A NOTE ON ELASTICOTAXIS IN MYXOBACTERIA

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When myxobacteria of the family *Myxococcaceae* are grown on agar slants, a striking abnormality in the production of fruiting bodies is often apparent. The fruits formed along the line of inoculation are not scattered at random, but are definitely oriented in roughly parallel lines *at right angles* to the line of inoculation. Sometimes each line is composed of a series of the usual discrete, approximately spherical fruiting bodies, but in other cases the individual fruits become confluent, so that a ridge of microcysts is formed. Due to the spread of the vegetative swarm, fruits are also ultimately formed on the agar surfaces on either side of the line of inoculation; such fruits always exhibit the customary random distribution and normal shape. In fig. 1 is shown a slant culture of *Myxococcus fulvus* which illustrates this phenomenon. It was first observed in this species, but is also exhibited by all the other members of the *Myxococcaceae* which I have studied.

In seeking for an explanation of this behavior, it was at once apparent that chemical stimuli could not be invoked, since they would also have affected the fruiting process as it occurred in the regions of the slant surface away from the line of inoculation. The only conceivable difference between the streaked and unstreaked areas of the agar was a physical one. Accordingly, a series of experiments was performed in order to clarify the nature of the physical forces involved.

MATERIALS AND METHODS

As a test organism, *Chondrococcus* (*Myxococcus*) *exiguus* (Kofler) *nov. comb.*[2] was selected since it possessed several marked advantages for the purposes of the investigation. The fruiting bodies of this species are very small and inconspicuous, but they are formed in extreme abundance and in a comparatively short period of time on dung agar. The medium employed throughout was dung-decoction agar. The dung decoction was prepared by boiling horse or rabbit dung with twice the volume of water for 15 or 20 minutes, filtering, and diluting to an appropriate concentration (usually a tenfold dilution; this has to be varied somewhat, however, from one lot of dung to the next). To the diluted dung decoction 2% agar was added.

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[2] A complete description of this organism, together with the reasons for its separation from *C. coralloides*, with which it was combined by Jahn (1924) will be given in a later publication.

EXPERIMENTS

Experiment 1. Using sterile precautions, a rectangular piece of dung agar was cut out of a poured plate and placed over a glass rod in another petri dish as shown in fig. 2. The upper surface of the agar lying directly over the glass

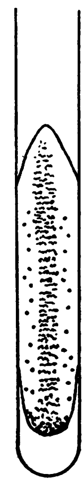

FIG. 1. MYXOCOCCUS FULVUS, SHOWING THE DISTRIBUTION OF FRUITING BODIES ON AN AGAR SLANT. EXPLANATION IN TEXT

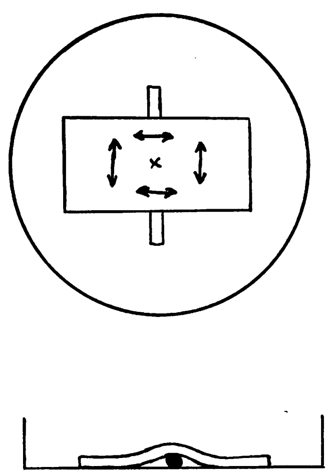

FIG. 2. THE SET-UP USED IN EXPERIMENT 1, VIEWED FROM ABOVE AND FROM THE SIDE
The arrows in the upper diagram indicate the directions of stress on the agar surface.

rod was thus brought into a state of tension, which gradually decreased on either side and changed into a state of compression.

Under ordinary circumstances, a myxobacterial swarm will spread evenly in all directions from the point of inoculation, thus forming a circular colony. However, in this experiment the swarm behaved in an entirely different manner.

From the point of inoculation (marked X in fig. 2) it spread rapidly along the lines of tension but hardly at all across them; then, as it reached the region where the agar was in a state of compression, the direction of movement shifted by 90 degrees, so that the final shape assumed was that shown in fig. 3. Microscopic examination showed that the individual rods in the swarm were oriented parallel to the direction of movement and to the lines of force in any given region. Fruiting body formation occurred in irregular parallel lines which lay at right angles to the direction in which movement had taken place (fig. 3). The marked difference between fruiting body formation on stressed and unstressed media is shown in fig. 4. This experiment was repeated a number of times and always gave similar results. Occasionally the inoculation was made on the two ends of the agar strip which, lying flat on the floor of the petri dish, are subjected neither to tension nor to compression. In such cases, normal

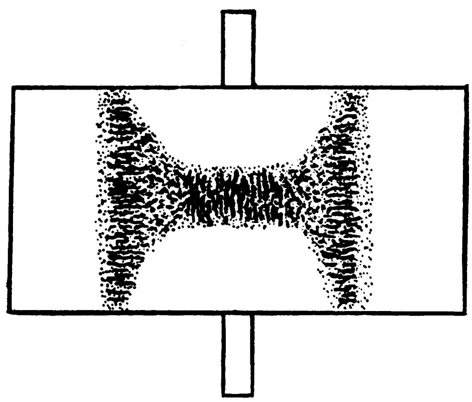

FIG. 3. GROWTH OF C. EXIGUUS IN EXPERIMENT 1
The stippled area represents the swarm; the black lines within this area represent the fruiting bodies.

fruiting body formation occurred in the areas surrounding the inoculum, but changed sharply to the oriented pattern in those parts of the swarm which had reached and fruited on the areas of compression (fig. 5).

