

Social Motility: Interaction between two sessile soil bacteria leads to emergence of surface motility

Lucy M. McCully¹, Adam S. Bitzer¹, Sarah C. Seaton^{2#}, Leah M. Smith^{1,#}, Mark W. Silby¹

¹Department of Biology, University of Massachusetts Dartmouth, North Dartmouth, MA 02747

²Department of Biology, University of North Carolina Asheville, Asheville, NC 28804

#Present addresses:

Leah M. Smith

Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand

Sarah C. Seaton

Indigo Agriculture, 500 Rutherford Avenue, Charlestown, MA, USA

Running title: Social motility as an emergent trait

For correspondence

Mark Silby

Department of Biology

University of Massachusetts Dartmouth

285 Old Westport Road

North Dartmouth

MA 02747

Phone: +1-508-999-8364

Fax: +1-508-999-8196

Email: mark.silby@umassd.edu

1 **Abstract**

2 Bacteria often live in complex communities in which they interact with other organisms.
3 Consideration of the social environment of bacteria can reveal emergent traits and behaviors
4 that would be overlooked by studying bacteria in isolation. Here we characterize a social trait
5 which emerges upon interaction between the distantly-related soil bacteria *Pseudomonas*
6 *fluorescens* Pf0-1 and *Pedobacter* sp. V48. On hard agar, which is not permissive for motility
7 the mono-culture of either species, co-culture reveals an emergent phenotype we term ‘social
8 motility,’ where the bacteria spread across the hard surface. We show that initiation of social
9 motility requires close association between the two species of bacteria. Both species remain
10 associated throughout the spreading colony, with reproducible and non-homogenous patterns of
11 distribution. The nutritional environment influences social motility; no social behavior is observed
12 under high nutrient conditions, but low nutrient conditions are insufficient to promote social
13 motility without high salt concentrations. This simple two-species consortium is a tractable
14 model system that will facilitate mechanistic investigations of interspecies interactions and
15 provide insight into emergent properties of interacting species. These studies will contribute to
16 the broader knowledge of how bacterial interactions influence the functions of communities they
17 inhabit.

18 **Introduction**

19 Within the soil live a plethora of microbial species that form complex communities
20 responsible for important ecological functions, such as nutrient cycling and plant health. Omics
21 approaches have given us a wealth of information on the composition, diversity, metabolic
22 potential, and ecology of plant- and soil-associated microbial communities (Reviewed in
23 Philippot *et al.*, 2013; Fierer, 2017). However, to get a complete understanding of microbial
24 functions and interactions within these environments, we must look at every layer, from the full
25 community *in vivo* to the individual microbe *in vitro* (Reviewed in Abreu and Taga, 2016).
26 Historically, research has focused on the study of single species in pure culture, but bacteria are
27 social organisms, and thus study of the mechanisms and consequences of multi-species
28 interactions is necessary for us to understand the function of microbial communities as a whole.
29 Investigating entire soil communities *in situ* presents considerable challenges because of
30 fluctuating soil conditions and the wide range of relevant scales, ranging from particulate to
31 ecological levels (Reviewed in Fierer, 2017). Reducing the microbial community to pair-wise
32 interactions or small consortia allows for a detailed mechanistic study and is an essential link
33 between from the study of isolated microbes in the laboratory to understanding the collective
34 activities of natural microbial communities (Blasche *et al.*, 2017).

35 Recent work has considered the social environment of bacteria, investigating altered
36 behaviors and production of secondary metabolites when co-cultured with other organisms.
37 Some bacteria exhibit emergent behaviors when presented with other species, likely the result
38 of induction of genes that are not expressed in pure culture. For example, *Pseudomonas*
39 *fluorescens* produce an antifungal compound during interactions with other species (de Boer *et al.*
40 *et al.*, 2007; Garbeva and de Boer, 2009; Garbeva *et al.*, 2011a, 2011b, 2014). The co-culture of
41 different actinomycete species results in the production of secondary metabolites, changes in
42 pigment, and sporulation (Seyedsayamdost *et al.*, 2011; Traxler *et al.*, 2012, 2013). The
43 presence of *E. coli* or *Pseudomonas* species effects sporulation and biofilm formation in *Bacillus*
44 *subtilis* (Powers *et al.*, 2015; Grandchamp *et al.*, 2017). One subset of social interactions are
45 those which alter the motility behaviors and capabilities of other species. For example, physical
46 association with *Saccharomyces cerevisiae* results in *Streptomyces venezuelae* consuming the
47 yeast and triggers 'exploratory growth' of the bacteria (Jones *et al.*, 2017). In another example,
48 *B. subtilis* moves away from a *Streptomyces* competitor across a solid surface (Stubbendieck
49 and Straight, 2015; Liu *et al.*, 2018). Other behaviors appear less competitive, where a motile
50 species will travel with a non-motile species that can degrade antibiotics, allowing the

51 consortium to colonize hostile environments (Venturi *et al.*, 2010; Finkelshtein *et al.*, 2015).
52 *Xanthomonas perforans* can even change the behavior of *Paenibacillus vortex*, producing a
53 signal that induces *P. vortex* to swarm towards it so it can hitchhike (Hagai *et al.*, 2014).

54 *Pseudomonas fluorescens* Pf0-1 and *Pedobacter* sp. V48 are known to interact through
55 diffusible and volatile signals, which induce changes in gene expression and production of an
56 antifungal compound by *P. fluorescens* (Garbeva *et al.*, 2011a, 2011b, 2014). Previous studies
57 with *Pedobacter* and a strain closely-related to *P. fluorescens* Pf0-1 (AD21) found that, in
58 addition to reciprocal gene expression changes and antagonistic behavior toward the plant
59 pathogen *Rhizoctonia solani*, the mixture of the strains also showed expansion on the plate
60 beyond the initial area of inoculation (de Boer *et al.*, 2007; Garbeva and de Boer, 2009). We
61 further investigated this observed behavior by moving from culturing *P. fluorescens* Pf0-1 and
62 *Pedobacter* without contact, as was done in the antagonism assays (Garbeva *et al.*, 2011a), to
63 mixing them together. We hypothesized that, while antibiotic production can be induced at a
64 distance through diffusible or volatile signals, the motility behavior requires close contact and is
65 therefore controlled in a manner distinct from the other two forms of communication.

66 In this study, we describe an interaction between two distantly-related soil bacteria, *P.*
67 *fluorescens* Pf0-1 (phylum: Proteobacteria) and *Pedobacter* sp. V48 (phylum: Bacteroidetes).
68 This interaction produces an emergent behavior, which we term “social motility,” in which the
69 bacteria move together across a hard agar surface. When grown in isolation, neither species
70 moves beyond the normal amount of colony expansion. In co-culture, both bacteria are present
71 throughout the motile colony, and fluorescent imaging shows a non-homogenous distribution.
72 We demonstrate that a close association between the colonies of both species is required for
73 motility to initiate and that the levels of nutrients and salts in the media affect the development of
74 the motile phenotype.

75 **Results**

76 **Social motility arises when mixing two distantly-related bacteria.**

77 In previous studies, antifungal activity was observed when *P. fluorescens* Pf0-1 and
78 *Pedobacter* sp. V48 were cultured 15 mm apart (Garbeva *et al.*, 2011a). In addition to this
79 interaction-induced trait, the possibility of motility was noted in a mixture of *P. fluorescens* AD21
80 and *Pedobacter* (de Boer *et al.*, 2007; Garbeva and de Boer, 2009). When we plated *P.*
81 *fluorescens* Pf0-1 and *Pedobacter* on TSB-NK medium solidified with 2% agar a mixed colony
82 of the two bacteria expanded across the surface of the agar, an environment in which neither
83 monoculture exhibited motility. The emergent social motility is shown in Fig. 1.

