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SHORT COMMUNICATION

Swimming bacteria promote dispersal of non-motile staphylococcal species

Tahoura Samad[1,3], Nicole Billings[1,3], Alona Birjiniuk[2,3], Thomas Crouzier[1], Patrick S Doyle[2] and Katharina Ribbeck[1]

[1]*Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA and* [2]*Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*

Swimming motility is considered a beneficial trait among bacterial species as it enables movement across fluid environments and augments invasion of tissues within the host. However, non-swimming bacteria also flourish in fluid habitats, but how they effectively spread and colonize distant ecological niches remains unclear. We show that non-motile staphylococci can gain motility by hitchhiking on swimming bacteria, leading to extended and directed motion with increased velocity. This phoretic interaction was observed between *Staphylococcus aureus* and *Pseudomonas aeruginosa, Staphylococcus epidermidis and P. aeruginosa,* as well as *S. aureus* and *Escherichia coli*, suggesting hitchhiking as a general translocation mechanism for non-motile staphylococcal species. By leveraging the motility of swimming bacteria, it was observed that staphylococci can colonize new niches that are less available in the absence of swimming carriers. This work highlights the importance of considering interactions between species within polymicrobial communities, in which bacteria can utilize each other as resources.

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Bacteria often exist as polymicrobial communities in a multitude of environments (Fernandez *et al*., 2000; Hosni *et al*., 2011; Burmølle *et al*., 2014). Here we investigate the ways in which bacteria in the same community may affect each other's motility in liquid environments. Swimming motility offers a considerable advantage for bacteria by enabling movement toward environments of favorable conditions (Stocker *et al*., 2008; Dennis *et al*., 2013), and movement away from toxins or predators (Adler, 1966; Berg, 1975). Non-flagellated bacteria do not have the capacity to independently translocate with this mechanism. The genus *Staphylococcus*, for example, is classically considered non-motile in fluid environments due to the lack of flagella (Kloos and Bannerman, 1994; Freney *et al*., 1999). Despite their limitations in motility, staphylococcal species effectively reach and thrive in their preferred ecological niches.

We tested if non-motile species may benefit from the swimming motility of flagellated bacteria. To address this, we studied two human pathogens that are found in the same ecological habitats, but rely on different mechanisms for translocation. *Staphylococcus aureus* is a Gram-positive, non-motile cocci species and *Pseudomonas aeruginosa* is a Gram-negative, flagellated rod species capable of swimming motility. Using a standardized biofilm assay (Ceri *et al*., 1999), a vertical insert was placed into a microwell containing bacterial inoculum. The insert does not touch the bottom or sides of the microwell, thereby creating two distinct niches for potential colonization: the first at the bottom of the well, and the second at the top of the inoculum (air–liquid interface) on the lateral surface of the insert (Figure 1a). We expected non-motile *S. aureus* to settle and form a biofilm at the bottom of the microwell and motile *P. aeruginosa* to build a biofilm at the air-liquid interface, which requires upward swimming. Figures 1b and c show that indeed, *P. aeruginosa* readily colonized the air-liquid interface while *S. aureus* was largely absent from this location in monoculture. However, when the two species were co-cultured in the same microwell, significantly more *S. aureus* cells were isolated from the air-liquid interface. On average, there were 6-fold more *S. aureus* cells isolated 30 seconds after initiation of biofilm formation, and 16-fold more after 16 hours (Figure 1b). Together, these results indicate that colonization of this niche was strongly enhanced by the presence of *P. aeruginosa.* A similar trend was observed with a 100 times higher inoculation density of *S. aureus* cells, with 30-fold more *S. aureus* cells at the air-