Experiment 2. Some sterile glass beads were scattered over the surface of a dung agar plate, which was then inoculated in the center. Normal random fruiting occurred in the regions free of beads, but around each bead the fruits were formed in irregular concentric rings, which gradually became less marked as the distance from the bead increased. The same phenomenon, although less sharply defined, is often observable in the immediate neighborhood of the inoculum, as can be seen in fig. 4.

Experiment 3. A dung-agar plate was inoculated in the center and incubated in a vertical position. Under these conditions no distortion of swarm or fruiting bodies took place, showing that the previously noted effects could not be attributed to gravitational forces.

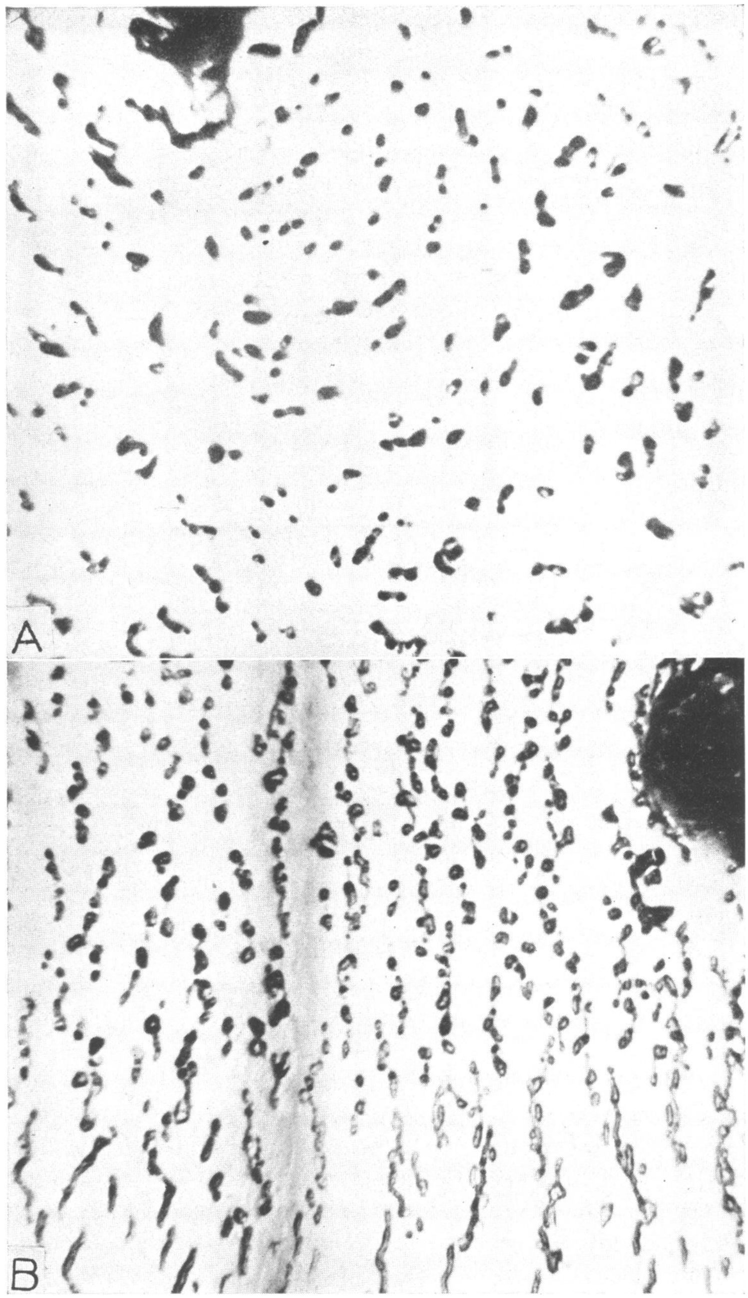

FIG. 4. FRUITING BODY FORMATION BY C. EXIGUUS

A. On an unstressed surface. Note the indications of a concentric arrangement around the inoculum (large dark patch). B. On a surface subjected to tension.

