The discovery of a third form of flagella-mediated motility in symbiotic bacteria

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Professor Takayuki Nishizaka and Dr. Yoshiaki Kinosita from Gakushuin University, together with Dr. Yoshitomo Kikuchi from AIST, have discovered an unforeseen form of flagella-mediated motility shown by pest bean bug symbionts, which entails swimming by wrapping their flagellar filaments around their cell bodies. Bacteria with this form of flagella-mediated motility were able to traverse glass surfaces, so this form of movement is most likely effective on extracellular matrix surfaces.

This is the first study to reveal a characteristic swimming motility adopted by bacteria upon going into a symbiotic relationship with bean bugs. It is hoped that the findings will lead to novel ways to control pests by preventing symbiosis. The findings of this study were published online in *The ISME Journal*.

Many species of swimming bacteria have a rotary structure called a flagellum consisting of more than 20 kinds of proteins. Propulsion via rotating their flagellar filaments allows the bacteria to swim freely in water. Flagella-mediated motility is essential for bacteria to move in search of habitats, and two forms have been known to date: (i) "run and tumbling," seen in peritrichous bacteria such as Escherichia coli; and (ii) "forward run-reverse-flick," seen in Vibrio alginoliticus. Such forms of flagella-mediated motility are adopted when moving in water, but they are also adopted by pathogenic bacteria to reach a host's internal organs. Thus, they are widely recognized as virulence factors.

Although bacterial flagella-mediated motility is closely linked to virulence, it is also has a significant role in the functioning of several symbiotic systems between animals and bacteria. For example, bobtail squids incorporate Aliivibrio fischeri, a bioluminescent bacteria species, to emit light, diffusing their appearance under moonlight to avoid predators. Motility of the bioluminescent bacteria is essential for this symbiotic relationship to work. Another example is bean bugs, recognized as agricultural pests, which are known to acquire insecticide resistance by incorporating bacteria of the Burkholderia genus. Flagellar motility of the bacteria plays an important role for this form of symbiosis to work as well.

There is an extremely narrow constricted passage filled with polysaccharide-rich mucous between a bean bug's gut and its symbiotic organ. Dr. Kikuchi and his team have previously reported that only Burkholderia symbiont with motility can pass through this constricted passage while mutant strain bacteria lacking motility cannot. Interestingly, previous studies have shown that motile non-symbionts such as E. coli are unable to pass through this constricted passage, and therefore cannot reach the symbiotic organ. A similar constricted passage also exists in bobtail squids, and it is known that only bioluminescent bacteria can selectively pass through it. However, researchers did not know why only symbionts could pass through. Upon starting the current study, the team hypothesized that "symbiotic Burkholderia symbiont have a unique motility mechanism that allows them to pass through the constricted area."

Flagella-mediated motility in the Burkholderia symbiont was directly captured in order to verify the hypothesis. Because a flagellar filament has a diameter of only approximately 20 nm (a 100,000th of 2 mm), it could previously be observed only via electron microscopy. The researchers discovered that flagellar filaments can be visualized under a fluorescence microscope when the cell body is treated with fluorescent dyes, solving this problem. In addition, with the use of an EMCCD camera, considered to be the most sensitive type of camera in the world, they were able to capture flagellar filament movement at a rate of 400 frames per second. From this observation, we found that the Burkholderia symbiont can swim at a speed of 25 m/s (about 10 times its body length) by rotating its flagellar filament at 150 rotations per second.

Only known forms of motility shown by other bacteria such as E. coli have been observed with the Burkholderia symbiont in normal liquid media. Dr. Yoshiaki Kinosita, a member of the research group, created an environment simulating the sticky internal condition of the constricted passage with methylcellulose. While normal motility forms were observed as in low-viscosity media, he was also able to observe flagellar filament-wrapping swimming under this condition. This flagellum-wrapping movement does not match any known forms of flagella-mediated motility and can be described as a third form of flagella-mediated motility. Motility efficiency was approximately halved, and was thus lower than other forms of motility when the Burkholderia symbiont moved through viscous liquid in this motility form like a drill bit.

Why does the Burkholderia symbiont display a unique motility form with low efficiency? When the researchers placed E. coli, a non-symbiont, in the same condition, they obtained interesting results. As time passed, E. coli became bound to the glass surface, unable to move. By contrast, no Burkholderia cells were observed to be similarly immobilized. Upon closer observation, the Burkholderia symbiont moving with normal motility forms were captured, but were able to wrap their flagella and free themselves, thereby crossing the glass surface. Furthermore, by using a total internal reflection fluorescence microscope, which allows high-resolution observation within the vicinity of the glass surface, the researchers showed that there is contact between the flagellar filament and the glass surface when gliding-like motility occurs. Considering that the glass surface is uneven, it can be assumed that the flagellar filament wrapped around the cell body perfectly fits into the surface grooves, rotates, and generates propulsion, allowing for effective motility on solid surfaces. Thus, this third form of motility could be described as essential to move effectively on extracellular matrix surfaces.

Is the flagellum-wrapping movement unique to the Burkholderia symbionts? To address this question, we looked at A. fischeri, a symbiont of bobtail squids. Like bean bugs, bobtail squids have a mechanism to incorporate symbiotic bacteria, and only motile A. fischeri are known to be able to reach the symbiotic organs. When A. fischeri were treated with fluorescent dyes and observed in the same way as the Burkholderia symbiont, the researchers found that A. fischeri also swim by wrapping their flagellar filaments around their bodies. This implies the possibility of the newly discovered third form of flagella-mediated motility being adopted across many different species of symbiotic bacteria.

This study revealed that the transition from normal forms of motility to flagellar filament-wrapping motility is achieved by reversing the direction of flagellum rotation from counterclockwise to clockwise. However, this change in rotational direction can also be seen in E. coli and other bacteria species that do not display flagellum wrapping. Therefore, the transition to the third form of motility cannot be explained by the change in rotational direction alone. At the next stage, the researchers will address this issue through structural analysis of proteins necessary to trigger the third form of flagellar motility and exhaustive gene analysis. Furthermore, they will analyze flagella-mediated motility inside the host body and see if swimming/gliding form-switching takes place area-specifically within the bean bug gut. This study will show whether the normal motility forms or the flagellum-wrapping form is more efficient when moving inside the constricted passage. By thoroughly revealing the relationship between symbiosis and the diverse forms of motility shown by bacteria at the gene level based on our studies, it is expected that the development of novel insecticides that prevent the infection and colonization of symbionts will follow.

More information: Yoshiaki Kinosita et al, Unforeseen swimming and gliding mode of an insect gut symbiont, Burkholderia sp. RPE64, with wrapping of the flagella around its cell body, *The ISME Journal* (2017). DOI: 10.1038/s41396-017-0010-z

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