Correspondence: K Ribbeck, Department of Biological Engineering, Massachusetts Institute of Technology, 25 Ames St, MIT, Cambridge, MA 02139, USA.
E-mail: ribbeck@mit.edu
[3]These authors contributed equally to this work.
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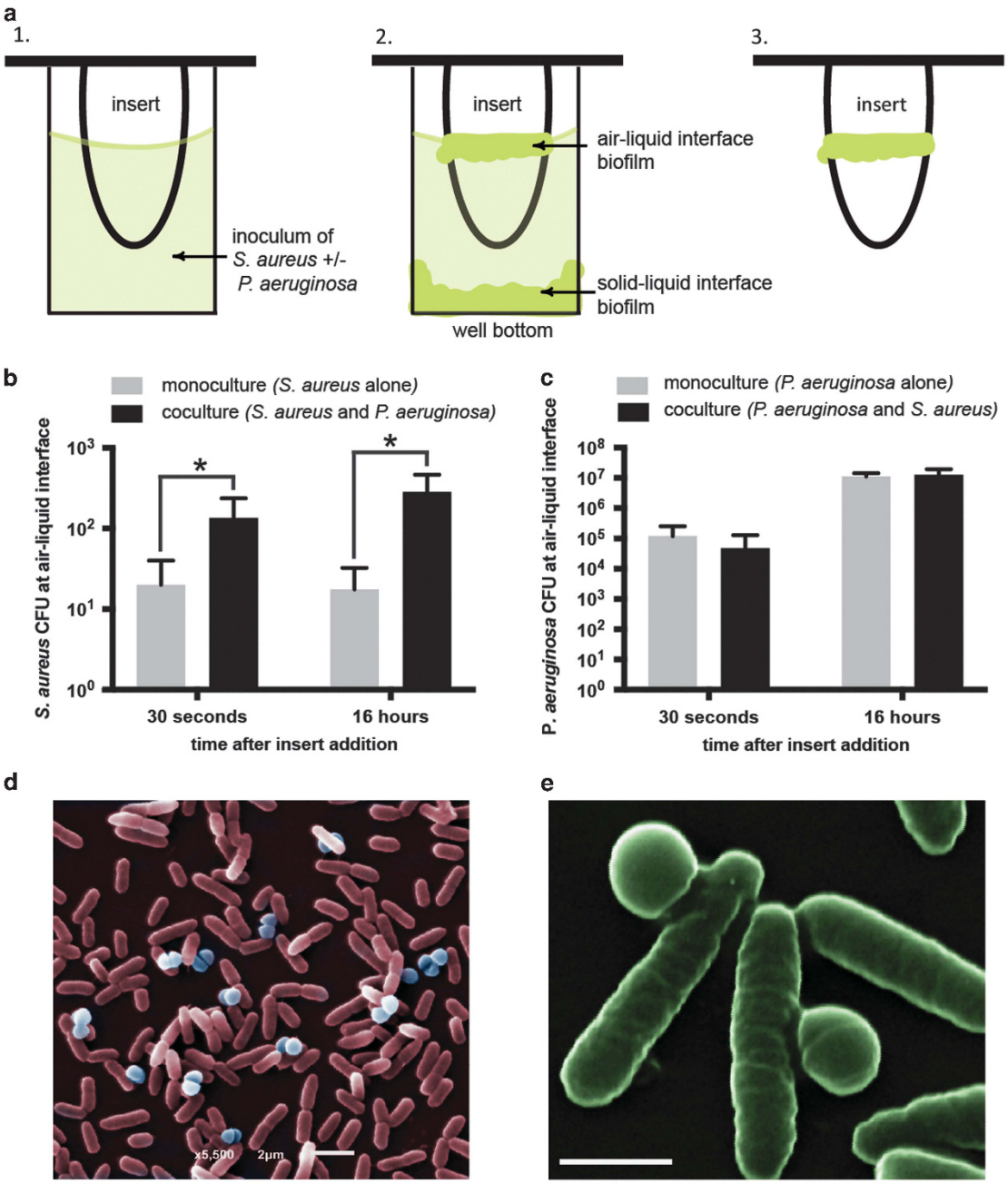

Figure 1 *P. aeruginosa* augments *S. aureus* localization to air-liquid interface. (**a**) Inoculum containing *S. aureus* with or without *P. aeruginosa* is added to a microwell and allowed to settle for one hour before a polystyrene insert is gently placed into the inoculum (**a**, panel 1). This set up provides two niches where the bacteria can colonize over time, the bottom of the well and the lateral surface of the insert at the air–liquid interface (**a**, panel 2). After 30 seconds or 16 hours, the insert is removed to isolate cells that colonize the air–liquid interface (**a**, panel 3) (**b**) *S. aureus* CFUs at the air-liquid interface at 30 seconds and 16 hours after addition of the insert. An initial inoculation density of ~ 10^6 cells per ml for *S. aureus* and ~ 10^8 cells per ml for *P. aeruginosa* (100:1 *P. aeruginosa* to *S. aureus* ratio) was used. The presence of *P. aeruginosa* increases colonization of the air-liquid interface by *S. aureus* at both time points. Mean ± s.d. ($n = 4$). *$P < 0.05$ (30 seconds $P = 0.0098$; 16 hours $P = 0.0016$). (**c**) *P. aeruginosa* CFUs are not affected by the presence of *S. aureus*. (**d**, **e**) False colored scanning electron micrographs of *P. aeruginosa* (rod cells) and *S. aureus* (spherical cells) at the air-liquid interface after 2 h revealed onset of co-localization during the initial attachment stage of biofilm development. Scale bars in **d** and **e** represent 2 and 1 µm, respectively.

