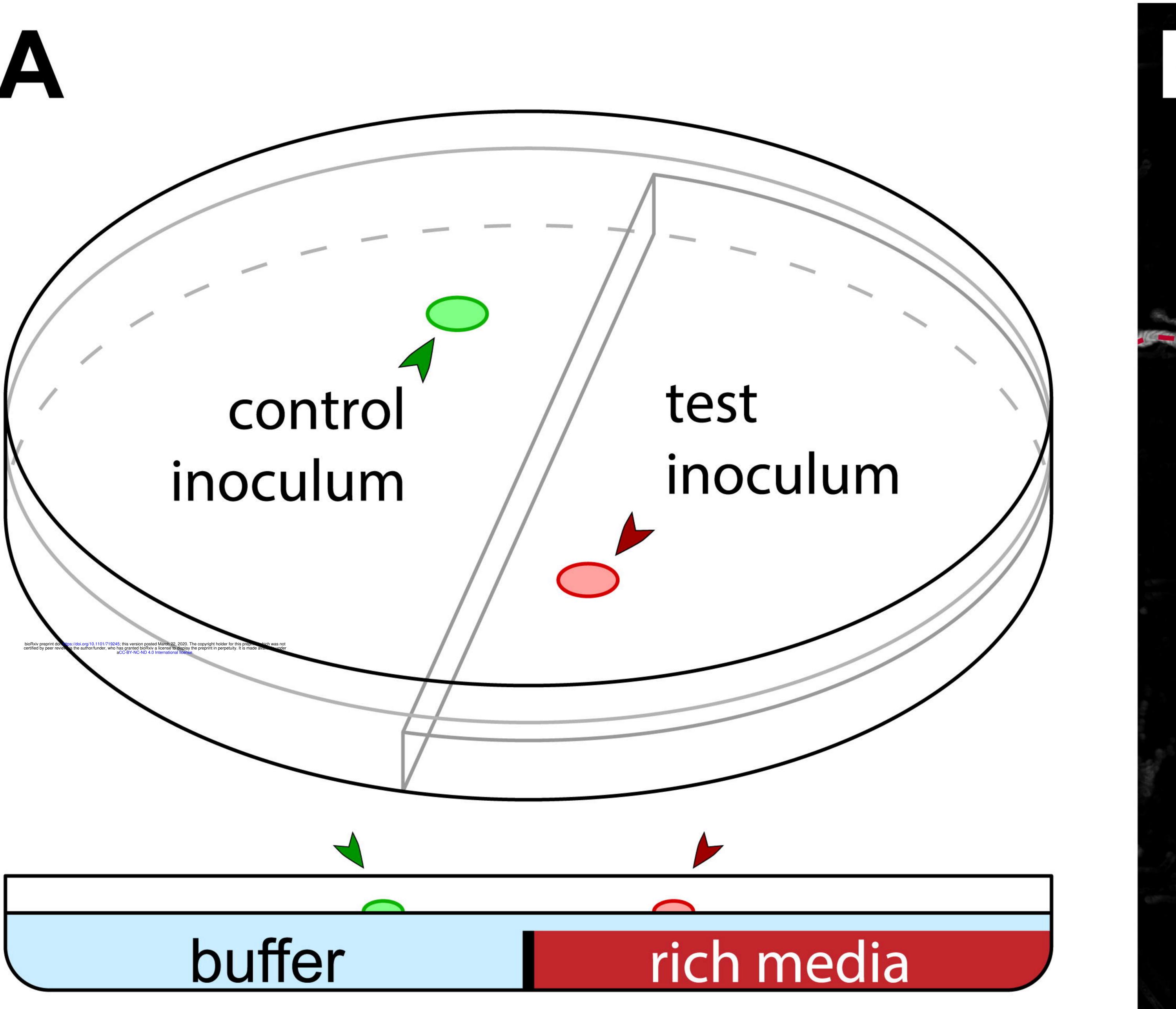