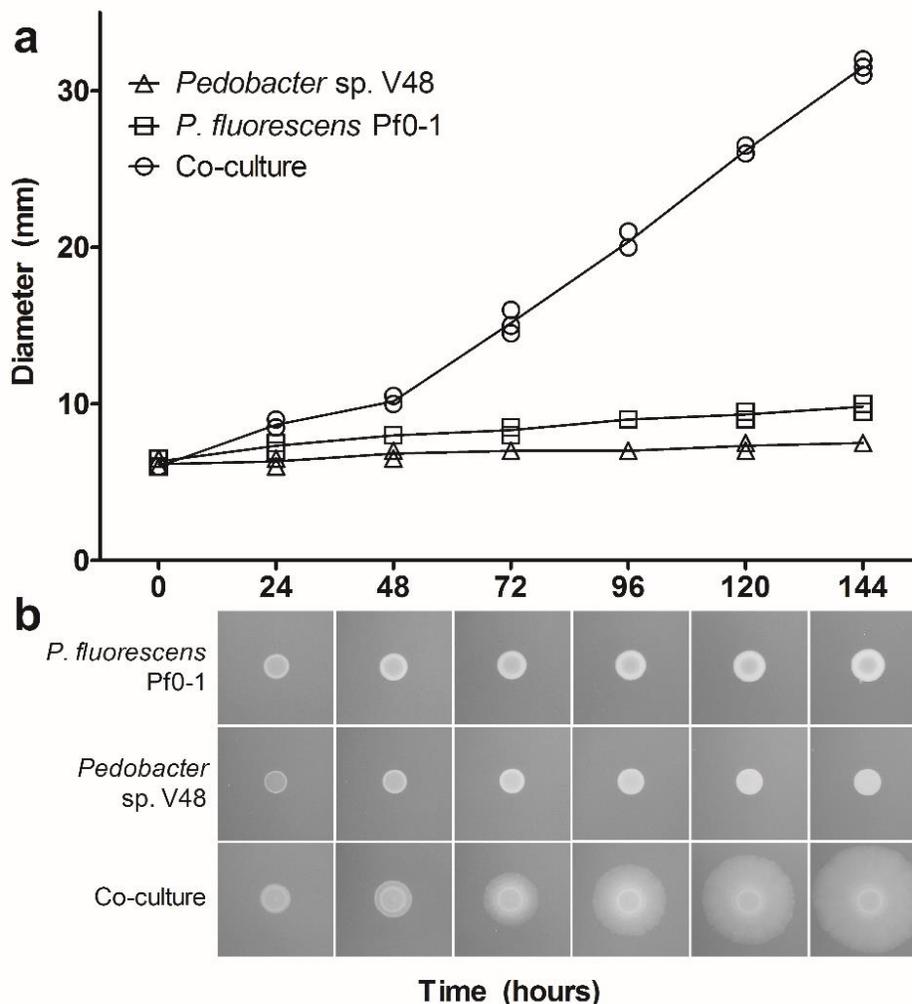


Figure 1. Mixed colony of *P. fluorescens* Pf0-1 and *Pedobacter* sp. V48 spreads across a hard agar surface (2%), a behavior not observed in the mono-culture of either species. a) Diameter of colonies at 24 h intervals. b) Phenotypes of mono- and co-cultures at 24 h intervals. Contrast and brightness levels were adjusted for optimal viewing.

84 Social motility becomes apparent between 24 and 48 h after inoculation, when the
85 colony begins to spread from the edge of the inoculum (Fig. 1b). The diameter of the motile co-
86 culture is significantly different from the colony expansion of the mono-cultures starting at the 24
87 h time point ($p < 0.001$) (Fig. 1a). Once the motility phenotype is fully visible (around 72 h), the
88 average speed of expansion is $1.69 \mu\text{m}/\text{min} \pm 0.09$ (s.e.m). At the onset of movement, the
89 leading edge has a visibly thicker front (Fig. 1b 48 h). As the colony spreads, the thick front
90 disappears and small 'veins' radiating from the center develop. Over time, the 'veins' become
91 more pronounced towards the leading edge, making a 'petal' pattern (Figs. 2a, b). The leading
92 edge is characterized by a distinctive, terraced appearance comprised of three to six layers (Fig.
93 2c).

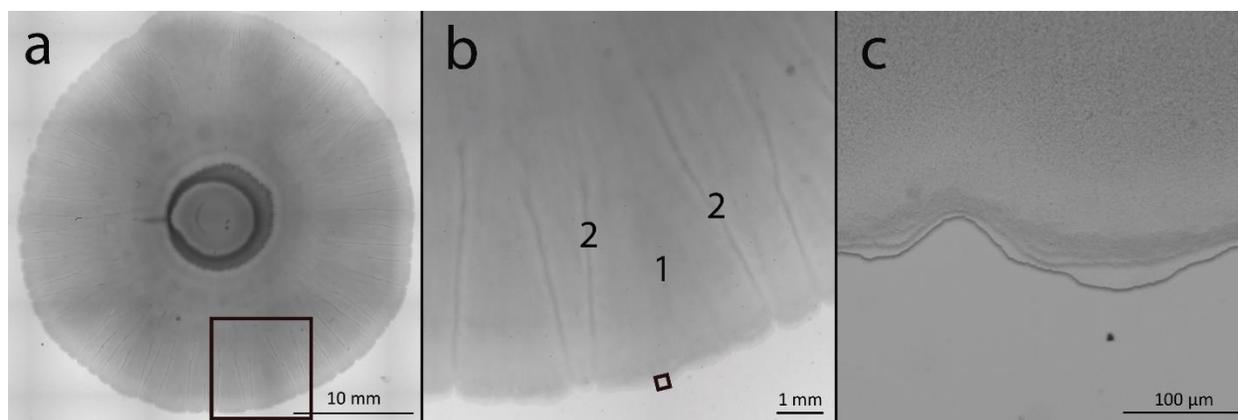


Figure 2. Mixed colony of *P. fluorescens* Pf0-1 and *Pedobacter* sp. V48 at different magnifications. a) Image of the whole co-culture colony created by stitching an 8X magnification mosaic. b) 16X magnification of the leading edge showing the patterns of 'petals' (1) in between 'veins' (2) visible near the edge of the colony c) 112X magnification shows a terraced appearance of the leading edge. Colony imaged 144 h after inoculation. White boxes indicate area enlarged in the adjacent panel. Scale bars are noted at the bottom of each image. Contrast and brightness levels were adjusted for optimal viewing.

94 ***P. fluorescens* Pf0-1 and *Pedobacter* sp. V48 co-migrate**

95 The previously observed 'bacterial expansion' in *Pedobacter* when interacting with
96 *Pseudomonas* sp. AD21 was suggested to be gliding motility, triggered as a mechanism to
97 escape competition from *P. fluorescens* (de Boer *et al.*, 2007; Garbeva and de Boer, 2009). We
98 examined the possibility that the motility observed when co-inoculating *Pedobacter* and *P.*
99 *fluorescens* Pf0-1 was a result of *Pedobacter* moving away from *P. fluorescens*. Bacteria were
100 collected from the center, middle, and edge of a seven-day-old motile colony. The presence or
101 absence of each species was tested by culturing these samples on selective media. We
102 recovered both species from each point in the motile colony (data not shown), showing co-
103 migration rather than an escape strategy by *Pedobacter*.

104 **Social motility shows reproducible spatial organization**

105 To obtain a more detailed look at the spatial relationships within the motile colony, we
106 tagged *P. fluorescens* with a cyan fluorescent protein (eCFP [Choi and Schweizer, 2006]) and
107 *Pedobacter* with a red fluorescent protein (dsRedEXPRESS [Choi and Schweizer, 2006]),
108 integrated into the chromosome. In *P. fluorescens*, eCFP carried by miniTni7 was integrated
109 upstream of *glmS* (Lambertsen *et al.*, 2004), creating Pf0-*ecfp*. In *Pedobacter*, dsRedEXPRESS
110 carried by the *HimarEm* transposon (Braun *et al.*, 2005) was integrated at random locations in
111 the chromosome, resulting in 16 independently-derived mutants with an insert. Each tagged
112 *Pedobacter* strain (V48-*dsRed*) was indistinguishable from the wild-type in social assays with *P.*
113 *fluorescens*, indicating no deleterious impact of the insertions. We picked one strain with an
114 insert in locus N824_RS25465 (GenBank accession NZ_AWRU01000034), and no apparent
115 defect in social motility. The initiation of social motility appeared slightly delayed in a mixture of
116 the tagged strains, but the visible patterns and stages of development looked identical, and
117 speed was not significantly different once movement initiated ($p = 0.0801$).