liquid interface after 30 seconds, and 3-fold more cells at the same location 16 hours after inoculation (Supplementary Figure 1A). For comparison, *P. aeruginosa* cell numbers at the air-liquid interface were largely unaffected by the presence of *S. aureus* (Figure 1c; Supplementary Figure 1B). Scanning electron micrographs of cells from vertical inserts confirmed co-localization of *P. aeruginosa* and *S. aureus* in the biofilm at the air-liquid interface after 2 h (Figures 1d and e). Taken together, these data suggest *S. aureus* has acquired, through *P. aeruginosa,* an increased capacity to travel longer

distances, allowing it to colonize niches that are relatively inaccessible in the absence of swimming carrier bacteria.

We hypothesized that the increased colonization of the air-liquid interface by *S. aureus* may be due to hitchhiking of *S. aureus* on *P. aeruginosa*. To evaluate this possibility, fluorescently labeled *P. aeruginosa* and *S. aureus* were combined at equal numbers and placed in a microchamber (Supplementary Figure 2) for observation by live confocal

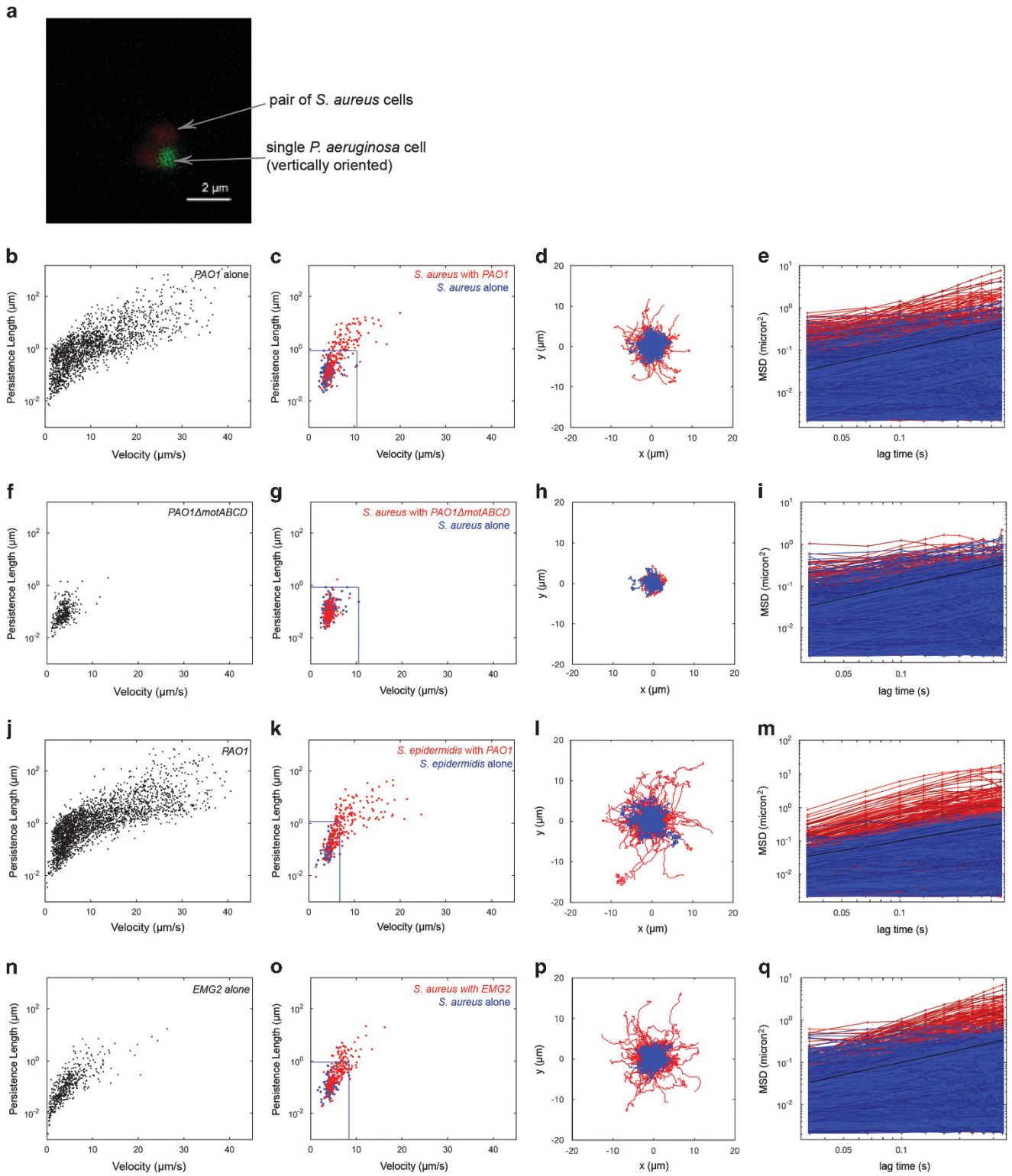

Figure 2 For caption see next page.

microscopy. When imaged concurrently, *S. aureus* cells were observed associated to *P. aeruginosa* cells (Figure 2a) and moving together for a period of time (Supplementary Video 1).

To further quantify the motility of *S. aureus* in the absence and presence of *P. aeruginosa,* single cell tracking was employed on videos obtained of the cells in single and dual species cultures. For tracking experiments, we used *P. aeruginosa* PAO1-eGFP and *S. aureus* stained with hexidium iodide. From the resultant cell trajectories, we calculated persistence length (defined as the length scale of decay for angular autocorrelation of a trajectory, which provides a measurement of how linear a trajectory is), velocity and mean squared displacement (MSD). *S. aureus* showed random motion patterns (Supplementary Video 2) with MSDs similar to a sphere undergoing Brownian motion in liquid medium (Supplementary Figure 3). For comparison, *P. aeruginosa* exhibited a run-reverse motility pattern consistent with previous accounts of swimming motility among this species (Supplementary Video 3). *P. aeruginosa* also exhibited greater velocities and persistence