INTERPRETATION AND DISCUSSION OF THE RESULTS

These experiments make it apparent that the peculiar manner of fruiting-body-formation first observed on slants is only a secondary effect, induced by oriented movement of the swarm. Furthermore, it is clear that the directive force which affects the vegetative cells is derived from the stresses in the agar gel which is acting as the substrate. Thus there are two distinct questions to be answered; what are the factors causing oriented movement of the vegetative cells? and how does the orientation of the vegetative cells alter the shape and arrangement of the fruiting structures?

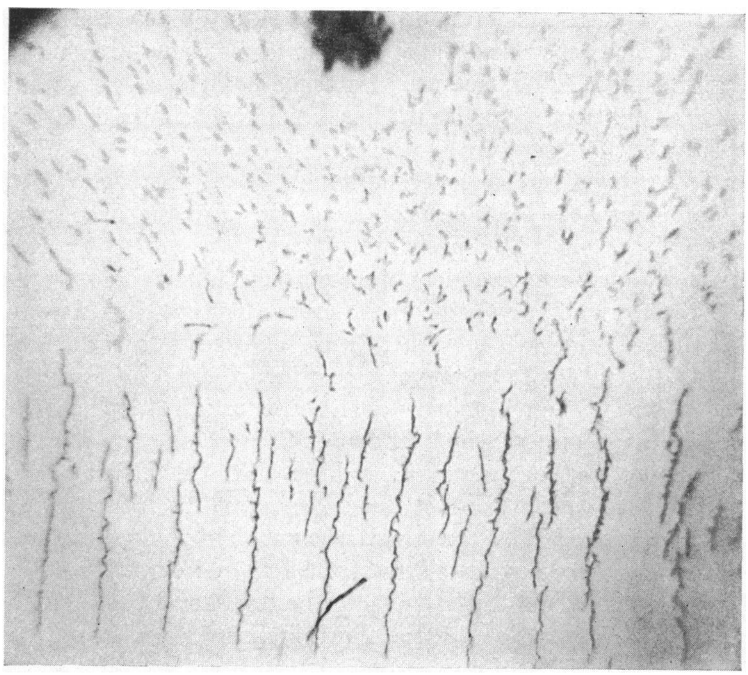

FIG. 5. FRUITING BODY FORMATION BY C. EXIGUUS, SHOWING THE CHANGE IN PATTERN AS THE SWARM SPREAD FROM AN UNSTRESSED SURFACE TO ONE SUBJECTED TO COMPRESSION

The orientation of *growth*, rather than *movement*, of living cells in response to stretching or compression of the substrate is a phenomenon which has long been known. In a masterly series of experiments, Jacobsen (1907) and Sergent (1906, 1907) showed that the growth pattern of *Kurthia* (*Zopfius*) *zopfii* in and on gelatin media is conditioned absolutely by the stresses which occur in the gelatin. In a number of ingenious ways they produced predictable stresses in the gelatin which were patterned in every detail by the growing *Kurthia* threads. Similar studies were later conducted by Kufferath (1911). More recently Weiss (1933, 1934, 1939) has studied extensively the oriented growth *in vitro* of vertebrate cells on coagulated plasma subjected to stresses. The patterns of development described by Weiss, Jacobsen, Sergent and Kufferath are broadly comparable to those found in the present work, and there can be little doubt that in all these cases the factors responsible are the same.

Weiss has explained the directive effect of tensions as being due to the production of oriented structures in the substrate whose pattern is retraced by the developing cells; to this "ultrastructural organization" he has ascribed a major organizational rôle in embryological development. In order for this explanation to be valid, the substrate (ground substance) must be composed of large molecules possessing elasticity and capable of assuming a long chain structure under the influence of mechanical tension. These requirements are fulfilled by all three substrates on which the phenomenon has been observed. In coagulated plasma and gelatin the chemical skeleton consists of polypeptide chains, in agar of polysaccharide chains—large polymeric molecules which can become oriented in the postulated manner. Particularly in the case of gelatin the potential orientability of the molecules has been clearly shown by X-ray analysis of stretched gels (for literature see Meyer and Mark (1930)). 2% agar gels under tension exhibit birefringence, although unstretched agar of the same concentration does not—an indication that here also the necessary oriented structures can be produced by mechanical forces.

Perhaps the most convincing piece of evidence in support of Weiss' hypothesis is the observation, made by Weiss, Jacobsen and myself, that after the release of tension oriented growth or movement will still occur, due to the fact that the orientation in the substrate has become semipermanent. In the present work this is best shown by the preliminary observation of oriented fruiting along the line of inoculation of an agar slant long after the tension caused by pulling the loop over the agar has been removed. I have observed the same phenomenon on plates streaked with *Cytophaga krzemieniewskae* and *C. diffluens*. The swarm spreads far more rapidly along the streaks than at right angles to them, although the pattern eventually becomes obliterated by the decomposition of the agar. That syneresis cannot be a causative factor here is shown by the fact that oriented growth continues as long as the third day after inoculation.

