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SHORT COMMUNICATION

Surface-motility induction, attraction and hitchhiking between bacterial species promote dispersal on solid surfaces

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The ability to move on solid surfaces provides ecological advantages for bacteria, yet many bacterial species lack this trait. We found that *Xanthomonas* spp. overcome this limitation by making use of proficient motile bacteria in their vicinity. Using *X. perforans* and *Paenibacillus vortex* as models, we show that *X. perforans* induces surface motility, attracts proficient motile bacteria and 'rides' them for dispersal. In addition, *X. perforan*s was able to restore surface motility of strains that lost this mode of motility under multiple growth cycles in the lab. The described interaction occurred both on agar plates and tomato leaves and was observed between several xanthomonads and motile bacterial species. Thus, suggesting that this motility induction and hitchhiking strategy might be widespread and ecologically important. This study provides an example as to how bacteria can rely on the abilities of their neighboring species for their own benefit, signifying the importance of a communal organization for fitness.

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Bacteria colonize surfaces of various environments and are often dependent on surface motility for survival. Such a mode of motility allows bacteria to escape local stresses, translocate to a better nutritional environment and efficiently invade host tissue (Fraser and Hughes, 1999; Rashid and Kornberg, 2000; Harshey, 2003). However, despite the benefits, for many bacterial species the ability to migrate on solid surfaces does not exist or is dependent on high surface wetness and thus, restricted to specific environmental conditions (Wang *et al*., 2005; Kearns, 2010). The latter is true for the phytopathogenic bacteria from the genus *Xanthomonas*, which rely on high relative humidity for movement (Diab *et al*., 1982). We examined the interaction of *X. perforans* with several bacterial species that posses the ability to migrate across solid surfaces, focusing on *Paenibacillus vortex*. *Paenibacillus* spp. are found in many diverse environments and are often associated with plants (McSpadden Gardener, 2004, this study). Many of them including *P. vortex* are proficiently motile, able to migrate on solid surfaces containing $\geqslant 1.5\%$ agar, with no dependence on media type (Ingham and Ben Jacob, 2008).

On co-inoculation *P. vortex* exhibited a strong migration toward *X. perforans.* Directional migration was observed on rich (Figures 1a and b), as well as poor media (Supplementary Figure S1). Examination of *P. vortex* movement over time indicated that the migration speed of both control and exposed colonies was not significantly different (Supplementary Table S1). However, in the presence of *X. perforans*, *P. vortex* colonies commenced movement earlier (Figures 1c–f) and spread to greater distances (2.56 ± 0.4-fold) than control colonies (Figures 1g, h and k). Notably, the induction of movement in exposed colonies compared with control also occurred when the two bacterial colonies were separated by a plastic barrier of a bipartite plate, indicating that the effect of *X. perforans* on *P. vortex* surface motility was mediated by an airborne substance (Figures 1i, j and l; Supplementary Figure S2).