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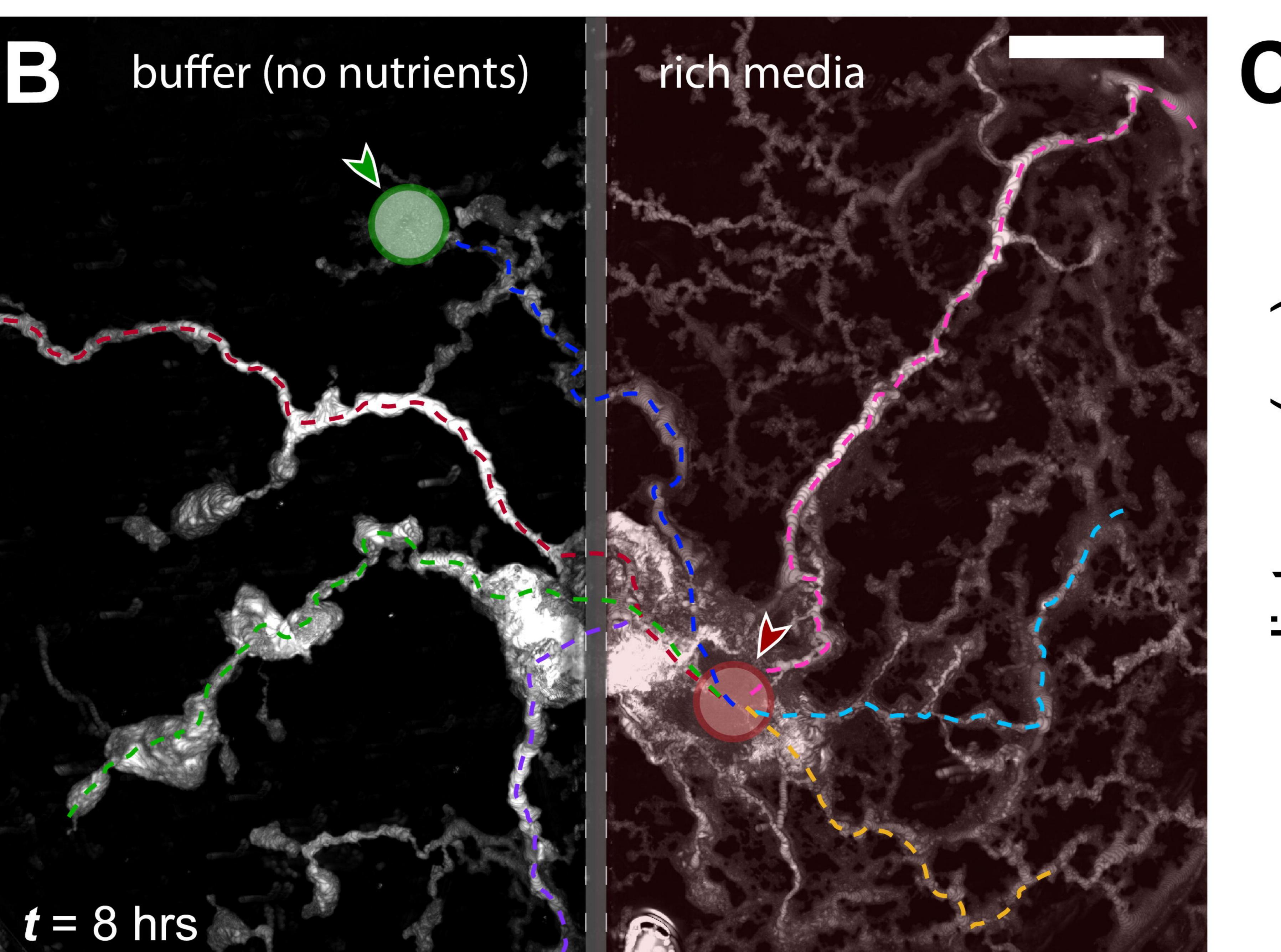