

## Mini Review

# An Overview on Bacterial Motility Detection

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## ABSTRACT

Motility by means of flagella is important for the identification of microorganisms. Motility can be observed satisfactorily both under microscope and on culture media. The essentials and some hints of the motility medium, craigie tube, hanging drop and capillary tube methods are presented here in a brief manner. It is believed that this review presentation, will be helpful for both young researchers or anybody dealing with microbiology.

**Key Words:** Motility; Motility medium; Craigie tube; Capillary tube; Hanging drop

## INTRODUCTION

The ability of an organism to move by itself is called motility. Motility is closely linked with chemotaxis, the ability to orientate along certain chemical gradients. The capacity for true motility or self-propulsion is present in a great variety of cells, but not all of them. Eucaryotic cells can move by one of the following locomotor organelles such as cilia, flagella, or pseudopods. Prokaryotes move by means of un-usual, propeller-like flagella unique to bacteria or by special fibrils that produce a gliding form of motility. Almost all spiral bacteria and about half of the bacilli are motile, whereas essentially none of the cocci are motile (Volk & Brown, 1997). Motility by means of flagella is of important for the identification of microorganisms, for instance *Bacilli* are motile except for the anthrax, which is instead capsular, therefore flagella could be produced to enable the organisms to run away from danger and to move from less favorable to more favorable environment. The same is observed with *Clostridia*, all are motile except *Clostridium perfringens*, which has a capsule. It's also been suggested that motility is an essential feature in the colonization hence pathogenicity of *Helicobacter pylori* (Eaton *et al.*, 1992).

Flagella are long, slender, helical and generally several times the length of the cell. Motile cells may have one flagellum, but most have several. Individual flagella of a single species are highly uniform in diameter, but the flagella of different bacterial species vary from about 12 nm to 30 nm in diameter. Because the size is below the limit of resolution of the light microscope, flagella are not visible in routine stained smear of bacteria. Flagella can be seen with the light microscope only if a special substance called a mordant is used to build up the diameter of the flagellum. After treatment with the mordant, a stain is applied to color the thickened flagellum. Flagella are visible in standard electron micrographs of bacteria; such micrographs show

flagella to be amazingly similar to lengths of rope, even down to the coiled strands found in robes.

The positions at which flagella are inserted into the bacterial cell are characteristic for a genus. For example, monotrichous cells have only a single flagellum found at one end of the cell. Amphitrichous cells have two flagella and the cells (e.g., in the genus *Proteus*) have peritrichous flagella meaning flagella all around the cell, thus they are extremely motile. These cells can even swim on top of agar, a characteristic called "swarming". The genus *Pseudomonas* contains cells that have lophotrichous flagella, or a tuft of flagella on one end of the cell making them next to *Proteus* in terms of motility (Talaro & Talaro, 1993).

Each flagellum has a very rigid, helical structure and actual motility results from the rotation of the flagellum in a manner similar to that of a boat propeller. Bacteria have shown that when the flagellum is rotating counter-clockwise, the bacterium travels in a more or less straight line; but if the direction of flagellar movement is reversed, the organism will tumble aimlessly.

When anticlockwise rotation is resumed, the cell moves off in a new direction. This ability is important, since it allows bacteria to change direction. Bacteria can sense nutrient molecules such as sugars or amino acids and move towards them - a process is known as chemotaxis. Additionally, they can also move away from harmful substances such as waste products and in response to temperature, light, gravity, etc.

When the bacterium is moving up a gradient of a chemotactic attractant, the straight-line movements continue longer and the tumbling occurs only briefly. However, if the bacterium is moving away from an attractant or accidentally toward a toxic chemical, the tumbling occurs frequently until the net movement is properly redirected.

Flagella are not the only means by which bacteria move about. Some bacteria exhibit a gliding motility by which they crawl over surfaces by waves of contraction

produced within the cytoplasm. Gliding is defined as the movement of a non-flagellated cell in the direction of its long axis on a surface (Hendrichsen, 1972). Such organisms are extremely important in the decaying of dead organic material but are not of medical importance, because they do not cause human disease. Axial filaments, which are found only in the spirochetes, consist of protein fibrils wound spirally around the organism and attached at the two poles of the cell. They are located just beneath the membrane, where they function to impart rapid motility to the spirochete (Volk & Brown, 1997). However this review deals only with flagellar motility detection even if other modes of motility like actine based gliding or twitching motility.

If identification of a bacterium requires detection of the actual number and placement of flagella, special stains or electron microscope preparations are required, as flagella are too minute to be seen in un-stained live preparations with an ordinary light microscope. Often it is sufficient to know simply whether a bacterial species is motile, which helps differentiation between genera and species. The focus of this work is to bring the detection of motility concerning flagellar mode together with some basic hints.

**Motility determination.** Beyond determination of motility by flagella staining, motility can be observed most satisfactorily both under microscope and on culture media.

**Use of culture medium.** Bacterial motility can be shown using different types of medium. The composition of these preparations gives freedom of movement comparable to that of broth culture.