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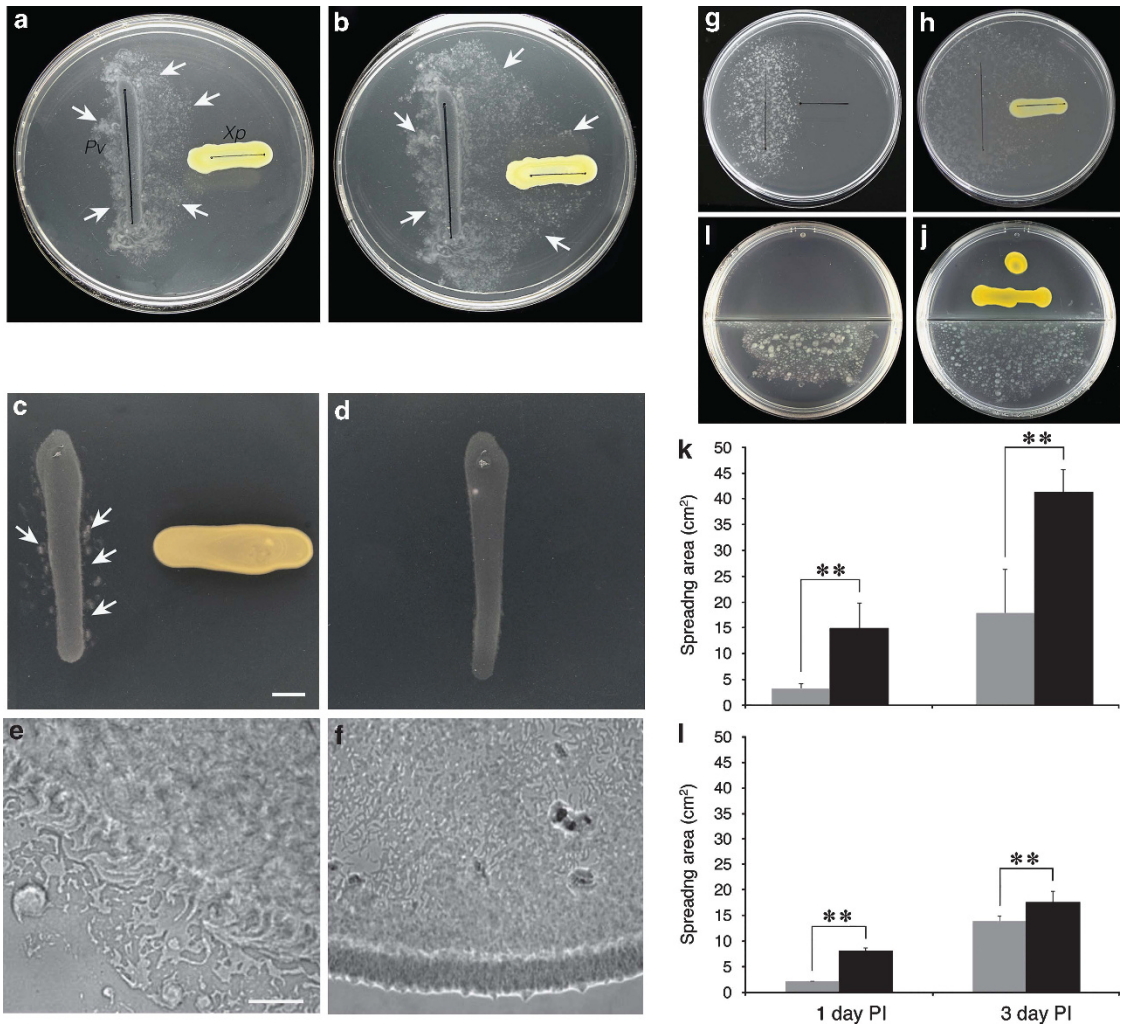

Figure 1 The effect of *X. perforans* on *P. vortex* surface motility: directional migration of *P. vortex* toward *X. perforans* at 20 (**a**) and 48 h (**b**) post *P. vortex* inoculation. White arrows designate *P. vortex* colony edges. (**c**–**f**) Movment commencement of *P. vortex* in the presence (**c**) or absence of *X. perforans* (**d**). Light microscope image of *P. vortex* colony's edge in the presence (**e**) or absence of *X. perforans* (**f**). Migrational area of *P. vortex* in the presence (**h**) or (**g**) absence of *X. perforans* in regular (**g**, **h** and **k**) and (**i**, **j** and **l**) bipartite petri plates. In (**k** and **l**) black bar, presence of *X. perforans*; gray bar, control. (**) in (**k**) 1 day $P = 0.00003$; 3 days $P = 0.007$. (**) in (**l**) 1 day $P = 0.02$, 3 days $P = 0.005$. Mean ± s.e.m. plotted; $n = 5$; two-tailed *t*-test performed. Plate diameter 9 cm. (**c** and **e**) Scale bars are 0.5 cm and 100 μm, respectively.