Thus the primary effect of an oriented ultrastructure on the development of myxobacteria consists of an induced orientation in the swarm of vegetative cells. How does this in turn lead to the observed orientation of the fruiting bodies?

Under normal circumstances when fruiting occurs on an unstressed medium the vegetative cells in any given region of the swarm move towards one or more points on the surface of the agar and aggregate there to form the fruiting locus. The cells in these loci then gradually become transformed into microcysts. The vegetative rods in the area surrounding a fruiting locus orient and move towards it from all sides, just as if they were iron filings being attracted to the pole of a magnet. As a result, the mature fruiting body tends to be circular, or approximately so, in cross section. This is most perfectly exemplified in *Myxococcus fulvus* and *M. virescens; Chondrococcus exiguus* and the other *Chondrococcus* species which I have studied form somewhat irregular fruits. The normal fruiting process is shown diagrammatically in fig. 6.

On a substrate which is under tension the free movement of the vegetative cells in all directions necessary for the formation of round, discrete fruiting bodies is no longer possible or is greatly reduced; the cells can move back and forth parallel to the oriented ultrastructure of the substrate, but their movement across the lines of force is impeded. Thus when the stimulus to fruiting occurs, the vegetative cells are prevented from moving directly to the locus, and aggregate instead in irregular lines, as shown in fig. 7. If the orientation is weak, sufficient movement across the lines of stress may occur to make possible the formation of a row of discrete fruiting bodies, but on more strongly oriented substrates the final result will be a continuous ridge of microcysts.

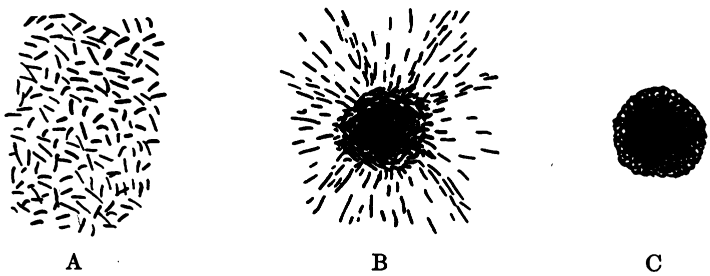

FIG. 6. DIAGRAMMATIC REPRESENTATION OF FRUITING BODY FORMATION IN THE FAMILY MYXOCOCCACEAE AS IT OCCURS ON AN UNSTRESSED MEDIUM

A. Undifferentiated swarm. B. Vegetative cells moving to a fruiting locus. C. Mature fruiting body. A completely random arrangement of vegetative cells as shown in 4A is actually rarely seen, since there are usually waves and ridges of oriented moving cells all through the swarm; however, the cells in such regions are potentially capable of moving in any direction.

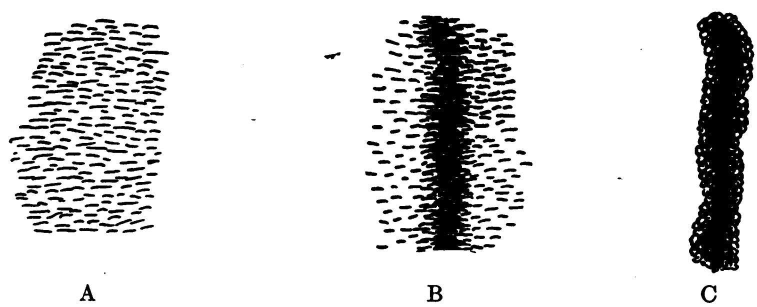

FIG. 7. DIAGRAMMATIC REPRESENTATION OF FRUITING BODY FORMATION IN THE MYXOCOCCACEAE AS IT OCCURS ON AN ORIENTED SURFACE

A. Undifferentiated swarm. B. Vegetative cells moving to a fruiting line. C. Mature fruiting body.

Jacobsen proposed the name *elasticotropism* for the directed growth of *Kurthia zopfii* through stretched gels; on the basis of his terminology, the directed movement of the myxobacterial swarm under similar conditions should be called an *elasticotaxis*.

SUMMARY

Swarm movement in myxobacteria belonging to the family *Myxococcaceae* is oriented parallel to stresses in the agar substrate, which results in a subsequent orientation of the fruiting bodies in irregular lines at right angles to the direction

of movement. The possible cause of this phenomenon, which may be termed an elasticotaxis, is discussed with particular reference to the hypothesis of Weiss.

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