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Fruiting Chemotaxis in Myxococcus fulvus (Myxobacteria)

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INTRODUCTION: Many myxobacteria are known for their fruiting behavior. Individual cells enmeshed in their slime will eventually come from all directions to a central spot (Fig. 1). At this spot, the accumulated cells become the fruit. A heap of cells is the fruit in *Myxococcus sp.*, and an elaborately branched fruit is characteristic of *Chondromyces sp.* Taxonomists now call the myxobacterial fruiting body the *myxosporangium.* The cells inside the myxosporangium are called *myxospores* in preference to the former designation of microcysts (John Holt, Iowa State University, Ames; personal communication). The myxospores are relatively resistant resting cells.

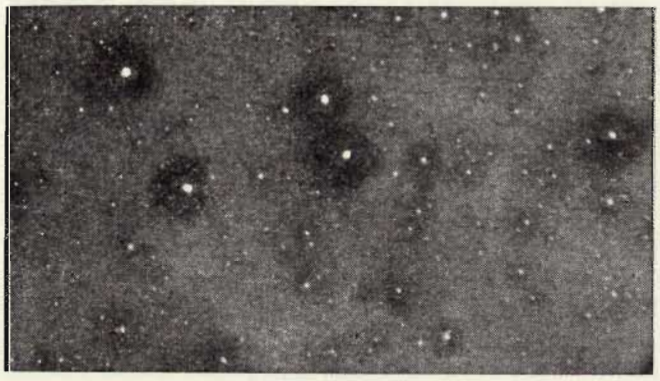

FIGURE 1. Myxosporangia with their fruiting zones. Casitone-grown and $CaCO_3$ induced. About 5X.

What causes the cells to come to the central spot? The formation of myxosporangia suggests behavior parallel with that of the myxamoebae, the *Acrasieae.* Their life history is well-documented (e.g., Bonner, 1959). The aggregative behavior is under the influence of chemical substances (Runyon, 1942; and Bonner, 1947; and Sussman, Lee, and Kerr, 1956). Is myxobacterial fruiting aggregation chemically controlled? Indirect evidence has been given by Bonner (1952) and Smith (1956), who used *Chondromyces crocatus.* Lev (1954) suggested fruiting chemotaxis in *Myxococcus sp.*, but the method he used does not make it clear whether nutritional or fruiting chemotaxis is involved.

Direct evidence has been given by McVittie and Zahler (1962) working with dispersed strains of *Myxococcus xanthus.* Their method consists of using a cellophane membrane on the surface of agar. *M. xanthus* cells growing and fruiting on the agar cause the cells that have been added on the top of the cellophane to aggregate in the same position as the myxosporangia below. McVittie and Zahler's type of evidence is similar to the evidence submitted by Runyon (1942) in his work with *Dictyostelium discoideum.* Runyon's findings were later confirmed in a number of ways by Bonner (1947), who showed that there must be a chemical substance involved.

In this paper an attempt is made to confirm and improve on McVittie and Zahler's work with myxobacteria. Rather than use dispersedly grown cells, the methods to be outlined take advantage of the adherent swarm grown in static, submerged culture. Advantage is taken of the property of the swarm to produce myxosporangia in the absence of nutrients (Fluegel, 1962).

MATERIALS AND METHODS: *Organism.* The myxobacterium, *Myxococcus fulvus* (MFC), was isolated from Duluth soil. *M. fulvus* makes a small spherical or hemispherical, salmon-pink myxosporangium on milk agar. It is visible to the unaided eye. The myxosporangium is a heap of slime and myxospores. At times the heap may have a constriction at the base. A few days after its formation, the structure collapses, thus facilitating dispersal of the myxospores. The swarm is strongly proteolytic but lacks amylolytic properties. The vegetative cells are gram negative. The swarm cells vary in size depending upon nutritive conditions but are about 0.5 to 0.8 μ wide and about 8 to 10 μ long. They have blunt ends. The myxospores are round and about 1.2 μ in diameter. When they glide, the cells make slime in the form of fine threads (Fluegel, J. Bacteriol., *in press*). The mode of this gliding locomotion is not known.