Bacterial cell density could have a profound effect on the time point of movement commencement. In order to examine whether the induced movement of exposed *P. vortex* colonies was due to an increased growth rate and thus a higher cell density, *P. vortex* cells of exposed and control colonies were extracted from agar and analyzed by an image-stream cytometer, measuring both cell number and length of thousands of cells in each run. Interestingly, although *P. vortex* cells exposed to *X. perforans* headspace commenced movement earlier than control, cell numbers of exposed samples were actually lower (2.7 ± 0.7-fold) than those of control samples (Supplementary Figure S3a). Conversely, mean cell length was higher in *P. vortex* colonies exposed to *X. perforans* headspace (Supplementary Figure S3b). Such attributes could result from incomplete cell division and elongation of a subpopulation, a phenomenon previously shown to be characteristic of migrating *P. vortex* colonies (Ingham and Ben Jacob, 2008). Indeed, image-stream cytometer analysis indicated that the fraction of cells longer than 15 μm was significantly higher (3.0 ± 0.2-fold) in exposed *P. vortex* colonies compared with control (Supplementary Figure S3c).

The fact that *X. perforans* active substances induced motility of *P. vortex* in rich as well as poor media and that *P. vortex* cell numbers were not higher in exposed colonies compared with control, suggest that the affecting substance is not involved in *P. vortex* metabolism and likely serves as a cue. This hypothesis was further strengthened by the fact that *X. perforans* was able to induce surface motility in *P. vortex* colonies that stopped exhibiting this mode of motility under laboratory conditions (Supplementary Movie S1). Loss of surface motility

after multiple growth cycles in the laboratory has been reported for several bacteria (Velicer *et al.*, 1998; Henderson *et al.*, 1999; Kearns *et al.*, 2004). The mechanisms responsible for this loss are beyond the scope of this study. Nonetheless, the fact that a non-motile culture regained this ability on exposure to *X. perforans* points out that the switch between the two phenotypes can be modulated by external factors, and can work in both directions as proposed to occur in phase variations (Velicer *et al.*, 1998; Henderson *et al.*, 1999; Kearns and Losick, 2003; Kearns *et al.*, 2004). The possibility that the external factors affecting surface motility can be signals produced by neighboring species raises many new questions regarding the mechanism that governs surface motility in nature.

In order to assess the prevalence of the described interaction, we examined the effect of additional bacteria from various genera, as well as of additional *Xanthomonas* species from differing pathovars on *P. vortex*. Response of additional bacterial species to *X. perforans* was also examined. All xanthomonads tested affected *P. vortex* surface motility similarly to *X. perforans* (Supplementary Table S1), but phytopathogenic bacteria from other genera had no effect (Supplementary Figure S4). Surface motility of the following bacterial species was not affected by *X. perforans: Bacillus subtilis* 3610, swrA/sfp complement strain of *B. subtilis* 168, *Escherichia coli* K-12 and *Azospirillum brasilense*, all of which typically swarm on surfaces containing less than 1% agar (data not shown). However, a positive effect was observed with *Paenibacillus dendritiformis* (data not shown) and *Proteus mirabilis* cells, which have the ability to disperse on relatively dry solid surfaces of $\geqslant$1.5% agar (Supplementary Figure S5). Moreover, the active substance in *X. perforans* headspace was able to restore swarming motility in *P. mirabilis* colonies that lost this phenotype under laboratory conditions, in a similar manner to that described for *P. vortex* (Supplementary Figure S6). We also performed isolations of bacteria from tomato plants. Only bacteria that were able to move on 1.5% agar plates were examined. Two bacterial strains were isolated and according to their 16SrDNA sequences were identified as *Flavobacterium* sp. and *Paenibacillus* sp. (Supplementary Table S1). Out of these two isolates, only *Paenibacillus* sp. exhibited enhanced surface motility in response to *X. perforans* (data not shown).

The induction of surface motility and attraction of such proficiently motile bacteria could hold a benefit, visible on a petri dish, for *X. perforans.* This bacterium lacks the ability to migrate across nutrient 1.5% agar plates (Shen *et al.*, 2001, also see Figure 1). However, in co-inoculation experiments with *P. vortex*, *X. perforans* began to massively spread on the plates (Figures 2a and c). In scanning electron microscope images of the co-migration areas on the plate, *X. perforans* cells (slightly wider in diameter and shorter in length) were visible on top of the *P. vortex* rafts appearing as single cells on top of these rafts at the edges of the colonies but more as aggregates in the denser areas of both colonies (Figures 2b and d).

