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Predatory *Bdellovibrio* Bacteria Use Gliding Motility To Scout for Prey on Surfaces▽§

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Bdellovibrio bacteriovorus **is a famously fast, flagellate predatory bacterium, preying upon Gram-negative bacteria in liquids; how it interacts with prey on surfaces such as in medical biofilms is unknown. Here we report that *Bdellovibrio* bacteria "scout" for prey bacteria on solid surfaces, using slow gliding motility that is present in flagellum-negative and pilus-negative strains.**

Surface-associated, Gram-negative bacterial biofilms are an increasing health problem along with resistance to commonly prescribed antibiotics (7). The small, naturally predatory, soil deltaproteobacterium *Bdellovibrio bacteriovorus* preys upon and kills a wide range of such pathogens in liquids and on biofilms (1). *Bdellovibrio* bacteria are famously fast and flagellate in liquids, swimming at high speeds (60 to 160 μm/s) by rotating a single polar flagellum, using chemotaxis to locate regions rich in prey bacteria (5, 9, 20). We showed previously that although flagellum-based swimming allows encounters with prey-rich regions, it is nonessential for prey entry once prey are encountered and cannot account for surface interactions with prey in biofilms where liquid is scarce (9). Prey bacteria are invaded in a process that involves type IV pili; *Bdellovibrio* bacteria pass through the outer membrane, which is then resealed (2, 10). They develop internally in the periplasm of the prey, sequentially degrading prey macromolecules, killing prey in a few minutes and digesting their contents over 2 to 4 h (16, 17). Escape of progeny *Bdellovibrio* cells from exhausted prey has been presumed to involve both lytic enzymes and flagellar rotation (13).

To study interactions between *Bdellovibrio bacteriovorus* HD100 and prey on surfaces, we applied 10-μl samples of predatory *Bdellovibrio bacteriovorus* HD100 and mutant strains (Δ*pilA*, Δ*fliC3*, and *mreB* monomeric teal fluorescent protein [mTFP] tagged) mixed with *Escherichia coli* prey bacteria on solid 1% agarose pad surfaces in 2 mM $CaCl_2$–25 mM HEPES (pH 7.6) buffer, and microscopic images were acquired over several hours at room temperature every 150 s as described previously (3). The *fliC3* mutant was constructed as described elsewhere (9), and the *mreB* mTFP fusions were constructed as described elsewhere (4). Silent, in-frame deletions of the *pilA* and *Bd0416* genes were constructed by a modification of the method of Steyert and Pineiro (19).

The microscopic images were then encoded into time-lapse movies at 7 frames per second (fps). Speeds of moving *Bdellovibrio* bacteria were measured by determining the path of specific cells using the montage function of the SimplePCI imaging software program (Compix Inc. Imaging Systems), and then the measuring tool and time signatures were used to acquire speeds. Standard deviations of speeds are shown in Table 1, and the Student *t* test was used to determine significance of differences using the Microsoft Excel software program.

When *Bdellovibrio* progeny lysed their digested prey and emerged to other immobilized prey cells on the agarose pad, they were unable to swim toward new prey by flagellum-driven motility due to the lack of a liquid environment (a result of evaporation over the incubation time of several hours under a coverslip on the microscope stage). However, on the agarose pad, the *Bdellovibrio* bacteria started to slowly move (Fig. 1) across the surface at variable but low speeds, which we measured at an average of 16 μm/h from cases of sustained unidirectional runs (Table 1; see also Movies S1 to S4 in the supplemental material), although many cells also exhibited stop-start or backward and forward motilities. Their speed increased significantly ($P = 0.0035$) from that on the agarose when they encountered, and moved over, the surfaces of prey bacteria, rising to an average speed of 35 μm/h, and they often circled round and round prey cells (Fig. 1) as if "scouting out" potential points on prey for invasion. On frequent occasions, *Bdellovibrio* bacteria exhibiting this surface motility directly infected nearby prey cells upon contact (see Movie S1). The rapid swimming movement in the bdelloplast observed previously (13) was not seen under these conditions, which may be because the *Bdellovibrio* bacteria detected that lower-moisture conditions in the bdelloplast were better suited to swarming and did not swim at all with flagella. We cannot exclude that a 150-s flagellar swimming phase may have existed, shorter than the acquisition time of the movie, but the fact that the *fliC3* mutant strain (which cannot swim) emerged from bdelloplasts on these surfaces in a gliding manner indistinguishable from that of wild type supports the former idea (see Movie S5).

