A CINEMATOGRAPHIC ANALYSIS OF THE MOTION OF COLONIES OF B. ALVEI

LAWRANCE E. SHINN

The Institute of Pathology, The Western Pennsylvania Hospital, Pittsburgh, Pa.

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At the 1937 meetings of the Society of American Bacteriologists, Smith and Clark reported (1938) on the motility of entire colonies of *Bacillus alvei* and other organisms. Being interested at the time in lapse-time motion picture studies of bacterial colonies, we obtained a culture through the agency of the American Type Culture Collection. The marking on this culture leads us to assume that it came originally from Smith.

The strain was found to be motile as described. Preparation for cinematographic study comprised seeding one point on the margin of a freshly poured plate of 2 per cent Proteose-Peptone, beef infusion agar (1.5 per cent agar) of pH 7.4. The exact amount of surface moisture was found to be highly critical, and several plates of varying degrees of dryness were always set up. These were incubated briefly until the plate showing rapid motion of discrete colonies across the fresh medium could be selected with the aid of a hand lens. This plate was then placed in the incubator (30°C.) of the photographic equipment and a record made of about two days' growth at 15 frames per hour. This resulted in a 3800 times magnification of speed when the film was projected at the normal rate. On one hundred feet of film we have recorded the action on 10 plates.

The development of a typical culture appears to fall into five general periods:

1. The first growth consists of minute colonies which move across the field at high speed in any direction. The path is rarely linear for any distance. Rather, it is usually a series of wide circling movements. The number of such active colonies increases rapidly until the second phase sets in.

2. The colonies now come to rest, not gradually, but very abruptly. Frequently the small stable colony so formed will have a residual motion of rotation. The abruptness of the halt eliminates any question of "running down," and I think it is to be explained by an occurrence which is frequently seen under the microscope, namely, a turning back of the head of the advancing colony so that it forms a sharp left hand loop which quickly progresses to form a spiral and then, as the spiral tightens, a compact colony which still possesses the rotary motion to which its linear motion has been transformed.

3. When nearly all the colonies have entered upon the second phase, the third begins. This is one of comparative rest with continued growth in size.

4. The fourth phase embraces a second active period in which colonies apparently at rest take on a slow rotary motion which accelerates and becomes more extensive as time goes on. This is photographically the most striking stage. As the rate increases, spiral arms are extended from many colonies so that they resemble spiral nebulae. Fragments are frequently detached to lead an independent existence and may depart entirely or continue to circle the parent as a satellite. Some colonies will detach a complete ring of growth which continues to rotate about the center at some distance from it. These changes resemble those caused by centrifugal force, but this is, of course, illusory.

5. In the last stage the culture "freezes" into a final stability. In contrast to the abrupt halt in the initial phase, this stoppage is gradual. The spirals and rings rotate more and more slowly until finally all motion is lost. This seems in general to be the result of overcrowding but may be in part due to loss of surface moisture.

This last point is borne out by an accident which took place in one series. During the run the temperature in the incubator fell for a short time with a resultant condensation of moisture on the glass cover of the petri dish. In viewing the film it was noted that, simultaneously the culture, which was in the early 4th stage, ceased its rotations. When the damage was repaired, with consequent rise in temperature and redistillation of the

condensed water from the glass back to the surface of the now cooler agar, the motion was abruptly resumed. Since this motion does not appear to be impaired by room temperature as opposed to 30 degrees Centigrade, moisture would seem to be the controlling factor in this instance.

The timed nature of the lapse-time picture permits ready measurement and comparison of velocities on numbers of colonies. The following average values are typical:

Rotations (phase 4):	
Colonies 1–1.25 mm. in diameter	1.4 r.p.h.
Colonies 1.25–1.75 mm. in diameter	0.8 r.p.h.
Colonies 1.75–2.25 mm. in diameter	0.5 r.p.h.
Linear motions (phase 1):	
Colonies 0.2–0.5 mm. in diameter	14 mm./hr.

It may be interesting to compare the last value with stated velocities for single motile cells of other species.

Eberthella typhi	65 mm./hr.
Bacillus megatherium	27 mm./hr.
Bacillus alvei (colonies)	14 mm./hr.

When we consider the bulk of the *B. alvei* colony, as compared to that of a single cell, the value 14 appears to be quite respectable.

Another interesting observation is that, of the probably 200–300 rotating colonies shown in the film, only two have been detected whose motion is clockwise. The remainder are uniformly counterclockwise. This brings up again the controversy which has been raised at numerous times in the past over the reason for such directed rotation in the biological world.

To return finally to the paper by Smith and Clark, I must call attention to two points: first, the statement that (as distinguished from the motion of *Bacillus circulans*) "in the *B. alvei* type the colony moves forward like a bullet and may take any direction." It would appear evident from the motion picture studies that Smith's statement is true only for phase 1 and that in the later stages the most characteristic feature is a rapid and extensive rotation with no motion of translation. It is only fair to state that we did not have more than a vague suspicion of the existence of this rotary motion until the pictures were available. Second,

while not in a position to suggest the true mechanism, I must question the conclusion that "the motility of the colonies is not a characteristic of any one species, but that it is the result of a strong motility of the individual cells combined with certain physical conditions of the medium." I doubt if this can be the entire answer, for, while we may grant that the motive power is derived from the motility of the single cell and that the condition of the surface is a highly critical factor, we still leave unexplained the unified action of the cells so that the colony moves as a whole rather than spreading in all directions as in the case with other highly motile strains. It would seem necessary to postulate some mechanism by means of which the individualists of the cell population are induced to submit to a regimentation.

It is my impression that colonial motility in *B. alvei* has been described only in Smith's report. It is difficult to understand why such an outstanding characteristic should have been overlooked. In an article by Muto (1904) there is described an organism with strikingly similar properties. The motions of this strain (*Bacillus helixoides*) are apparently identical with those described here for *B. alvei*. The question is then raised as to whether the strain in my possession is *B. alvei* or *B. helixoides* or whether, in last analysis, these two species, which according to reported descriptions are distinctly different in morphology and staining, are one and the same. The strain in my possession would, on cursory examination, answer best to Muto's description.

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