118 Fluorescent microscopy verified culturing data that showed both bacteria are present
119 throughout the motile colony, but we also found that population density varies across distinct
120 areas within the colony. These distribution patterns were highly reproducible and show six
121 distinct zones (Fig. 3). At zone 1, the point of inoculation, fluorescent imaging shows a
122 homogenous mix of both bacteria (Fig. 3b, c). Zone 2, the coffee ring formed at the edge of the
123 point of inoculation (Deegan *et al.*, 1997; Sempels *et al.*, 2013; Yanni *et al.*, 2017), is bright
124 orange, indicating that *Pedobacter* dominates this region (Fig. 3b). *Pedobacter* spreads out from
125 this dense area into zone 3, in a starburst pattern (Fig. 3b). Just outward from the starburst, we
126 see a blue ring in multiple experiments (zone 4), where *P. fluorescens* appears more abundant
127 (Figs. 3c, d). In the main body of the co-culture, a thin motile section spreads out, making
128 'petals' (Zone 5), with 'veins' (Zone 6) between them (Figs. 2b and 3a). The 'veins' between the
129 'petals' appear to have high *Pedobacter* populations (Fig. 3b), while the areas directly
130 surrounding them are dominated by *P. fluorescens* (Fig. 3c). The flat areas of the 'petals'
131 appear more well-mixed, though the red signal becomes difficult to detect toward the edge of
132 the colony (Fig. 3d). Overall, imaging data show that we can find both species throughout the
133 colony, but the distribution is not homogenous. Rather, we observed reproducible patterns with
134 some well-mixed areas and others of high spatial assortment.

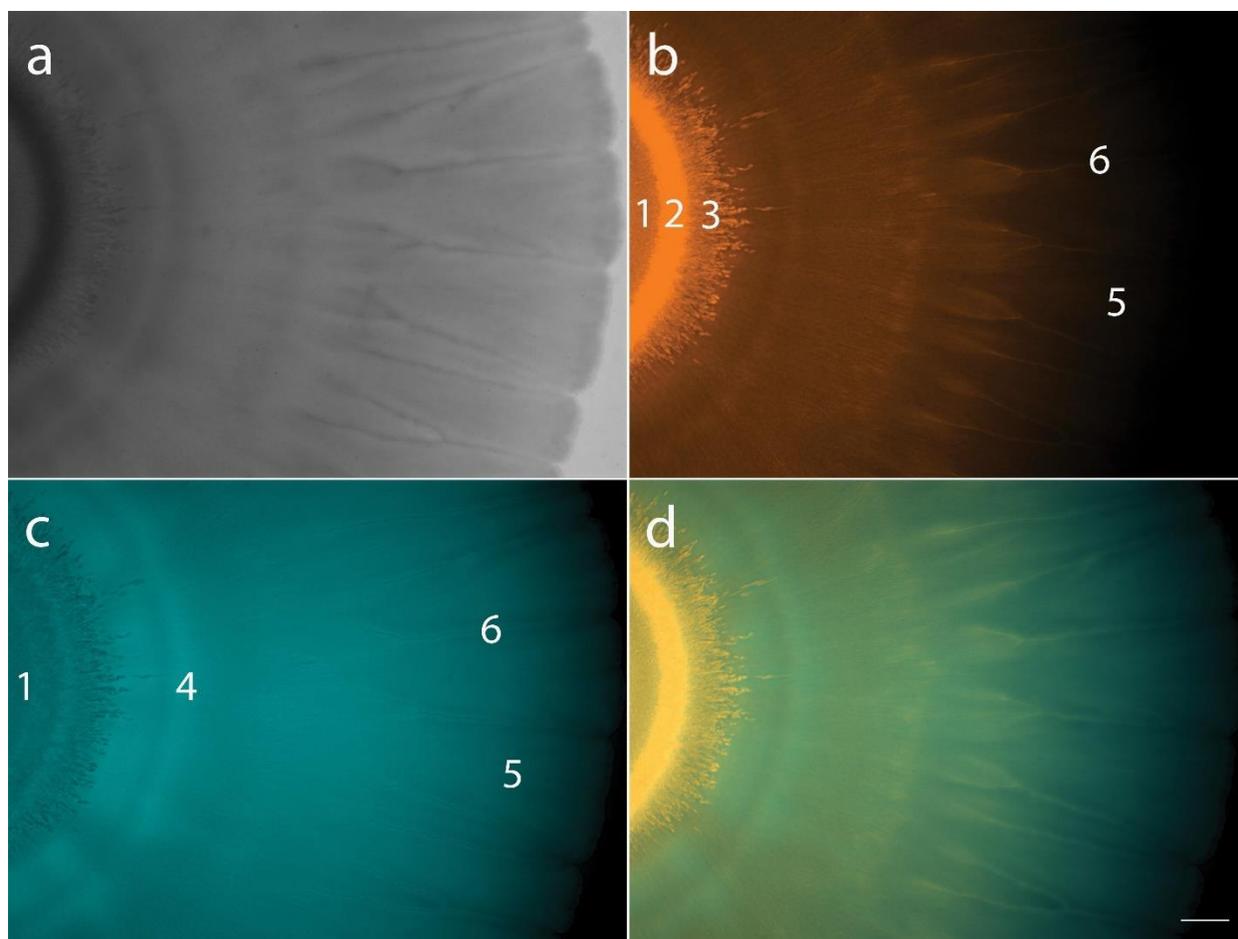


Figure 3. Mixed colony of Pf0-*ecfp* and V48-*dsRed*. a) Co-culture colony viewed with white light. b) Co-culture imaged using DsRed filter (filter set 43 HE), pseudo-colored in orange, showing V48-*dsRed* distribution throughout the colony. c) Co-culture imaged using CFP filter (filter set 47 HE), pseudo-colored in turquoise, showing Pf0-*ecfp* distribution throughout the colony. d) Merged images of DsRed and CFP filters. Numbers on panels b and c indicate six zones of distinct patterns: 1. Point of inoculation, 2. Coffee ring, 3. Starburst, 4. *P. fluorescens* ring, 5. Petals, 6. Veins. Colonies imaged at 7X magnification, scale bar represents 1 mm. Colony imaged 144 h after inoculation.

135 ***Physical association of P. fluorescens Pf0-1 and Pedobacter V48 is required for social***
136 ***motility***

137 Previous studies demonstrated interactions between *P. fluorescens* and *Pedobacter*
138 were mediated via both diffusible and volatile signals (Garbeva *et al.*, 2011a, 2011b, 2014). We
139 asked whether a close association between the two bacteria was a necessary condition for
140 social motility or whether signaling via diffusible compounds could trigger the movement. To
141 answer this question, we used assays in which the bacterial participants were plated side-by
142 side with no physical barrier and in which they were separated by semi-permeable membranes.

143 When colonies were adjacent, rather than mixed, no social motility was observed while
144 the *P. fluorescens* and *Pedobacter* colonies were visibly separate (data not shown). However,

145 once the colonies grew sufficiently to make contact (Fig. 4 24 h), the colony started to spread
146 out from the point of contact (72 h). The spreading front radiates outward (96 h), first developing
147 around the *P. fluorescens* colony (144 h), then proceeding to surround the *Pedobacter* colony
148 (192 h). At this level of resolution, contact between the colonies appears to occur before any
149 spreading can be seen.

150 Samples were collected from the edge of the moving front every 24 hours after contact,
151 both on a y-axis from the point of contact and following the moving front as it wrapped around
152 the *P. fluorescens* colony (Fig. 4). The presence of each species was tested by culturing these
153 samples on selective media. Both species were culturable at every point sampled (data not
154 shown), showing that *Pedobacter* is present in the moving front behind the *P. fluorescens*
155 colony (Fig. 4, 144 h), on the opposite side of where they initially came into contact. This
156 indicates that *Pedobacter* moves around the *P. fluorescens* colony on the motile front.

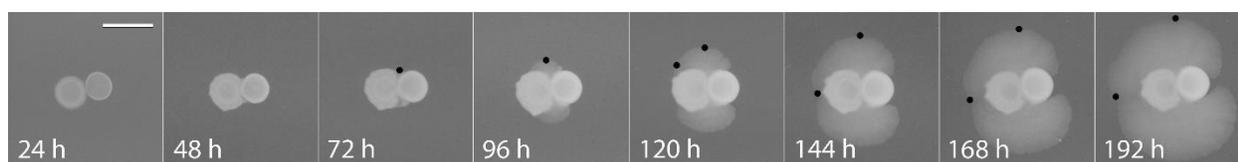


Figure 4. Social motility emerges after contact between colonies of *P. fluorescens* Pf0-1 (left) and *Pedobacter* sp. V48 (right). Colonies come into contact 24 hours after inoculation; the motile front becomes visible 48 hours after contact and spreads outward and around the *P. fluorescens* colony before surrounding the *Pedobacter* colony. Spots indicate sampling locations. Pictures taken every 24 h. Scale bar represents 10 mm.