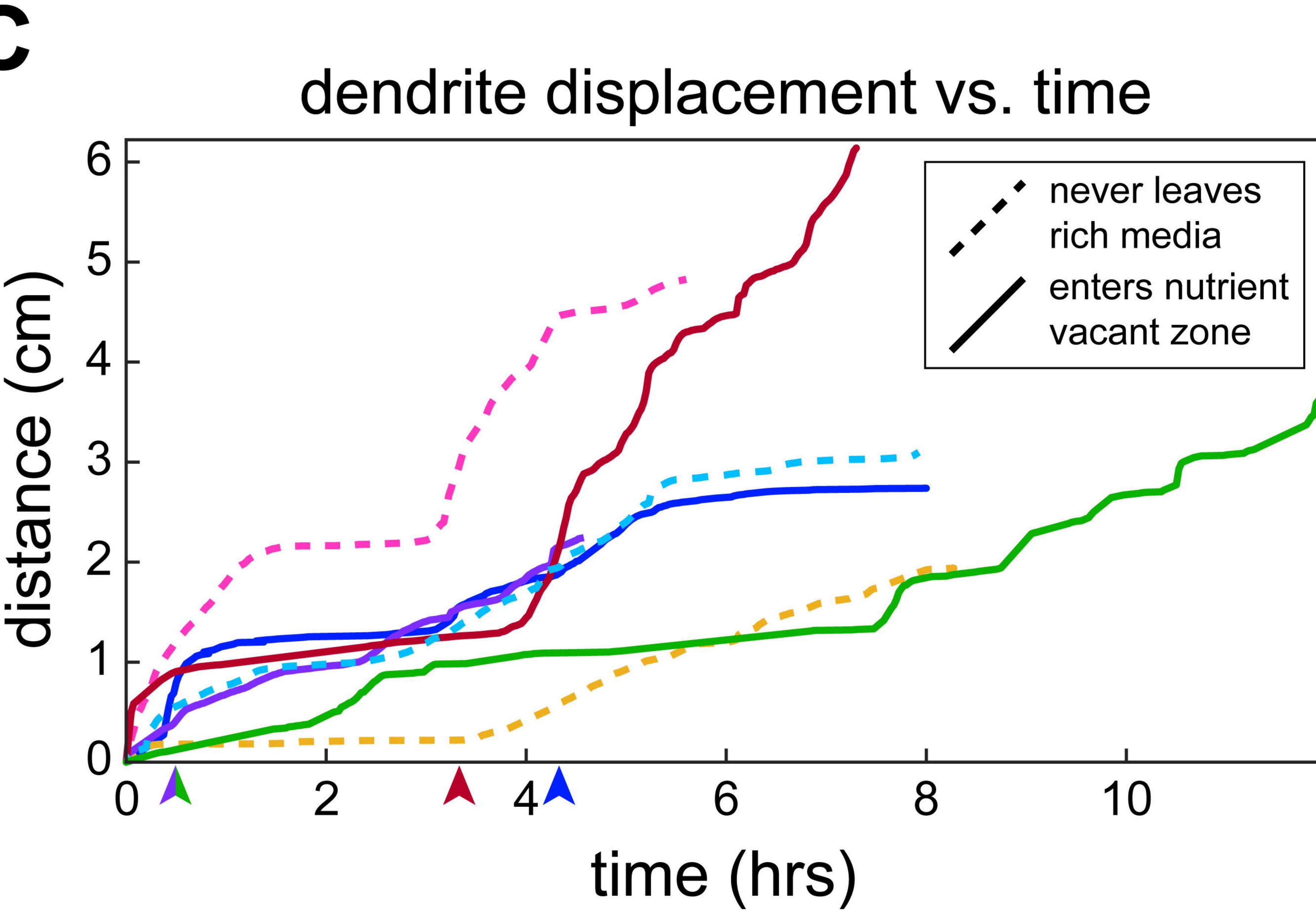
A