In addition, scanning electron microscope analysis of *X. perforans* and *P. vortex* colonies on detached tomato leaf surface (major hosts for *X. perforans* infection) indicated that when both species were co-inoculated on leaves, a massive spread of cells occurred between the two colonies by 6 days post inoculation, (Supplementary Figure S7). Whereas when *X. perforans* cells were inoculated alone on the leaf, no bacterial cell spreading was observed at that time (data not shown). Notably, scanning electron microscope analysis could not provide definite proof for migration of both species, as when on leaves, both bacterial species were covered with a thick matrix, making species differentiation based on cell size and spatial organization not accurate (Supplementary Figures S7a and c). However, by using membrane-fluorescent stained *X. perforans* cells inoculated on tomato leaves, we were able to show *X. perforans* cell dispersion dependent on *P. vortex* presence (Figures 2e–m; Supplementary Figure S8). When inoculated alone or in the presence of *E. coli* bacteria, fluorescent *X. perforans* cells remained at the point of inoculation, even during prolong incubations of up to 7 days (Figures 3i and j; Supplementary Figures S8a and b). However, after 5 days of co-inoculation with non-fluorescent *P. vortex* on the major leaf vein, a spread of fluorescent *X. perforans* cells was visible between the two colonies (Figures 2l and m). When co-inoculation was performed between the minor leaf veins, a dispersal zone of fluorescent *X. perforans* cells was visible after 7 days (Supplementary Figure S8d).

The importance of surface motility for epiphytic survival and host infection was demonstrated in several studies (Haefele and Lindow, 1987; Harshey, 2003; Lindow and Brandl, 2003). Thus, the benefit that this interaction could provide to *X. perforans* is clear. As to the responding bacteria the consequences of this relationship is less clear. It was shown that resident bacteria on leaves could enhance the survival of immigrant bacteria by modifying the microenvironment of the leaf surface (Monier and Lindow, 2005; Poza-Carrion *et al.*, 2013). It is possible that by 'helping' *X. perforans* reach openings on the leaf surface, *P. vortex* might obtain more nutrients owing to the increased activity of plant-degrading exoenzymes employed by *X. perforans* on infection. This, yet to be proven, could determine whether this interaction is commensal or mutualistic.

Surface motility is a costly trait; this study suggests that in natural environments the interactions between bacteria that are able to migrate on solid surfaces and those unable, are not random but involve coordinated events, which enable a group of bacteria to spare the cost but obtain the end product