157 To further evaluate the requirement that *P. fluorescens* and *Pedobacter* be physically
158 associated, we inoculated both strains immediately adjacent to each other but separated by
159 either semi-permeable mixed-ester cellulose or PES (polyethersulfone) membranes. When
160 inoculated this way, individual colony growth continued as normal, but these bacteria were
161 unable to trigger social motility despite their close proximity. After six days of growth, no sign of
162 social motility was observed (Fig. 5).

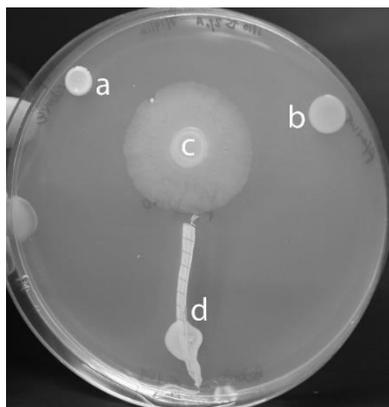


Figure 5. A semi-permeable barrier prevents development of the social motility phenotype. a) *Pedobacter* sp. V48 monoculture, b) *P. fluorescens* Pf0-1 monoculture, c) a mixed colony, and d) *P. fluorescens* and *Pedobacter* separated by a mixed-ester cellulose membrane. Pictures taken 144 h after inoculation. Colonies were grown on a 100 mm petri dish.

163 **Nutritional environment influences social motility**

164 Conditions in soil and rhizosphere environments fluctuate, with bacteria subjected to a
165 wide range of environmental stressors, including limited nutrient and water availability (Fierer,
166 2017). Because such fluctuations may influence expression of traits, we examined the effect of
167 nutrient level on social motility. Our standard assay condition, TSB-NK, consists of 10% strength
168 Tryptic Soy (3 g/L) supplemented with NaCl (5 g/L) and KH_2PO_4 (1 g/L) (Figs. 1b and 6b).

169 We first asked if social motility could initiate under richer nutrient conditions. No social
170 motility was apparent when *P. fluorescens* and *Pedobacter* were mixed on full-strength TSB (30
171 g/L) (Fig. 6a), with the co-culture exhibiting the same characteristics and colony expansion as
172 the *P. fluorescens* mono-culture. We next asked whether the salt amendments to TSB-NK
173 influence social motility, using assays without the addition of salts, and with the addition of NaCl
174 and KH_2PO_4 individually. When grown on 10% TSB, the co-culture is motile, but the distance
175 moved is modest compared to when the medium is supplemented with both salts (Fig. 6c). The
176 individual *P. fluorescens* colony expands similarly to the co-culture, suggesting minimal social
177 behavior under these conditions. Growth on TSB-K changes neither pattern nor rate of mono-
178 and co-culture expansion compared to 10% TSB (data not shown). On TSB-N, the mixed
179 culture spreads and develops the patterns characteristic of social motility, while the *P.*
180 *fluorescens* mono-culture does not expand (Fig. 6d). The phenotype and diameter of the
181 spreading colony are most similar to those observed in TSB-NK conditions (Fig. 6b).

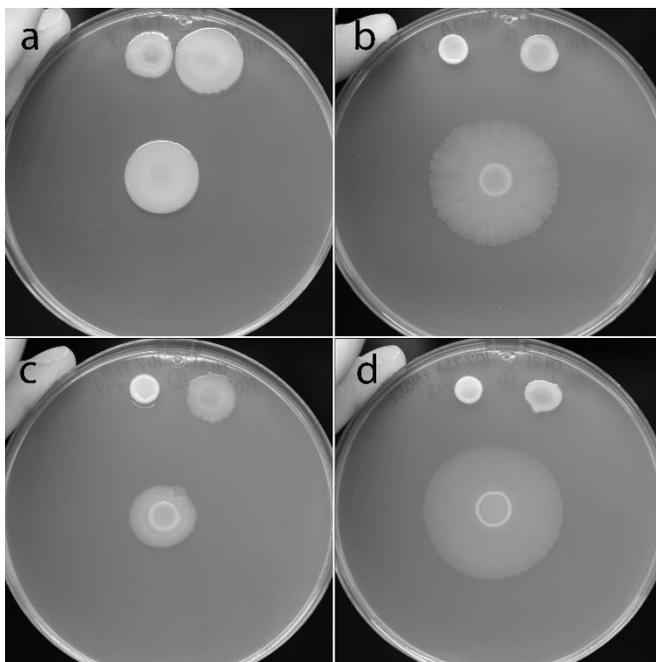


Figure 6. Low nutrient and high salt conditions are required for social motility. a) Mixed colony on full-strength TSB does not show social motility. b) Mixed colony on TSB-NK (10% Tryptic Soy supplemented with both NaCl and KH_2PO_4) shows social motility. c) Mixed colony on 10% strength TSB shows impaired social motility. d) Mixed colony on TSB-N (supplemented with NaCl) exhibits the social motility phenotype. For all panels *Pedobacter* sp. V48 mono-culture is on the upper left corner of the plate, *P. fluorescens* Pf0-1 is on the upper right corner of the plate, and the mixed colony is in the center. Pictures were taken 144 h after inoculation. Colonies were grown on a 100 mm petri dish.

182 In the previous experiment, we observed that variations of Tryptic Soy media led to
183 altered social phenotypes. To assess the influence of each component of TSB on social motility,
184 we utilized a medium in which these were individually manipulated. We made eight
185 combinations of media to vary D-glucose, tryptone, and NaCl in concentrations equivalent to
186 those in full-strength and 10% TSB. On media with D-glucose or tryptone at full-strength
187 concentrations, we did not observe social motility regardless of the concentration of the other
188 components (Figs. 7a-f). In these conditions, the appearance and expansion of the co-culture
189 resembled that of the *P. fluorescens* mono-culture, with notably greater biomass in media with
190 full-strength tryptone (Figs. 7a-d). When the concentration of all three components was reduced
191 to 10% we observed social motility, but the migration distance of the co-culture was modest,
192 and *P. fluorescens* mono-culture expanded to a similar extent (Fig. 7h). On media containing
193 10% strength D-glucose, 10% strength tryptone, and full-strength NaCl, social motility emerged
194 when *P. fluorescens* and *Pedobacter* were co-cultured (Fig. 7g). Unique to this condition, the
195 mono-cultures of both strains are immotile, indicating a dramatic change in behavior when
196 strains are mixed. The observations under this condition are most similar to those observed on
197 TSB-N and TSB-NK (Figs. 6b, d).

198 Based on these results, we conclude that full social motility expansion was only
199 observed in low nutrient medium supplemented with NaCl (Figs. 6b, d, and 7g). We observed
200 reduced social motility on low nutrient media without salt supplementation (Figs 6c and 7h), and
201 an absence of social behavior on rich media (Figs. 6a and 7a-f). While we can implicate salt as
202 an important factor in social motility, high salt concentrations alone are not sufficient to induce
203 social behavior, as we don't see social behavior under rich media conditions. This indicates that
204 there may be more than one important nutritional factor in the decision of these bacteria to
205 socialize.

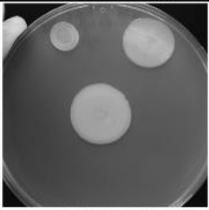
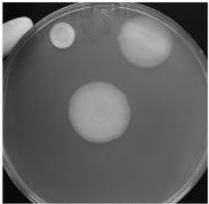
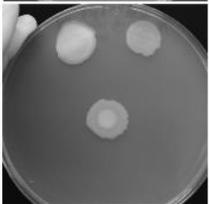
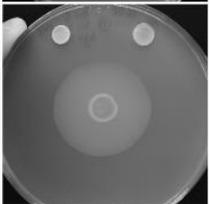
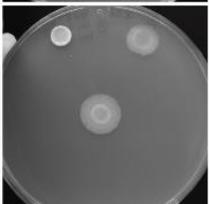
Glucose	Tryptone	NaCl	Phenotype
a High	High	High	
b High	High	Low	
c Low	High	High	
d Low	High	Low	
e High	Low	High	
f High	Low	Low	
g Low	Low	High	
h Low	Low	Low	

Figure 7. Role of nutrient levels in interaction between *P. fluorescens* Pf0-1 and *Pedobacter* sp. V48, looking at 3 core components of TSB: tryptone (20 g/L), D-glucose (2.5 g/L), and NaCl (5 g/L) for 'high' concentrations. Components were reduced to 1/10 for 'low' concentrations. For all panels *Pedobacter* mono-culture is on the upper left corner of the plate, *P. fluorescens* is on the upper right corner of the plate, and the mixed colony is in the center. Pictures were taken 144 h after inoculation. Colonies were grown on 100 mm petri dishes.

