

# Genetic and genomic analysis of *Flavobacterium johnsoniae* adhesion, motility, and protein secretion

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## Abstract

*Flavobacterium johnsoniae* crawls rapidly over surfaces in a process called gliding motility [1]. *F. johnsoniae* does not have flagella or pili and instead relies on a novel motility apparatus comprised of Gld and Spr proteins. SprB is a cell surface protein that appears to be propelled rapidly by the gliding 'motors' [2]. It is thought to transmit the force generated by the motors to the surface over which the cells move. Some of the Gld and Spr proteins are components of the Por secretion system (PorSS) needed for secretion of SprB [3–5] whereas others may propel SprB along the cell surface. We identified another cell surface adhesin, RemA, that interacts with polysaccharides produced by the cell and is propelled by the motility machinery. The presence of alternative motility adhesins may account for the ability of *Flavobacterium* cells to crawl over diverse surfaces.

Disruption of *sprB* results in a defect in gliding on agar, but cells retain limited ability to move over glass [2]. Analysis of the *F. johnsoniae* genome revealed numerous paralogs of SprB, which could be semi-redundant components of the motility apparatus and could account for the residual motility. SprB is thought to be a bacteriophage receptor and disruption of *sprB* results in resistance to some bacteriophages. To identify other cell-surface motility proteins, transposon mutagenesis was conducted starting with a strain deleted for *sprB*, and eight bacteriophage resistant mutants were identified. Each mutant had a more severe motility defect than did the parent strain, suggesting that the transposons had inserted in genes associated with motility. The sites of transposon insertions were determined, and four mutants had independent insertions in the same gene, which we named *remA* (*re*dundant *m*otility gene *A*). RemA exhibits sequence similarity to SprB. A recombinant gene encoding RemA carrying a ten amino acid tag (myc-tag) was constructed and introduced into the genome in place of wild type *remA*. The cells expressed RemA-myc-tag and the protein was found on the cell surface as determined by labeling live cells with anti-Myc-tag coated latex spheres, or with fluorescently labeled antibodies against the Myc-tag peptide. The spheres and the fluorescently labeled antibodies moved rapidly on the cell surface at speeds of 1 to 2  $\mu\text{m}$  per sec, suggesting that like SprB, RemA is propelled by the gliding motor. RemA travelled the length of the cell, looped around the pole, and returned to a location near its starting point, all within a few seconds, and this process was repeated many times. Such rapid and continuous movements over long distances are unprecedented for bacterial proteins.

RemA has sequence similarity with galactose/rhamnose-binding lectins, suggesting that RemA may be a polysaccharide-binding adhesin. Interestingly, of the four other genes identified in the phage resistance screen described above, three (*remC*, *wza*, and *wzc*), are predicted to encode proteins involved in polysaccharide synthesis and secretion. RemA and cell-surface polysaccharides appear to be involved in cell-cell interactions as well as in motility. Wild type cells formed aggregates that were rapidly dispersed by galactose or rhamnose, but were not dispersed by a variety of other sugars. Cells with mutations in *remA*, *remC*, *wza*, or *wzc* failed to aggregate. However, when cells of a *remA* mutant and cells of a *remC* mutant were mixed together they formed cell aggregates, suggesting that RemA on one cell binds to polysaccharides on the surface of another cell. Wild type cells and cells overexpressing RemA were stained with fluorescently labeled galactose-binding lectin *Ricinus communis* agglutinin I (RCA<sub>120</sub>). RCA<sub>120</sub> attached to cells and was rapidly propelled on their surfaces, but cells of a *remA* mutant did not propel RCA<sub>120</sub>. Cells of a *remC* mutant failed to bind or propel RCA<sub>120</sub>, suggesting that the putative glycosyltransferase, RemC, is involved in formation of a polysaccharide that is recognized by RCA<sub>120</sub> and by RemA.

Secretion of SprB to the cell surface requires the PorSS, a novel protein secretion system that is not closely related to the more well-studied Type I-Type VI bacterial protein secretion systems [5]. Genome analyses suggest that PorSSs are common among members of the phylum *Bacteroidetes*, but they have not been found in other bacteria. The *F. johnsoniae* PorSS is needed for secretion of SprB and of an extracellular chitinase, and the PorSS of *Porphyromonas gingivalis* is needed for secretion of virulence factors [5]. RemA exhibits similarity to a domain identified in proteins secreted by the PorSS. Cells of *porSS* mutants produced RemA but failed to secrete it to the cell surface, suggesting that RemA, like SprB, is secreted by the PorSS.