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TABLE 1. Speeds of moving *Bdellovibrio* bacteria

Strain	Speed on 1% agarose pad (μm/h)[a]
B. bacteriovorus HD100	16.3 ± 7.6
B. bacteriovorus HD100 on *E. coli* prey cell surface	35.0 ± 16.8
B. bacteriovorus HD100 with ≥60 μg/ml A22	0
B. bacteriovorus HD100 Δ*fliC3*	19.7 ± 9.3
B. bacteriovorus HD100 Δ*pilA*	19.7 ± 8.9
B. bacteriovorus HD100 Δ*Bd0416*	19.1 ± 5.2
B. bacteriovorus HD100 *mreB1*::*mTFP*	14.5 ± 5.5
B. bacteriovorus HD100 *mreB2*::*mTFP*	21.2 ± 8.9

[a] Means and SDs are given.

This slow surface motility was conserved in different *Bdellovibrio* wild-type strains; two different wild-type strains of host-dependent predatory *Bdellovibrio* (HD100 and one freshly isolated from the environment) and three different derivatives (HID2, HID13, and HID26) (8) of host-independent *Bdellovibrio* all showed the same behavior (data not shown). Among the morphologically heterogeneous host-independent *Bdellovibrio* bacteria, mainly the smaller "attack-phase-like" cells were motile, but multiple examples of longer, more morphologically diverse cells were also motile (see Movie S2 in the supplemental material), showing that all *Bdellovibrio* morphotypes within a host-independent population are capable of this surface motility. There was often a delay of 1 to 2 h after attachment to the agarose surfaces before the motility commenced in the majority of cells, although some started immediately (see Movie S3). This delay suggests that the surface motility components of *Bdellovibrio* may be induced when needed in most cells, with a possible small stochastic proportion of the population expressing them constitutively.

To verify that the surface motility we observed was gliding and was not driven by any traditional bacterial appendages, we tested *Bdellovibrio* mutants with nonfunctional flagella due to a deletion of the gene for the FliC3 protein and *Bdellovibrio* with no PilA pilus fiber protein (2, 9). Both of these strains showed gliding motility on the 1% agarose pads at rates similar to wild-type rates (Table 1).

Myxobacteria are large-celled deltaproteobacterial "cousins" of *Bdellovibrio* and are "famously slow movers," having no flagella but using twitching of type IV pili to move at average speeds of 264 μm/h (4.4 μm/min) in social streams or wolf packs in search of prey, which they digest from the outside by secreting extracellular enzymes (18). Recently the Mignot and Zusman laboratories also reported details of a non-pilus-dependent, adventurous gliding motility of *Myxococcus xanthus*, which uses Tol-like protein complexes, thought to interact with the actin-like cytoskeleton to cause movement estimated variously from 24 to 36 μm/h (6) and 120 to 240 μm/h (11, 12). This movement is reminiscent of that which we have seen for *Bdellovibrio*, and furthermore, *Bdellovibrio* bacteria were often seen to be swarming together in pairs or trios on agarose surfaces (see Movies S3 and S4).