221 **Discussion**

222 In this study we investigate social motility, a phenomenon that emerges from the
223 interaction of two distantly-related soil bacteria. Neither species moves on its own, but a mixture
224 of the two species can spread across a hard agar surface (2%). Contact between the two
225 bacterial colonies is required for motility to initiate, and this association is maintained as the co-
226 culture expands. The social phenotype could be observed only under specific nutritional
227 conditions, indicating an interplay between environmental and biological factors. Our research
228 contributes to the growing body of work studying bacteria in social contexts to investigate
229 emergent traits and behaviors.

230 Surface motility is a trait that could be beneficial to bacteria under a range of
231 environmental conditions, particularly circumstances which are not permissive for moisture-
232 dependent translocation. Species related to *Pedobacter* sp. V48 use gliding motility on 1% agar
233 or glass surfaces (McBride, 2001, 2004; McBride and Zhu, 2013). However, V48 has not been
234 observed to engage in motility behaviors on its own. *P. fluorescens* Pf0-1 is capable of flagella-
235 driven swimming in and swarming motility on semi-solid agar (0.3% and 0.6% respectively)
236 without the need for a partner bacterium (Deflaun *et al.*, 1990; Seaton *et al.*, 2013). Social
237 motility is distinct from *Pseudomonas* flagellar motility in its requirement of the presence of a
238 second species. Additionally, media with higher agar percentages form environments that are
239 non-permissive for flagella-driven motility in *P. fluorescens*, as well as most species, but
240 together, Pf0-1 and V48 appear to employ an alternative strategy for movement across hard
241 surfaces.

242 De Boer *et al.* (2007) suggested that in water agar, the sporadic occurrence of
243 movement they observed indicated a strategy by *Pedobacter* to escape competition. However,
244 the co-migration under our conditions does not support this hypothesis, as the two species
245 remain associated throughout the colony. Our contact experiments provide further evidence, as
246 the presence of *Pedobacter* in the motile areas surrounding the *P. fluorescens* colony shows it
247 has moved towards its partner, rather than away from it. The pattern of *Pedobacter* migration
248 clearly indicates that it is not escaping.

249 Evidence, both from culturing and fluorescent imaging, shows that *P. fluorescens* and
250 *Pedobacter* co-migrate across the hard agar surface. Initiation of the process requires physical
251 contact, as motility is precluded when a semi-permeable membrane is placed between the two

252 colonies. We suggest that the nature of this interaction is distinct from contact-dependent toxin
253 delivery systems, such as type VI secretion and contact-dependent growth inhibition, as they
254 commonly mediate signal exchange between closely-related species, and are involved in
255 competition between more distantly-related strains (Saak and Gibbs, 2016; Gallique *et al.*, 2017;
256 Garcia, 2018). While our results do not rule out quorum sensing for communication between the
257 two species (Juhas *et al.*, 2005), a diffusible signal (if it exists) does not appear to be sufficient to
258 trigger the motility response. These data indicate that physical association is required for social
259 motility between *P. fluorescens* and *Pedobacter*.

260 Bacteria dwelling in soil experience variations in a wide range of abiotic conditions,
261 including the key parameters we have tested: salinity and available carbon and nitrogen
262 (Reviewed in Fierer, 2017). Environmental conditions have previously been shown to affect
263 motility of individual species; gliding motility in some *Flavobacterium* species increases with
264 reduced nutrient concentration (Pérez-Pascual *et al.*, 2009; Laanto *et al.*, 2012). Changes in
265 behavior resulting from environmental fluctuations can affect how species interact with one
266 another. The ability of *P. fluorescens* and *Pedobacter* to move socially is dependent upon the
267 conditions in which they are growing. In general, high concentrations of glucose and amino
268 acids led to a build-up of biomass and no apparent social movement. Lower glucose and amino
269 acid concentrations were associated with social motility across the plate, but decreasing the salt
270 concentration of the media slowed expansion of the colony. Social motility resulting from the
271 interaction is conditional, with alteration of just a subset of environmental factors resulting in
272 dramatic changes in behavior. It is tempting to speculate that the consortium of *P. fluorescens*
273 and *Pedobacter* can integrate signals from each other's presence and from the nutrient
274 conditions of their environment to determine whether to behave socially. We see similar
275 examples of intraspecies social behaviors being influenced both by biotic factors (quorum
276 sensing) and by abiotic factors (nutrient conditions) in *P. aeruginosa* (Boyle *et al.*, 2015),
277 *Bacillus subtilis* (Lazazzera, 2000), and yeast (Chen and Fink, 2006).

278 There is a wide variety of examples of motility resulting from interspecies interactions,
279 where the presence of a motile partner fosters the motility of an immotile participant. Non-motile
280 *Staphylococcus aureus* hitchhikes on swimming *P. aeruginosa* (Samad *et al.*, 2017) and
281 *Burkholderia cepacia* co-swarms with *P. aeruginosa* in environments where it cannot do so
282 independently (Venturi *et al.*, 2010). *X. perforans* induces motile *P. vortex* to swarm towards it,
283 which allows it to hitchhike on top of *P. vortex* rafts (Hagai *et al.*, 2014). *P. vortex* is also capable
284 of carrying fungal spores or antibiotic-degrading cargo bacteria to cross unfavorable

285 environments (Ingham *et al.*, 2011; Finkelshtein *et al.*, 2015). In an even more complex system,
286 *Dyella japonica* can migrate on fungal hyphae, but some strains can only do so in the presence
287 of a *Burkholderia terrae* helper (Warmink and van Elsas, 2009; Warmink *et al.*, 2011). All of
288 these examples of ‘hitchhiking’ phenomena stand in contrast to the behavior we have
289 investigated, where social motility emerges from two non-motile participants. The fact that both
290 species are present at the edge of the moving colony suggests that both have an active role in
291 the behavior, though it doesn’t rule out the possibility of one species inducing motility in the
292 other and hitchhiking, as seen in other systems (Hagai *et al.*, 2014).

293 In addition to describing a new mode of motility, this discovery highlights the possibility
294 that many functions and behaviors of bacteria in complex communities may be triggered by
295 interactions between different species or even domains. Studying interactions between two or
296 more microorganisms may lead to the discovery of emergent traits that would be impossible to
297 predict based on the study of each organism in isolation. Alongside approaches that
298 characterize the members and connectedness of microbial communities, tools to decipher the
299 phenotypic outcomes of interactions are needed in order to develop a full appreciation of
300 microbiomes. Studies of this type are important for understanding the role of microbial
301 communities within an ecological context.

302 We have investigated an interaction-dependent trait which emerges under particular
303 nutritional conditions when distantly-related bacteria come into close physical contact. This
304 social motility gives the participating bacteria the ability to spread on a hard agar surface, which
305 neither can do alone. This strategy of co-migration may serve as an additional mechanism by
306 which plant- and soil-associated bacteria can move in their natural environments, when the
307 conditions do not favor the modes of single-species motility previously described. Given the
308 distant and different locations from which these two strains were isolated, we hypothesize this is
309 not a unique interaction between this pair, but rather has evolved between various *Pedobacter*
310 and *Pseudomonas* species. To understand the phenomenon, several lines of investigation
311 should be pursued: mechanistic studies which explore the factors each species is contributing to
312 social motility, the process by which contact triggers motility, and the way in which
313 environmental conditions are integrated into the decision to move together. Such studies will
314 enable the application of our findings to the search for new examples of interaction-mediated
315 behaviors among bacteria.