The results of these and previous studies [2,6,7] suggest a model to explain gliding motility of *F. johnsoniae* and related bacteria (Fig. 1). Gld proteins anchored in the cell envelope comprise the 'motors' that propel SprB, RemA and perhaps other adhesins, resulting in cell movement. Some of the adhesins, such as RemA, interact with specific polysaccharides produced by the cell. These polysaccharides may coat the surface, forming a 'road' over which cells travel. *F. johnsoniae* cells glide on agar, glass, Teflon, polystyrene, and presumably many other surfaces in nature. Genome analyses suggest that *F. johnsoniae* has the ability to make many different adhesins and polysaccharides, which may explain the ability of cells to crawl over these diverse surfaces.

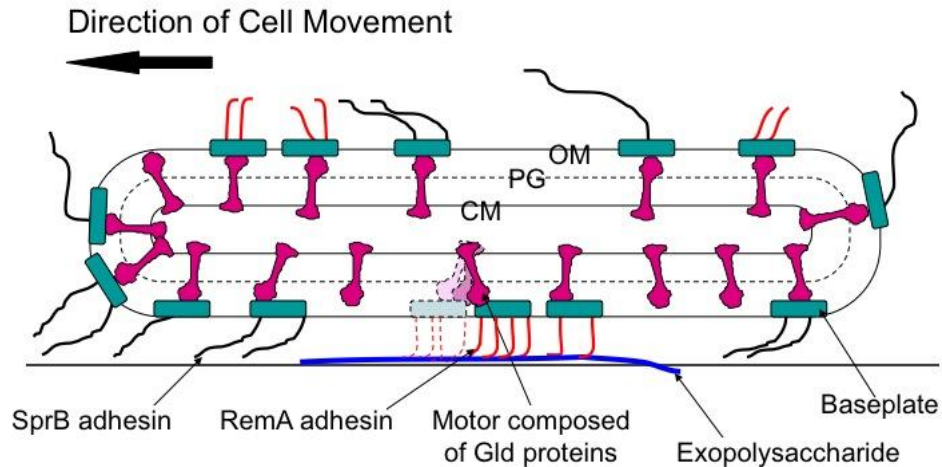


Figure 1. Model of *F. johnsoniae* gliding motility. Gld proteins anchored in the cell envelope form the motors (pink) that propel baseplates (turquoise) attached to adhesins such as SprB (black) and RemA (red) along the cell surface. Exopolysaccharides (blue) enhance motility by coating the substratum and interacting with specific adhesins such as RemA. CM- cytoplasmic membrane; OM- outer membrane; PG- peptidoglycan.

Many relatives of *F. johnsoniae* within the phylum *Bacteroidetes* exhibit rapid gliding motility, and these bacteria have homologs for the *gld* and *spr* genes. Some of these bacteria, such as *Flavobacterium columnare*, *Flavobacterium psychrophilum*, and *Capnocytophaga canimorsus* are pathogens, and adhesins such as SprB and RemA may be involved in binding to and movement over host surfaces. Gliding motility is found in other bacteria that do not belong to the phylum *Bacteroidetes*, such as the delta proteobacterium *Myxococcus xanthus*, the mollicute *Mycoplasma mobile*, and the cyanobacterium *Phormidium uncinatum* [1], but these organisms have their own unique motility machineries.

The results suggest that SprB and RemA are mobile cell surface adhesins involved in *F. johnsoniae* gliding, but many mysteries remain regarding the motors that propel these adhesins, and the sensory transduction (chemotaxis) system that allows cells to control their movements. Most of the Gld and Spr proteins lack sequence similarity to proteins of known function, and typical components of bacterial chemotaxis systems are absent in *F. johnsoniae*, so it is likely that novel aspects of *Flavobacterium* motility await discovery. SprB and RemA provide convenient handles to examine the functioning of the motility machinery in living cells, and will facilitate studies to understand the functioning of the *Flavobacterium* gliding machinery.

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