11 min — 1 mm

27 min — 1 mm

43 min — 1 mm

49 min — 1 mm — fluidized — jammed

53 min — 1 mm

62 min — 1 mm — monolayer — bilayer — trilayer

B

0.18 um/s
0.15 um/s

0.24 um/s
0.26 um/s

emergence of bilayers

coalescence of bilayers

10 min

200 um

A dehydration

0 mins   2 mins   4 mins
6 mins   8 mins   10 mins

B upon dehydration

C rehydration

0 mins   4 mins   8 mins
12 mins   16 mins   20 mins

D upon rehydration

A

100 um

t_1

t_2

t_3

t_4

B

frame movement

Island Area (um²)

t_1 t_2 t_3 t_4

Time (s)

0 seconds 254

1 mm

Average Speed (um/s)

0   10   20   30   40   50   60

parent colony

A control inoculum, test inoculum; buffer, rich media

B buffer (no nutrients), rich media; *t* = 8 hrs

C dendrite displacement vs. time

distance (cm)

time (hrs)

- - - never leaves rich media
——— enters nutrient vacant zone

Figure 1. Isogenic cells exist in motile and immotile phases within the same colony. (A) Time-lapse imaging of wild-type *B. subtilis* spreading dendritically on a nutrient-rich soft-agarose surface. We applied a computational image filter that uses local intensity fluctuations over time to report on the degree of movement. Green regions are high motile activity and stationary regions are left gray. Transient motile regions and fluctuating boundaries between motile and stationary regions emerged and dissipated on timescales shorter than (potential) phenotypic changes (see Movie S1). **(B)** Kymographs along the colored dashed lines in (A) (time is downward). Transitions from large-scale motility (green) to immobilized (jammed) states are denoted by the red arrows. Regions could transition from jammed back to fluidized if they came in contact with a fluidized region (see boundary movements in yellow). At later times, once the entire population had become immotile, cells grew 'upward' into bilayers that frequently coalesced. See Movie S6.
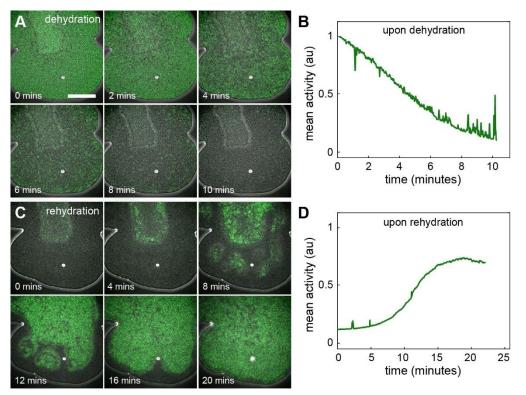
Figure 2. Water availability controls a jamming-like transition. **(A)** Time-lapse imaging of the tip of a wild- type *B. subtilis* dendrite on a nutrient-rich low-agarose surface. At *t* = 0 the plate was unsealed and its surface was exposed to air. Subsequent evaporation reduced water availability at the surface, causing both collective motion (green color) and the computational measure of mean motile activity **(B)** to drop as the cellular group entered the jammed state. Across the entire dendrite tip collective motility halted. **(C - D)** The same plate was then immediately resealed and imaged over 20 minutes. The punctuated drop in evaporation rate allowed cells to retain more water from the gel. Fluidized domains of motile cells subsequently emerged and coalesced until the entire dendrite was, once again, collectively motile. In both sets of images, the rectangular structure toward the top is a cellular bilayer (see Fig. 3). Noise in the activity measurement (B) was (in part) due to manual refocusing required by the retraction of the surface as water evaporated. Scale bar is 200 μm. See Movies S7A and S7B.
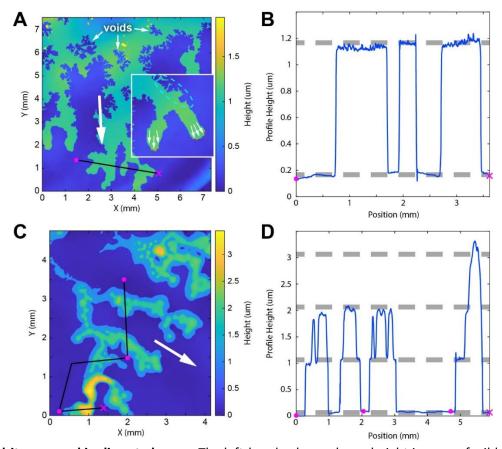
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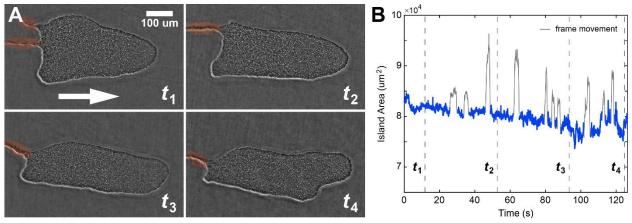
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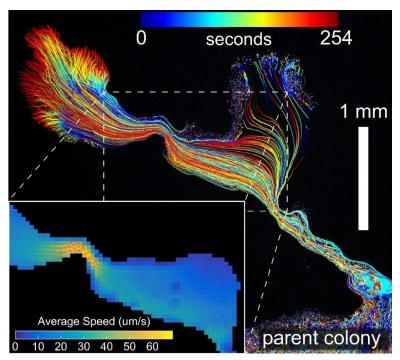
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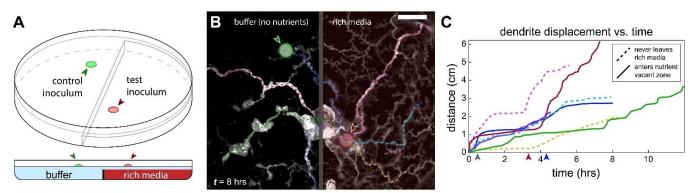
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