316 **Materials and Methods**

317 ***Bacterial strains, primers, plasmids, and culture conditions.*** Bacterial strains and plasmids
318 are described in Table 1. *E. coli* was grown at 37°C in LB Broth, Miller (Fisher Scientific,
319 Hampton, NH, U.S.A.). *Pseudomonas fluorescens* Pf0-1 and *Pedobacter* sp. V48 were routinely
320 grown at 30°C or 20°C respectively, in 10% strength Tryptic Soy Broth (BD Difco™, Franklin
321 Lakes, NJ, U.S.A.) amended with NaCl and KH₂PO₄, as described by de Boer (2007). This
322 medium is referred to throughout the text as TSB-NK. To differentiate the two species from
323 mixed cultures we used *Pseudomonas* minimal medium (PMM) with 25 mM succinate (Kirner *et*
324 *al.*, 1996) for *P. fluorescens* and 14.6 mM lactose for *Pedobacter*. Media were solidified with
325 1.5% BD Difco™ Bacto™ agar (w/v) when required, except for social motility assays, for which
326 2% agar was used. For experiments with variations in nutrients, we used full-strength TSB (30
327 g/L), 10% TSB (3 g/L), and 10% TSB amended with NaCl or KH₂PO₄ (called TSB-N or TSB-K,
328 respectively), and a medium composed of D-glucose (2.5 g/L), tryptone (20 g/L), and NaCl (5
329 g/L). These individual components were used at those concentrations or reduced to 10%
330 concentration in all eight combinations. For selection of transposon insertions carrying
331 fluorescent protein genes, Kanamycin (50 µg/mL), Gentamicin (50 µg/mL), or Erythromycin (100
332 µg/mL) was added to the growth medium.

333 ***Social Motility Assays.*** *P. fluorescens* and *Pedobacter* for use in social motility assays were
334 incubated in 2mL TSB-NK at 25°C for 24 hours, with shaking (160rpm). Social assays were
335 carried out on TSB-NK solidified with 2% agar. Plates were poured at a temperature of 62°C in
336 a single layer and allowed to set for ~15 minutes before inoculation. Inoculation was done on
337 freshly-poured plates.

338 (i) Mixed inoculum assays. Assays were started by combining 5 µL of each participant in one
339 spot on the agar surface. As controls, 10µL spots of each bacterial isolate were plated distant
340 from each other and the co-culture, all on the same plate. Once the inoculation liquid had dried,
341 plates were incubated at 20°C. Measurements of the colony diameter were taken every 24
342 hours. Experiments were performed in triplicate.

343 (ii) Direct contact assay - adjacent plating. *P. fluorescens* and *Pedobacter* were grown as
344 described above. The aliquots of bacteria were plated adjacent but without the drops touching.
345 Once the inoculation liquid had dried, plates were incubated at 20°C and monitored daily to

346 determine the time at which colony growth led to contact between the isolates, and when
347 motility phenotypes developed.

348 (iii) Direct contact assay - separation by membranes. *P. fluorescens* and *Pedobacter* were
349 plated close together, separated only by a membrane. Either Millipore Polyethersulfone (PES)
350 Express Plus® Membrane (0.22µm pores) (Darmstadt, Germany), or Gelman Sciences mixed-
351 ester cellulose Metrical Membrane (0.45µm pores) (East Lansing, MI, U.S.A.) were cut into
352 rectangular strips and sterilized by autoclaving. These strips were then embedded into the agar
353 by suspending them perpendicular to the bottom of petri dishes with forceps, as agar was
354 poured into plates. Once set, the filters protruded approximately 5mm above the agar surface.
355 Bacteria were inoculated on either side of the filter, with 5µL spots of each species, close
356 enough to touch the filter.

357 **Fluorescent protein tagging**

358 (i) eCFP labeling of *P. fluorescens*. pUC18T-mini-Tn7T-Gm-*ecfp* was a gift from Herbert
359 Schweizer (Addgene plasmid # 65030). A constitutively-expressed fluorescent protein gene
360 carried by pUC18T-mini-Tn7T-Gm-*ecfp* was transferred to *P. fluorescens* by conjugation from *E.*
361 *coli* S17-1, with transposase being provided by pUX-BF13 introduced from a second *E. coli*
362 S17-1 donor, as previously described (Monds *et al.*, 2006). Transposon-carrying strains were
363 selected by growth on Gentamicin (50 µg/mL), and transposition of the miniTn7 element into the
364 target site in the *P. fluorescens* genome was confirmed by PCR using primers Tn7-F and *glmS*-
365 R (Table 2). Pf0-1 with fluorescent inserts were tested for alteration in social motility by co-
366 culturing with *Pedobacter*, as described above.

367 (ii) dsRedEXPRESS labeling *Pedobacter*. pUC18T-mini-Tn7T-Gm-*dsRedExpress* was a gift
368 from Herbert Schweizer (Addgene plasmid #65032). To express *dsRedEXPRESS* in
369 *Pedobacter*, a *Pedobacter* promoter was cloned upstream of the *dsRedEXPRESS* coding
370 sequence. A highly expressed gene from an unpublished RNAseq experiment was identified
371 (N824_RS25200) and the upstream 320 bp were amplified from *Pedobacter* genomic DNA
372 using primers *PompA* and *dsRed*, designed for splicing-by-overlap extension-PCR (SOE-PCR)
373 (Table 2). The promoter was then spliced with the amplified *dsRedEXPRESS* coding sequence
374 using SOE-PCR (Horton *et al.*, 1989). Flanking primers were designed with *KpnI* restriction
375 sites, enabling cloning of the spliced product into a *KpnI* site in *pHimarEm1* (Braun *et al.*, 2005).
376 To join compatible ends between the plasmid and the amplicons, we used T4 DNA ligase (New

377 England Biolabs, Inc. Ipswich, MA, U.S.A.). The ligated plasmid was introduced into *E. coli* S17-
378 1 competent cells by electroporation (BioRad Micropulser™, Hercules, CA, U.S.A.). S17-1
379 colonies carrying the plasmid were selected by plating on LB medium containing Kanamycin (50
380 µg/mL), and the presence of the *dsRedEXPRESS* gene was confirmed by PCR, using *pHimar*
381 *KpnI*-flank primers (Table 2). The resulting plasmid is called *pHimarEm1-dsRed*.

382 *pHimarEm1-dsRed* was transferred to *Pedobacter* by conjugation using a method adapted from
383 Hunnicutt and McBride, 2000. Briefly, 20 hour old cultures of *E. coli* S17-1 (*pHimarEm1-dsRed*)
384 and *Pedobacter* were subcultured 1:100 into fresh LB, and grown to mid-exponential phase (*E.*
385 *coli*) or for 7 hours (*Pedobacter*). Cells were collected by centrifugation, suspended in 100µL of
386 LB, and then mixed in equal amounts on TSB-NK with 100 µL of 1M CaCl₂ spread on the
387 surface. Following overnight incubation at 30°C, cells were scraped off the surface of the plate,
388 and dilutions were plated on TSB-NK with Erythromycin (100 µg/mL) to select for strains that
389 received the plasmid (*ermF* is not expressed in *E. coli*). Transconjugants were incubated at
390 25°C for 3-4 days. Presence of the transposon in *Pedobacter* was confirmed using *ermF*
391 primers (Table 2).

392 The transposon insertion sites in the *Pedobacter* chromosome were amplified by arbitrarily-
393 primed PCR (Caetano-Anollés, 1993), using a method adapted from O'Toole *et al.*, 1999 (see
394 table 2 for primers), and identified by sequencing the arb-PCR products. Nucleic acid
395 sequencing was performed by Massachusetts General Hospital CCIB DNA Core (Cambridge,
396 MA, U.S.A.). Sequences were analyzed using CLC Genomics Workbench Version 10.1.1
397 (QIAGEN, Hilden, Germany) to find location of transposon integration.

398 **Imaging**

399 Still pictures were taken using an EOS Rebel T3i camera (Canon, Tokyo, Japan) and processed
400 using Photoshop CC 2017 Version: 2017.0.1 20161130.r.29 (Adobe, San Jose, CA, U.S.A.).
401 Using this software, the levels of some images were adjusted to improve contrast, and pictures
402 were converted to greyscale.

403 For microscopy, motile colonies were examined using an Axio Zoom.V16 microscope (Zeiss,
404 Oberkochen, Germany). To visualize fluorescent strains, filter set 43 HE DsRed was used with a
405 1.5 s exposure, shown with pseudo-color orange, as well as filter set 47 HE Cyan Fluorescent
406 Protein, with a 600 ms exposure, shown with pseudo-color turquoise. Images were captured

407 using Axiocam 503 mono camera, with a native resolution of 1936x1460 pixels. For image
408 acquisition and processing we used Zen 2 Pro software (Zeiss).