lengths than non-motile *S. aureus* (Figure 2b; Supplementary Figure 4). *S. aureus* trajectories changed distinctly when *P. aeruginosa* was present, with persistence lengths increased by an order of magnitude compared to values obtained for *S. aureus* in monoculture (Figures 2c and d; Supplementary Video 4). Cell trajectories set to start at coordinate (0,0), plotted for *S. aureus* alone (Figure 2d; blue) and *S. aureus* mixed with *P. aeruginosa* (Figure 2d; red) illustrate the extended and directed motion of *S. aureus* when *P. aeruginosa* was present. Comparing the MSD of *S. aureus* in the absence (Figure 2e; blue) and presence (Figure 2e; red) of *P. aeruginosa,* a shift was observed toward increased directed motility for *S. aureus* when *P. aeruginosa* was present. These *S. aureus* trajectories are superdiffusive (Supplementary Figure 5), which has previously been suggested for bacteria undergoing flagellar motility (Matthäus *et al*., 2009). For comparison, the trajectories of *S. aureus* in the presence of non-motile *P. aeruginosa* mutants, PAO1Δ*motABCD* and PAO1Δ*flgE,* did not measurably deviate from those of *S. aureus* alone (Figures 2f–i; Supplementary Figure 6; Supplementary Videos 5 and 6), further suggesting that the extended motility of *S. aureus* is dependent on the swimming motility of *P. aeruginosa*.

It was also observed that *P. aeruginosa* can carry another staphylococcal cargo*, Staphylococcus epidermidis. S. epidermidis* trajectories in the presence of *P. aeruginosa* were characterized by longer persistence lengths and increased velocity compared with *S. epidermidis* alone (Figures 2j–m; Supplementary Figures 5B and E; Supplementary Video 7). Moreover, *P. aeruginosa* was capable of transporting carboxylated polystyrene beads (Supplementary Videos 8–11). Together, these results suggest that *P. aeruginosa* can engage with a variety of cargos with distinct biochemistries, presumably through different types of chemical interactions. Another motile carrier species, *E. coli* EMG2 also has the ability to carry staphylococci as cargo (Figures 2n–q; Supplementary Figures 5C and F; Supplementary Video 12). Collectively, these data may support a generalized mechanism for translocation among staphylococcal species via interaction with flagellated bacteria.

