Velocity Measurements of Motile Bacteria by Use of a Videotape Recording Technique

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A method has been developed to measure accurately the velocity of motile bacteria.

In working on problems of bacterial motile behavior, it is essential that one be able to measure velocity accurately and quickly. A method is described here utilizing a 1-inch (2.54-cm) television tape recorder (Panasonic NV-504) to store the microscope image scanned by a television camera (Concord MTC-21). A television monitor (Electrohome EMV-23AG) coupled with the recorder displayed either "live" microscope pictures or the recorded playback image.

The television camera was joined to a Zeiss II photomicroscope supplied with a 12-V 60-W illumination source. The "phase 2" system and a ×40 Neofluar objective lens furnished the best picture on the monitor screen, since it yields an image of a dark organism against a light background. If dark-field or "phase 3" phase-contrast optics are used, the bright images of objects in the field "burn" into the camera screen, producing a black bar across the corresponding scan lines. The automatic controls of the camera will set the exposure for the black background of these images, and thus the bacteria observed will be "overexposed."

For calibration purposes, a Bausch and Lomb 0.1-mm ruled stage micrometer was recorded before each experiment. The audio track on the tape was used to identify subsequent stages of each experiment, and both hands were free for manipulation of the microscope and recorder. Each specimen was recorded for approximately 10 s or longer, if necessary, to observe motile behavior of the organisms under investigation.

The tape was played back to measure velocities, and the paths of a given bacterium were traced on a grid of heavy transparent plastic sheet with a wax marking pencil in real time or slow motion as necessary. To provide a flat surface for the plastic sheet, a ⅛-inch (0.32-cm) Plexiglas screen was mounted over the monitor face. Motility path lengths were then measured with a calibrated planimeter.

The tape then was rewound and played back, and the time required for an organism to traverse the marked path was recorded with a 1/100-s stopwatch. The velocity could then be calculated as follows: Velocity (micrometers per second) equals [distance (centimeters) on screen/time(s)] × [stage micrometer (micrometers)/stage micrometer on screen (centimeters)].

For example, an organism was observed to travel 38.5 cm on the screen in 3.4 s. A distance of 200 μm on the grid was magnified to 41.5 cm on the screen; therefore, the velocity of the organism was calculated to be 54.6 μm/s.

The parameters of this system were examined for reproducibility. Possible sources of error were tracing of the path of organisms and measurement of this with the planimeter. A fixed distance (0.2 mm on the stage micrometer) was recorded, played back, marked on the monitor screen, and measured (Table 1). A stationary "single frame" picture was shown to have 2.4% more magnification than when measured in motion. This is evidently an unavoidable idiosyncrasy of the recording process, and all subsequent measurements were therefore taken from moving tape. There is no reproducibility error within limits of the calibrated planimeter which is read to the nearest 0.5 cm.

Individual bacterial velocities differ; thus, sufficient measurements must be made to ob-

| Distance measured (mm) (stage micrometer) | Mode | Average of 10 measurements* (cm) on monitor screen |
|-----------------------------------------|------|--------------------------------------------------|
| 0.2                                     | Single frame | 42.25                                          |
| 0.2                                     | Moving image | 41.50                                          |

*In each case, all 10 measurements were identical to within planimeter limitations (±0.25 cm).
tain a statistically significant average. The 10
greatest observed velocities of *Serratia
marcescens* in 0.2% (wt/vol) methyl cellulose
varied from 36.67 to 46.25 μm/s, and averaged
42.71 μm/s (Table 2). A second measurement
made on the same culture at the same temperature
(21 C), 2 min later, averaged 44.06 μm/s,
with a minimum of 37.50 μm/s and a maximum
of 47.62 μm/s. The error in average velocity was
±1.5%. In the course of experiments using this
method, velocity averages that one would expect
to be similar have consistently been within
±1.5% of each other.

Several other techniques have been developed
to measure the velocity of bacterial motion. The
method of Shoesmith (3) involved counting the
number of bacteria crossing an ocular aperture
in a given time period. This is a convenient
method, but it gives results only in terms of a
percentage of the motility control. This figure
only indicates relative motile activity of a
culture and does not give absolute velocities.

Another method for measuring velocities is
that of "motility tracks" as developed by Vaitu-
zis and Doetsch (4). This involves taking photo-
graphic time exposures of motile organisms.
The bacteria will record their motility path as a
track on the film. This method gives absolute
velocity measurements; however, organisms
that stop, or which double back 180° and retrace
their original paths (such as in *Pseudomonas*),
or those which move in and out of focus may give erroneously short tracks on
the film. A variation of this technique using
stroboscopic illumination was reported by Mac-
nab and Koshland (2).

A more complex method is that of Berg (1),
who devised a moveable microscope stage which
keeps an individual bacterium centered and
records its velocity by recording the motion of
the stage. This method, however, can be used to
follow only one organism, and too much time is
needed for studying a preparation in order to
obtain sufficient data to arrive at a significant
average velocity for a culture.

The videotape recording method described
here avoids the problems of other methods,
although measuring the marked paths with a
planimeter, rewinding the tape, and timing the
motility paths may be tedious if a great number of
measurements are required. One obtains,
however, an accurate measurement of individual
velocities, and it requires only 10 to 15 s to
record the behavior of a number of organisms.
This is especially advantageous in reducing
extraneous effects of oxygen deprivation in-
duced by leaving organisms under a cover slip
too long, or temperature changes. Another ad-
vantage of this method is that the number of
bacteria sampled per unit volume is not critical.
The method could be improved by interfacing the
recorder with a digital computer program-
med to plot paths of bacteria and analyze
all parameters of their motion. Writing and
"debugging" the program would be a formidable
task, but is within the bounds of present
technology.

**LITERATURE CITED**

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**TABLE 2. Reproducibility of velocity measurements**

| Sample | Distance* (cm) | Time* (s) | Velocity* (μm/s) |
|--------|----------------|-----------|------------------|
| 1st    | 18.5           | 2.0       | 46.25            |
|        | 20.0           | 2.3       | 43.48            |
|        | 15.5           | 1.9       | 40.79            |
|        | 10.0           | 1.25      | 40.00            |
|        | 16.0           | 1.75      | 45.71            |
|        | 10.0           | 1.1       | 45.46            |
|        | 22.0           | 3.0       | 36.67            |
|        | 18.0           | 2.0       | 45.00            |
|        | 11.0           | 1.3       | 42.31            |
|        | 17.0           | 2.05      | 41.46            |
| 2nd    | 20.0           | 2.1       | 47.62            |
|        | 11.0           | 1.2       | 45.83            |
|        | 15.0           | 1.7       | 44.12            |
|        | 24.0           | 3.2       | 37.50            |
|        | 13.0           | 1.5       | 43.33            |
|        | 22.0           | 2.4       | 45.83            |
|        | 18.0           | 2.25      | 40.00            |
|        | 34.0           | 3.9       | 43.59            |
|        | 31.0           | 3.45      | 44.93            |
|        | 22.5           | 2.35      | 47.87            |

* Path length as measured from monitor screen with planimeter.
* Time for organism to traverse path length, measured with 1/100-s stopwatch.
* Average velocity was 42.71 for sample 1 and 44.06 for sample 2.
* *Serratia marcescens* in 0.2% (wt/vol) methyl cellulose, magnification ×2,000, at 21 C.
* A second slide, prepared and recorded 2 min later.