409 **Statistics**

410 We measured the amount of colony expansion of the mono-cultures of both *P. fluorescens* and
411 *Pedobacter* and the expansion of social motility in co-culture. Colony diameter of three
412 independent experiments was measured every 24 hours. To compare the diameter of mono-
413 cultures and co-cultures at each time point, we performed a two-way ANOVA followed by a
414 Bonferroni post-hoc test.

415 We compared the movement speed between a combination of wild type *P. fluorescens* and
416 *Pedobacter* to a combination of fluorescently-tagged Pf0-ecfp and V48-dsRed. Colony diameter
417 of six independent experiments were measured every day, and speed was calculated by
418 dividing the distance traveled by the amount of time elapsed since the last time point. To
419 calculate average speed, we only used time points after social motility phenotype developed. To
420 compare the means of the speed of the wild-type and tagged strains, we conducted an
421 unpaired, two-tailed, Student's t-test.

422 **Acknowledgements**

423 This study was supported in part by the Agriculture and Food Research Initiative Competitive
424 grant 2010-65110-20392 from the USDA's National Institute of Food and Agriculture. LMM was
425 supported by a University of Massachusetts Dartmouth Distinguished Doctoral Fellowship.

426 **Table 1. Bacterial strains and Plasmids**

Strain	Genotype or Description	Source or Reference
<i>E. coli</i>		
S17-1	<i>recA thi pro hsdR</i> RP4-2-Tc::Mu-Km::Tn7 λ pir Sm ^r Tp ^r	Simon, Prierer and Puhler, 1983
<i>P. fluorescens</i>		
Pf0-1	Wild type, Ap ^r	Compeau <i>et al.</i> , 1988
Pf0-ecfp	Pf0-1::mini-Tn7 <i>ecfp</i> Gm ^r	This study
<i>Pedobacter</i>		
V48	Wild type	De Boer <i>et al.</i> , 2003
V48- <i>dsRed</i>	V48 N824_RS25465T899::HimarEm1 <i>PompA-dsRed</i> EXPRESS Em ^r NCBI Accession: NZ_AWRU00000000.1, as of September 25, 2017	This study
Plasmids		
pUC18T-mini-Tn7T-Gm- <i>dsRed</i> EXPRESS	Gm ^r	Choi & Schweizer, 2006
pUC18T-mini-Tn7T-Gm- <i>ecfp</i>	Gm ^r	Choi & Schweizer, 2006
pUX-BF13	R6K replicon-based helper plasmid carrying Tn7 transposase genes	Bao <i>et al.</i> , 1991
pHimarEm1	Plasmid carrying <i>HimarEm1</i> ; Km ^r (Em ^r)	Braun <i>et al.</i> , 2005
pHimarEm1- <i>dsRed</i>	pHimarEm1 Ω (3.529kb::PompA- <i>dsRed</i> EXPRESS)	This study

427

428 **Table 2. Primers**

Primers	Sequence (5'-3')	Purpose	Source or Reference
Tn7 F	5'-CAGCATAACTGGACTGATTCAG - 3'	Verify integration of transposon into chromosomal <i>glmS</i> locus	Monds <i>et al.</i> , 2006
<i>glmS</i> R	5'-TGCTCAAGGGCACTGACG-3'	"	Monds <i>et al.</i> , 2006
<i>PompA-dsRed</i> F	5'ACGTTCTCGGAGGAGGCCATCAAC GCAACAAAAGAACTGC 3'	Amplification of N824_RS25200 promoter to join with <i>dsRed</i> gene	This study
<i>PompA</i> R	5'-TATGGTACC AGTCATCTAGCGGCTGTAG-3'	" Includes KpnI-site for inserting into pHimarEm1	This study
<i>dsRed</i> F	5'-TACTCAGGAGAGCGTTCACC-3'	Amplification of <i>dsRed</i> gene with no promoter, to join with V48 N824_RS25200 promoter by SOE PCR	This study
<i>dsRed</i> R	5'-GCAGTTTCTTTTGTTCGTTGATGGC CTCTCCGAGAACGT-3'	" Includes KpnI-site for inserting into pHimarEm1	This study
pHimar KpnI-flank F	5'-CTGCCCTGCAATCGACCTCG-3'	Verify ligation of <i>dsRed</i> into pHimarEm1	This study
pHimar KpnI-flank R	5'-CAGATAGCCCAGTAGCTGAC-3'	"	This study
<i>erm</i> F	5'-CCGCACCCAAAAAGTTGCAT-3'	Verify integration of transposon into V48 chromosome.	This study
<i>erm</i> R	5'-GACAATGGAACCTCCCAGAA-3'	"	This study
ARB1	5'-GGCCACGCGTCGACTAGTACNNNNN NNNNNGATAT-3'	Find location of transposon integration in V48 chromosome.	O'Toole <i>et al.</i> , 1999
ARB6	5'-GGCCACGCGTCGACTAGTACNNNNN NNNNACGCC-3'	"	O'Toole <i>et al.</i> , 1999
ARB2	5'-GGCCACGCGTCGACTAGTAC -3'	"	O'Toole <i>et al.</i> , 1999
Himar Arb1 (TnExt)	5'-GTGTTGTTCCAGTTTGAGATC-3'	"	This study
Himar609 Arb2 (TnInt)	5'-TGGGAATCATTGAAGGTTGG-3'	"	Braun <i>et al.</i> , 2005

429

430 References

- 431 Abreu NA, Taga ME. (2016). Decoding molecular interactions in microbial communities. *FEMS*
432 *Microbiol Rev* **40**: 648–663.
- 433 Blasche S, Kim Y, Oliveira AP, Patil KR. (2017). Model microbial communities for ecosystems
434 biology. *Curr Opin Syst Biol* **6**: 51–57.
- 435 de Boer W, Wagenaar A-M, Klein Gunnewiek PJA, van Veen JA. (2007). In vitro suppression of
436 fungi caused by combinations of apparently non-antagonistic soil bacteria. *FEMS Microbiol*
437 *Ecol* **59**: 177–185.
- 438 Boyle KE, Monaco H, van Ditmarsch D, Deforet M, Xavier JB. (2015). Integration of metabolic
439 and quorum sensing signals governing the decision to cooperate in a bacterial social trait.
440 *PLoS Comput Biol* **11**: 1–26.
- 441 Braun TF, Khubbar MK, Saffarini DA, McBride MJ, Aacteriol JB. (2005). *Flavobacterium*
442 *johnsoniae* gliding motility genes identified by *mariner* mutagenesis. *J Bacteriol* **187**: 6943–
443 6952.
- 444 Caetano-Anollés G. (1993). Amplifying DNA with arbitrary oligonucleotide primers. *PCR*
445 *Methods Appl* **3**: 85–94.
- 446 Chen H, Fink GR. (2006). Feedback control of morphogenesis in fungi by aromatic alcohols.
447 *Genes Dev* **20**: 1150–1161.
- 448 Choi KH, Schweizer HP. (2006). mini-Tn7 insertion in bacteria with single attTn7 sites: Example
449 *Pseudomonas aeruginosa*. *Nat Protoc* **1**: 153–161.
- 450 Deegan RD, Bakajin O, Dupont TF, Huber G, Nagel SR, Witten TA. (1997). Capillary flow as the
451 cause of ring stains from dried liquid drops. *Nature* **389**: 827–829.
- 452 Deflaun MF, Tanzer AS, Mcateer AL, Levy SB, Marshall B, Levy SB. (1990). Mutant of
453 *Pseudomonas fluorescens* development of an adhesion assay and characterization of an
454 adhesion-deficient mutant of *Pseudomonas fluorescens*. *Appl Environ Microbiol* **56**: 112–
455 119.
- 456 Fierer N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome.
457 *Nat Rev Microbiol* **15**: 579–590.
- 458 Finkelshtein A, Roth D, Ben Jacob E, Ingham CJ. (2015). Bacterial swarms recruit cargo
459 bacteria to pave the way in toxic environments. *MBio* **6**: 1–10.