The findings presented here quantitatively indicate that staphylococcal species, classically defined as non-motile, have altered motility patterns in the presence of flagellated *P. aeruginosa* and *E. coli.* Specifically, *P. aeruginosa* and *E. coli* may function as microbial carriers for staphylococcal species and result in enhanced dispersal range in fluid environments. The carrier-dependent movement described here appears mechanistically distinct from previously described spreading of *S. aureus* on surfaces, which occurs independent of a second, swimming bacterium (Kaito and Sekimizu, 2007; Pollitt *et al*., 2015).

While there exists a wealth of information regarding flagella-mediated bacterial self-propulsion

Figure 2 Swimming bacteria alter the motility of non-motile Staphylococci. (**a**) Confocal image of *S. aureus* attached to *P. aeruginos*a taken from a time-lapse confocal microscopy series. Scale bar represents 2 μm. (**b**) Individual trajectories of *P. aeruginosa* alone in culture plotted on axes of persistence length vs velocity. (**c**) The trajectories of *S. aureus* alone (blue), and of *S. aureus* in the presence of *P. aeruginosa* (co-culture) (red) were also plotted on axes of persistence length vs velocity. The blue box indicates the upper bound of persistence length and velocity for *S. aureus* alone. There is a cluster of trajectories in the co-culture condition with persistence lengths several orders of magnitude higher than those in the central cluster for the *S. aureus* alone. (**d**) The actual traces represented in (c) are re-centered to begin at coordinate (0,0), using the same color code as **c**. There is a set of trajectories in the co-culture condition that are further reaching and more linear than those of *S. aureus* alone. (**e**) The MSD of all individual trajectories, using the same color code as **c**. There is an increase in motility of the *S. aureus* in co-culture, as seen by the higher MSDs. (**f**) Individual trajectories of PAO1Δ*motABCD,* a non-motile *P. aeruginosa* mutant, alone in culture, exhibiting smaller persistence lengths and velocities than *P. aeruginosa*. (**g**) The trajectories of *S. aureus* alone (blue), and of *S. aureus* in the presence of PAO1Δ*motABCD* (red). The motility pattern of the *S. aureus* is preserved in the co-culture with PAO1Δ*motABCD*, confirming that the altered *S. aureus* motility is dependent on the swimming motility of *P. aeruginosa*. The blue box indicates the upper bound of persistence length and velocity for the *S. aureus* alone (**h**) The actual traces represented in **g** are re-centered to begin at coordinate (0,0), using the same color code as **g**. *S. aureus* trajectories in the presence of *P. aeruginosa* do not look different then trajectories of *S. aureus* alone in terms of extent and linearity (**i**) MSDs of *S. aureus* in co-culture with PAO1Δ*motABCD* compared to *S. aureus* alone are not appreciably different. (**j–m**) Repeating the analysis in **a–d** for another non-motile staphylococci cargo, *S epidermidis*, alone (blue) and in the presence of *P. aeruginosa* (red). *S. epidermidis* trajectories were more linear and further reaching in the presence of *P. aeruginosa*, suggesting that the hitchhiking observed is generalizable to members of genus Staphylococcus. (**n–q**) Repeating the analysis in **a–d** with *S. aureus* as the cargo, but with a different motile carrier, *E. coli* EMG2. *S. aureus* alone is shown in blue, while *S. aureus* in the presence of *E. coli* is shown in red.

(Lauga and Powers, 2009), in most natural environments, bacteria are part of polymicrobial communities (Sibley *et al.*, 2008; Consortium, 2012) and the contributions of interspecies phoretic interactions to bacterial dispersal in aqueous environments are not well understood. The ability of non-motile bacterial species to leverage motility from other bacteria has been observed to occur between microbes on the surface of plants, and in soil (Hagai *et al.*, 2014; Finkelshtein *et al.*, 2015). The primary motivation of this work is to highlight the impact of microbial hitchhiking on translocation and distribution of non-motile bacteria in liquid. We found that this phoretic mobility could directly change the localization patterns of staphylococci and open new niches for colonization, as observed in biofilm formation at the air-liquid interface. Looking forward, such behavior could influence community diversity, microbial dispersal, and perhaps enhance transmission of non-motile pathogenic strains. From a clinical perspective, our observations may have important implications on how non-motile pathogens disseminate.

Conflict of Interest

The authors declare no conflict of interest.

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