Motility media (Barrow & Feltham, 1993) is the one medium utilized for the motility detection, the tubes are stab-inoculated with a straight wire to the depth of about 5 mm, where-in only motile bacteria can move away from the line of inoculation in the low (0.4% or less) concentration of agar; descendants of cells, which have migrated throughout the medium show up as evidenced by turbidity (cloudiness) in the medium. The higher concentration of agar makes the gel too firm to permit organisms to spread (Koneman *et al.*, 1997). Growth of non-motile organisms is confined to the stab inoculum. One advantage of using motility medium is that a culture of any age can be used for inoculation as long as it is pure and viable. SIM (Sulfur, Indole, Motility) medium is another combined test medium used for (Barrow & Feltham, 1993). Hugh and Leifson's OF medium (Hugh & Leifson, 1953) and Voges-Proskauer reaction tubes (Furniss *et al.*, 1978) are also could be used to test motility. Both medium is semisolid and motile strains show diffuse growth. The list could be extended as MIL/MILS (Motility-Indole-Lysine-H<sub>2</sub>S), MIO (Motility-Indole-Ornithine) etc.

**Craigie tube technique.** The Craigie tube is used to demonstrate motility. This method consists of a wide screw capped tube or bottle containing semi solid agar (0.2 - 0.4%) in the centre of which is embedded a short, narrow tube open at both ends, in such a way that it projects above the agar. The strain to be tested is inoculated carefully into the

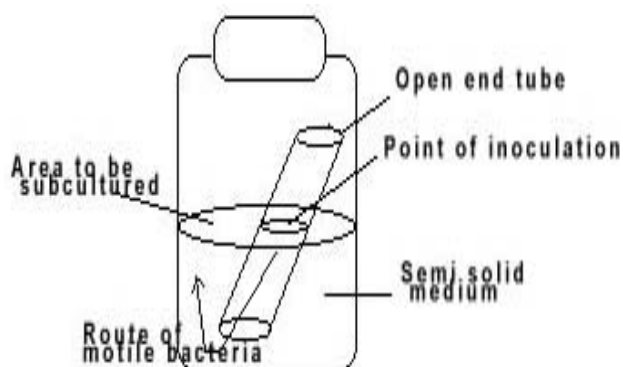
inner tube. After incubation, sub-cultures withdrawn from the top of the agar outside the central tube will yield a population of motile cells (Craigie, 1931). For contamination control, a known non-motile microorganism such as *Staphylococcus sp.* could be mixed with test organism prior to inoculation and sub-culturing from the outside of the central tube will define the validity of the test. This is also a method of separating motile and non-motile organisms. Instead of the Craigie's tube, a U-tube of soft agar may be employed, inoculation being made into one limb and sub-culture taken from the other limb. Motility may be inferred by observing the spreading growth in a semisolid (Tittler & Sandholzer, 1936), which may be seen better; as the organisms grow the dye, tetrazolium, is reduced and the medium changes colour (Kelly & Fulton, 1953). However, the use of tetrazolium may inhibit most fastidious bacteria and should not be used in all cases (Koneman *et al.*, 1997).

When a medium used for motility detection, the temperature of incubation is important; most motile organisms are motile at lower temperatures (e.g. 15 - 25°C) and may not be motile at the temperature (e.g. 37°C) optimal for growth (Barrow & Feltham, 1993). In other words, motility improves with lower temperature. If there is any suspicion about an organism, which may exhibit motility at a lower temperature, it should be inoculated two tubes simultaneously one at 35°C and the other at room temperature (22 - 25°C) (Ewing, 1986).

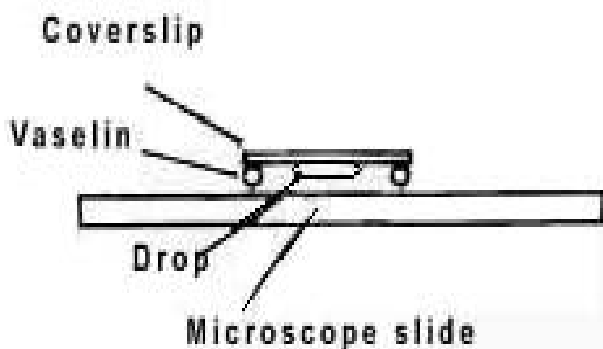
**Microscopic examination.** If we assume that bacterial flagella confer motility, flagella staining can then be used indirectly to denote bacterial motility. Since flagella are very thin (20 - 28 nm in diameter), they are below the resolution limits of a normal light microscope. They cannot be seen unless one first treats them with special dyes and mordants, which build up as layers of precipitate along the length of the flagella, making them microscopically visible. This is a delicate staining procedure and will not be attempted here.