- 460 Gallique M, Bouteiller M, Merieau A. (2017). The type VI secretion system: A dynamic system
461 for bacterial communication? *Front Microbiol* **8**: 1–10.
- 462 Garbeva P, de Boer W. (2009). Inter-specific interactions between carbon-limited soil bacteria
463 affect behavior and gene expression. *Microb Ecol* **58**: 36–46.
- 464 Garbeva P, Hordijk C, Gerards S, de Boer W. (2014). Volatile-mediated interactions between
465 phylogenetically different soil bacteria. *Front Microbiol* **5**: 1–9.
- 466 Garbeva P, Silby MW, Raaijmakers JM, Levy SB, de Boer W. (2011a). Transcriptional and
467 antagonistic responses of *Pseudomonas fluorescens* Pf0-1 to phylogenetically different
468 bacterial competitors. *ISME J* **5**: 973–985.
- 469 Garbeva P, Tyc O, Remus-Emsermann MNP, van der Wal A, Vos M, Silby M, *et al.* (2011b). No
470 apparent costs for facultative antibiotic production by the soil bacterium *Pseudomonas*
471 *fluorescens* Pf0-1. *PLoS One* **6**: e27266.
- 472 Garcia EC. (2018). Contact-dependent interbacterial toxins deliver a message. *Curr Opin*
473 *Microbiol* **42**: 40–46.
- 474 Grandchamp GM, Caro L, Shank EA. (2017). Pirated siderophores promote sporulation in
475 *Bacillus subtilis*. *Appl Environ Microbiol* **83**: e03293-16.
- 476 Hagai E, Dvora R, Havkin-Blank T, Zelinger E, Porat Z, Schulz S, *et al.* (2014). Surface-motility
477 induction, attraction and hitchhiking between bacterial species promote dispersal on solid
478 surfaces. *ISME J* **8**: 1147–51.
- 479 Horton RM, Hunt HD, Ho SN, Pullen JK, Pease LR. (1989). Engineering hybrid genes without
480 the use of restriction enzymes: gene splicing by overlap extension. *Gene* **77**: 61–68.
- 481 Hunnicutt DW, McBride MJ. (2000). Cloning and characterization of the *Flavobacterium*
482 *johnsoniae* gliding-motility genes *gldB* and *gldC*. *J Bacteriol* **182**: 911–918.
- 483 Ingham CJ, Kalisman O, Finkelshtein A, Ben-Jacob E. (2011). Mutually facilitated dispersal
484 between the nonmotile fungus *Aspergillus fumigatus* and the swarming bacterium
485 *Paenibacillus vortex*. *Proc Natl Acad Sci* **108**: 19731–19736.
- 486 Jones SE, Ho L, Rees CA, Hill JE, Nodwell JR, Elliot MA. (2017). Streptomyces exploration is
487 triggered by fungal interactions and volatile signals. *Elife* **6**: 1–21.
- 488 Juhas M, Eberl L, Tümmler B. (2005). Quorum sensing: The power of cooperation in the world
489 of *Pseudomonas*. *Environ Microbiol* **7**: 459–471.

- 490 Kirner S, Krauss S, Sury G, Lam ST, Ligon JM, Pee K V. (1996). The non-haem
491 chloroperoxidase from *Pseudomonas fluorescens* and its relationship to pyrrolnitrin
492 biosynthesis. *Microbiology* **142**: 2129–2135.
- 493 Laanto E, Bamford JKH, Laakso J, Sundberg LR. (2012). Phage-Driven Loss of Virulence in a
494 Fish Pathogenic Bacterium. *PLoS One* **7**. e-pub ahead of print, doi:
495 10.1371/journal.pone.0053157.
- 496 Lambertsen L, Sternberg C, Molin S. (2004). Mini-Tn7 transposons for site-specific tagging of
497 bacteria with fluorescent proteins. *Environ Microbiol* **6**: 726–732.
- 498 Lazazzera BA. (2000). Quorum sensing and starvation: signals for entry into stationary phase.
499 *Curr Opin Microbiol* **3**: 177–182.
- 500 Liu Y, Kyle S, Straight PD. (2018). Antibiotic stimulation of a *Bacillus subtilis* migratory
501 response. *mSphere* **3**: e00586-17.
- 502 McBride MJ. (2001). Bacterial gliding motility: multiple mechanisms for cell movement over
503 surfaces. *Annu Rev Microbiol* **55**: 49–75.
- 504 McBride MJ. (2004). *Cytophaga-flavobacterium* gliding motility. *J Mol Microbiol Biotechnol* **7**:
505 63–71.
- 506 McBride MJ, Zhu Y. (2013). Gliding motility and por secretion system genes are widespread
507 among members of the phylum *Bacteroidetes*. *J Bacteriol* **195**: 270–278.
- 508 Monds RD, Newell PD, Schwartzman JA, O'Toole GA. (2006). Conservation of the Pho regulon
509 in *Pseudomonas fluorescens* Pf0-1. *Appl Environ Microbiol* **72**: 1910–1924.
- 510 O'Toole GA, Pratt LA, Watnick PI, Newman DK, Weaver VB, Kolter R. (1999). Genetic
511 approaches to study of biofilms. *Methods Enzymol* **310**: 91–109.
- 512 Pérez-Pascual D, Menéndez A, Fernández L, Méndez J, Reimundo P, Navais R, *et al.* (2009).
513 Spreading versus biomass production by colonies of the fish pathogen *Flavobacterium*
514 *psychrophilum*: Role of the nutrient concentration. *Int Microbiol* **12**: 207–214.
- 515 Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH. (2013). Going back to the
516 roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol* **11**: 789–799.
- 517 Powers MJ, Sanabria-Valentin E, Bowers AA, Shank EA. (2015). Inhibition of cell differentiation
518 in *Bacillus subtilis* by *Pseudomonas protegens*. *J Bacteriol* **197**: 2129–2138.
- 519 Saak CC, Gibbs KA. (2016). The self-identity protein IdsD is communicated between cells in

- 520 swarming *Proteus mirabilis* colonies. *J Bacteriol* **198**: 3278–3286.
- 521 Samad T, Billings N, Birjiniuk A, Crouzier T, Doyle PS, Ribbeck K. (2017). Swimming bacteria
522 promote dispersal of non-motile staphylococcal species. *ISME J* **11**: 1933–1937.
- 523 Seaton SC, Silby MW, Levy SB. (2013). Pleiotropic effects of GacA on *Pseudomonas*
524 *fluorescens* Pf0-1 in vitro and in soil. *Appl Environ Microbiol* **79**: 5405–5410.
- 525 Sempels W, De Dier R, Mizuno H, Hofkens J, Vermant J. (2013). Auto-production of
526 biosurfactants reverses the coffee ring effect in a bacterial system. *Nat Commun* **4**: 1757.
- 527 Seyedsayamdost MR, Traxler MF, Zheng S-L, Kolter R, Clardy J. (2011). Structure and
528 biosynthesis of amyachelin, an unusual mixed-ligand siderophore from *Amycolatopsis* sp.
529 AA4. *J Am Chem Soc* **133**: 11434–11437.
- 530 Stubbendieck RM, Straight PD. (2015). Escape from lethal bacterial competition through
531 coupled activation of antibiotic resistance and a mobilized subpopulation. *PLoS Genet* **11**:
532 e1005722.
- 533 Traxler MF, Seyedsayamdost MR, Clardy J, Kolter R. (2012). Interspecies modulation of
534 bacterial development through iron competition and siderophore piracy. *Mol Microbiol* **86**:
535 628–644.
- 536 Traxler MF, Watrous JD, Alexandrov T, Dorrestein PC, Kolter R. (2013). Interspecies
537 interactions stimulate diversification of the *Streptomyces coelicolor* secreted metabolome.
538 *MBio* **4**: e00459-13.
- 539 Venturi V, Bertani I, Kerényi Á, Netotea S, Pongor S. (2010). Co-swarming and local collapse:
540 Quorum sensing conveys resilience to bacterial communities by localizing cheater mutants
541 in *Pseudomonas aeruginosa*. *PLoS One* **5**: e9998.
- 542 Warmink JA, van Elsas JD. (2009). Migratory response of soil bacteria to *Lyophyllum* sp. strain
543 Karsten in soil microcosms. *Appl Environ Microbiol* **75**: 2820–2830.
- 544 Warmink JA, Nazir R, Corten B, van Elsas JD. (2011). Hitchhikers on the fungal highway: The
545 helper effect for bacterial migration via fungal hyphae. *Soil Biol Biochem* **43**: 760–765.
- 546 Yanni D, Kalziqi A, Thomas J, Ng SL, Vivek S, Ratcliff WC, *et al.* (2017). Life in the coffee-ring:
547 how evaporation-driven density gradients dictate the outcome of inter-bacterial competition.
548 *arXiv*. <http://arxiv.org/abs/1707.03472>.
- 549