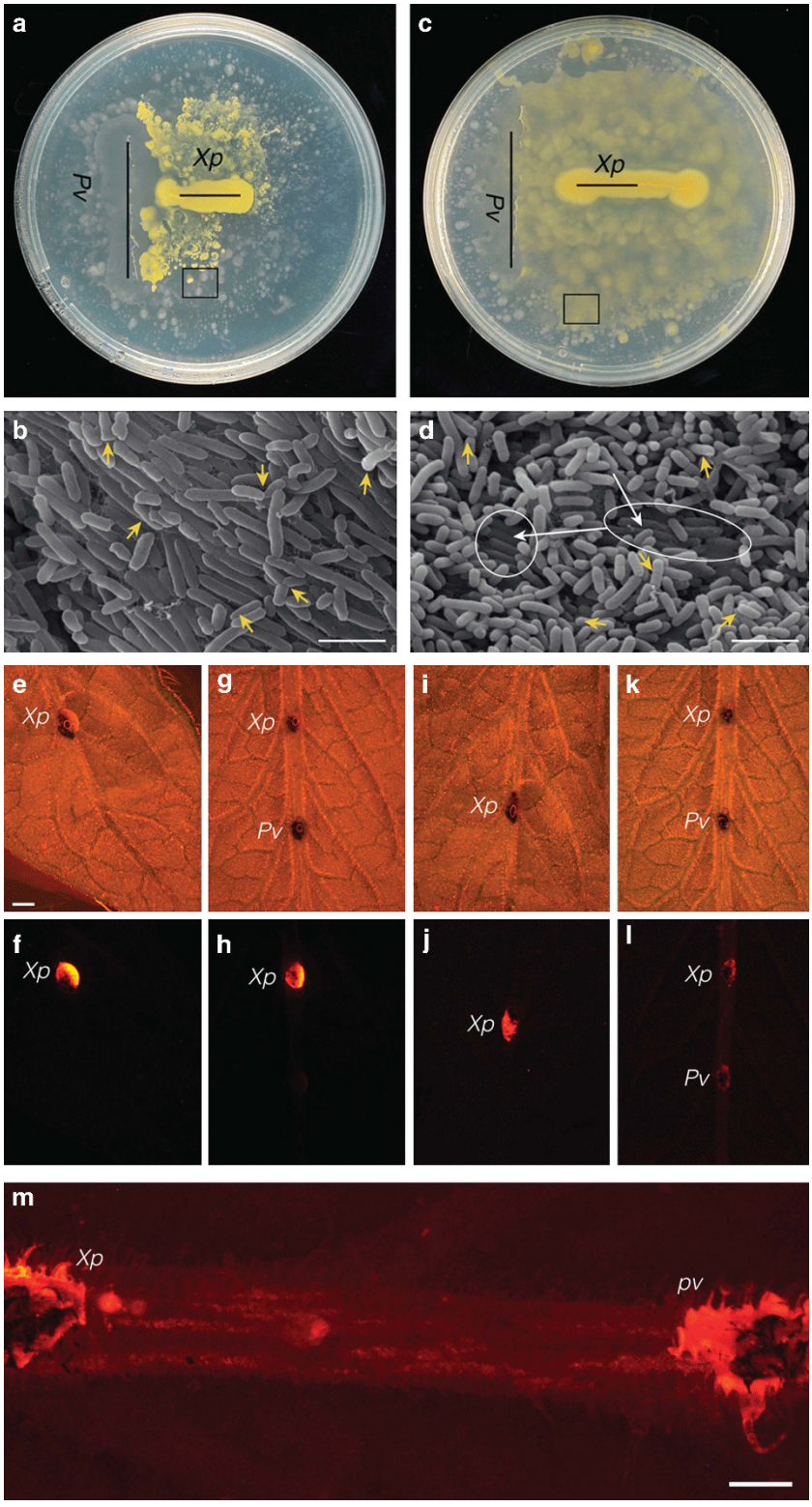

Figure 2 Co-inoculation of *X. perforans* and *P. vortex* results in dispersal of *X. perforans* cells. Co-inoculation on the 1.5% nutrient agar plate (**a** and **b**) at 60 and 90 h (**c** and **d**) post *P. vortex* inoculation. (**b** and **d**) Scanning electron microscope images of areas in squares depicted in (**a**) and (**c**), respectively. Scale bar, 2.5 μm. White arrows indicate *P. vortex* rafts; yellow arrows indicate *X. perforans* 'riders'. Co-inoculation on tomato leaves. Bright-field (**e**, **g**, **i** and **k**) and fluorescent (**f**, **h**, **j**, **l** and **m**) images of fluorescent stained *X. perforans* (*Xp*), inoculated on the surface of the major vein of tomato leaves, in the presence (**g**, **h**, **k**, **l** and **m**) or absence (**e**, **f**, **i** and **j**) of *P. vortex* (*Pv*). (**e**–**h**) Images taken at 2 days post inoculation. (**i**–**m**) Images taken at 5 days post bacterial inoculation. (**e**) Scale bar, 2 mm; (**m**) scale bar, 1 mm. (**m**) A higher magnification of (**l**) showing the fluorescent trail between the colonies. Brown dots visible in the bright-field images are colors marked on the leaf to indicate the point of inoculation.

of surface motility. Such interactions emphasize the importance of a community's phenotypic richness in addition to that of the individual's for the benefit of its members.

Conflict of Interest

The authors declare no conflict of interest.

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Author contributions

YH led the project, designed experiments and wrote the paper together with SS. EH designed and performed experiments and wrote the paper. RD, TH-B EZ and ZP designed and performed experiments.

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