**The wet mount.** Preparation is the simplest way to determine motility by placing a few loopful of the organism on a clean slide and cover with a cover-slip. The best way to examine the slide is with a phase-contrast microscope, utilizing high-dry magnification. A phase-contrast microscope uses special phase-contrast objectives and a condenser assembly to control illumination and give an optical effect of direct staining. The special optics convert slight variations in specimen thickness into corresponding visible variation in brightness. Thus, the bacterium and its structures appear darker than the background. In case of a dark-field microscope, which uses a special condenser to direct light away from the objective lens. However, bacteria lying in the transparent medium will scatter light so that it enters the objective. This gives the optical effect of an indirect stain. The organism will appear bright against the dark background. Dark field microscopy is especially valuable in observing the very thin spirochetes. If a bright-

**Fig. 1. Craigie tube**



**Fig. 2. Hanging Drop preparation with an ordinary slide**



**Fig. 3. Capillary tube preparation on a slide**



field microscope is used it is necessary to reduce the lighting to a bare minimum to achieve contrast. Once the organisms are in focus under high-dry, it could be placed immersion oil on the cover-slip and swung the oil immersion lens into position (Benson, 1981).

The advantage of a wet mount is that it is the quickest means for determining motility. Such a preparation also is useful for determining cellular shape and arrangement.

One disadvantage of this method is that the slide quickly dries out, rendering the organisms immotile. Another disadvantage is that if the organism is pathogenic, there is the possibility of danger to the person in handling viable organisms on a slide.

When it is necessary to study viable organisms on a slide for a longer period of time than is possible with a wet mount, one must resort to a hanging drop slide. The preparation could be the most used method in some motility determination cases unless the use of depression slide.

Basically, hanging drop is prepared by applying a little vaseline or stopcock grease to the four corners of the coverslip and transferring a loopful of fresh liquid culture to the centre of the coverslip. Finally depression slide (deep-well slide) is placed over the cover slip so that contact is made with the vaseline but not with the droplet of culture. Inversion of the preparation quickly provides the droplet of culture hangs below the coverslip (Benson, 1981). The preparation of this method is almost similar in all published material to date. But an ordinary microscope slide is also suitable for this preparation instead of depression slide, which is not expensive as depression slide, not have to clean it back and possible to work at the same time in a laboratory especially during the laboratory classes.

The advantage of hanging drop is to observe motility of living bacteria as well as true size and shape and disadvantages is difficult to see bacteria. So the focusing should be into the edge of the droplet.

When bacteria are viewed down microscope, some motion is seen due to brownian motion and convection currents in the fluid, even if the bacterium is a sessile organism. Brownian motion is the oscillation of bacteria about a mean position caused by bombardment of water molecules. Convection current may cause to change the shape of the drop and all the bacteria move. To avoid the false result, it should be noted that in convection current all bacteria move in one direction and brownian motion is occurred simultaneously in one place without any progress. If the motion is true movement, the type of the movement could be classified into four different motilities. Darting motility is the characteristic of polar flagellates as *Vibrio sp.*, active motility is the characteristic of peritricate flagellates like *Salmonella sp.*, and sluggish motility is known also stately motility as in *Bacillus* and *Clostridia* genus. Lastly Thumbling motility is the characteristic of *Listeria*, which has peritricate flagella.

The problem pertaining to motility is: should all strains be tested or only rods? If we only examine the rods we shall overlook the motility of many strains of *Enterococcus faecium*, of *Micrococcus agilis* and other cocci. When these test become part of the daily routine they do not take up much extra time; they are only time-consuming and up-setting of routine when they are special tests. Another important criteria is using fresh culture for microscopic examination (Barrow & Feltham, 1993). Because bacteria tend to become non-motile in older

cultures, an old culture may become so crowded with inert living and dead bacteria that it is difficult to find a motile cell. Production of acid may result in loss of bacterial motility. If the pH of the medium gets acidic during the growth, the protein of the flagella dissociates into a large number of identical protein molecules (Volk & Brown, 1997).

Anaerobes present special problems in that motility will be inhibited by the air present in hanging drop preparations. Capillary tube preparations are more likely to show motility in *Clostridia* (Barrow & Feltham, 1993). Some of the liquid from Cooked Meat Medium is taken up into the capillary tube, which can be cut from a Pasteur pipette and sealed both ends on a flame. This could be placed on a microscope slide with the aid of glue tag or something similar to that and observed with high dry objective.

Many species of bacteria, including *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae* and *Myxococcus xanthus*, move their bodies, not by rotating flagella to swim, but by pulling on a solid surface with their polar type pili, called gliding motility. (Merz *et al.*, 2000) directly measured a retractile force on pili using laser tweezers but this motility could not be detected easily in a routine basic laboratories.

## CONCLUSION

Apparently, this report brings together some known records of motility detection method in basics with some un-mentioned facilities. It is clear that this presentation will be helpful for both young researchers and teaching assistant for laboratory classes especially for investigators from other disciplines dealing with bacteriology. Especially when depression slide is not available for the preparation of hanging drop, the use of ordinary microscope slide will be

of use as being cheap and disposable. Capillary tube and Craigie tube methods are also basic, cheap and effective methods for complete motility understanding.

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