There are 4 operons, of 8 to 10 genes each, in the *B. bacteriovorus* HD100 genome which are homologous to *tolRQAB-tonB exbBD*-like genes implicated by Mignot and Zusman in myxobacterial motility (11, 14, 15), but as yet there are no defined key players from these gene products in the Myxobacterial motility process (where several operons of these genes also exist) to target in *Bdellovibrio*, a bacterium where directed mutagenesis is very time-consuming to achieve. Thus, we tested the motility of a single mutant, in the *tolB*-like gene *Bd0416*, from the *Bd0412-420 tol*-like operon. We chose this operon since it contains genes most highly upregulated in *B. bacteriovorus* HD100 upon incubation with prey (8). Our finding that this single mutant did not have an altered motility phenotype (Table 1) suggests only that functional redundancy between homologous *tol*-like gene products may be an issue, and we have embarked upon an extensive study of combinatorial mutagenesis which may reveal which proteins drive gliding.

We did, however, test for involvement in the *Bdellovibrio* surface motility of the bacterial actin-like MreB proteins, of which there are two in *Bdellovibrio* (4), by using strains where single MreB functions were attenuated by C-terminal TFP tags (since deletion of *mreB* is lethal) and by adding different concentrations of the MreB-specific inhibitor A22 (Table 1) (4). We found that the gliding motility continued in each of the two single MreB-TFP strains tested but that it was inhibited by 60 μg/ml A22 in wild-type *Bdellovibrio*. Previous work using A22 showed that concentrations up to 100 μg/ml were not toxic to *Bdellovibrio* (4). This demonstrates that MreB cytoskeletal integrity is required for the surface gliding to occur, an observation concurrent with those of Nan and coworkers for myxobacteria (14).

In conclusion, while *Bdellovibrio* bacteria use fast flagellum-

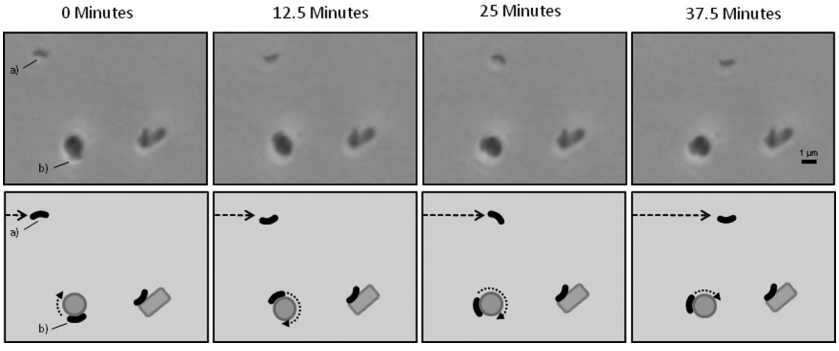

FIG. 1. Stills from time-lapse capture of gliding *Bdellovibrio* bacteria showing both lone gliding motion on an agarose surface and scouting motion around the prey cell surface.

driven motility for long-range chemotaxis to prey on rich regions in liquid media and pilus-driven motility for prey cell entry, we have discovered that they use slow gliding motility (at 15 to 20 μm/h, or 15 to 20 *Bdellovibrio* body-lengths per hour) to leave lysed prey cells in low-moisture environments, to encounter prey bacteria in biofilms on surfaces, and for "scouting" round prey surfaces at close range on solid media. We have observed that this slow gliding is biologically useful, seeing, in the movies on the agarose pads, that gliding brings *Bdellovibrio* effectively up to prey, where they enter and kill them. The gliding machinery may be similar to that in their larger nonflagellate, deltaproteobacterial "cousins," the myxobacteria, and further comparative genetic and functional studies will discern how the machinery is constructed and regulated.

Now that we understand that *Bdellovibrio* glides to bacterial prey on surfaces, we can hope to optimize this process when trying to apply *Bdellovibrio* practically to clear pathogenic bacteria from biofilms.

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C.L. and A.K.F. carried out all of the time-lapse microscopic experiments and measurements, designed parts of the experimental program, and coauthored the manuscript. L.H. constructed the *pilA* and *Bd0416* knockout mutant strains. R.E.S. supervised the research, designed parts of the experimental program, and coauthored the manuscript.

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