Growth and induction methods. Liquid media used were (1) non-fat dry milk (Carnation) medium (8 g in a liter of water), or (2) casitone medium (Difco-casitone, 5 g; $MgSO_4$, 1 g; 100 ml of .1 M, pH 7.2, phosphate buffer and water to make 1000 ml).

Myxospores from two 2 to 3 week old milk agar slants (0.8% milk, 2.0% agar) were dispersed in 500 ml of either liquid medium. The medium with the myxospores was then transferred to plastic or glass petri dishes and incubated at 31 C.

Myxosporangia do not appear in *rapidly growing,* submerged swarms unless induced with Ca++ (Fluegel, 1962). When there was a desired amount of growth on the petri dish floor, the growth medium was discarded. Either saturated $CaCO_3$ or .01 M $CaCl_2$ solution was used to wash the swarm. Ten ml of the same solution was then placed in the petri dish, and it was incubated at 31 C. Within 24 hr there were numerous pinkish to

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white myxosporangia on the dish floor in the swarm slime. When India ink was added to milk, the developing swarm was black. Upon induction with calcium solution, the myxosporangia arise from beneath the slime, break through, and mature (Fig. 2). The difference in colors makes this obvious. If water is used for wash and induction, the process takes 2 to 3 days.

FIGURE 2. The formation of the myxosporangium from the slime.

TECHNIQUES AND RESULTS: *Water induction.* Chemotactic response occurred when water was used to induce myxosporangia in milk-grown swarms. In 0.8% non-fat dry milk, swarms develop clavate columns that are about 120 μ high and 50μ wide at the upper portion before they branch. They branch as shown in Fig. 3A. At this time the swarm was washed in water, and water replaced the discarded medium. A myxosporangium (M) begins to form in 2 to 3 days among many columns. The branches of the columns point in random directions; some (e.g., #3) point directly at M. Those branches which point in the opposite direction (e.g., #1) after M is formed eventually supply cells (as in B) to branches #3. Branch #3 grows for 2 to 3 days until the myxobacteria become myxospores. The extended branch resembles a miniature carrot. If any nearby branch (e.g., #2) does not point directly at M, then this branch exhibits a bent growth toward M. In gross view there may be five or six miniature carrot-shaped structures pointing toward the myxosporangium from various branched columns surrounding the myxosporangium.

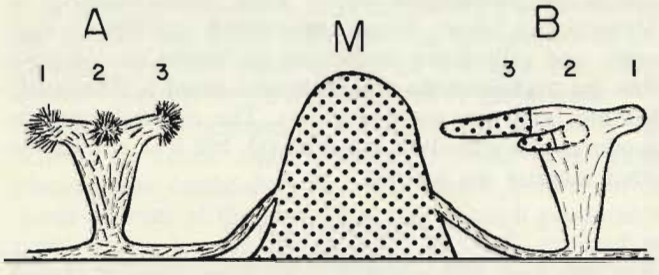

FIGURE 3. Column response toward myxosporangium.

The process exhibited by Fig. 3 does not happen when calcium inducer is used. The calcium induction takes place in 14 to 18 hr, and all of the fruits are as illustrated in Fig. 2. But, other strains which are slow fruiters in calcium inducer do exhibit the process shown in Fig. 3. Therefore, the phenomenon is probably a function of time and the reaction of the cells in the columns toward the established myxosporangium.

Circulation currents. One method used by Bonner (1947) to test for chemotactic response in *D. discoideum* was to provide water circulation. He showed that when an aggregative center produced a chemical substance, those amoeba in the lee of the center responded positively by migrating toward the stimulus. Those upstream from the center would not receive the stimulus, and hence could not respond.

Because myxobacterial aggregative centers originate from beneath the swarm slime, a chemical stimulus carried by water currents must first get to the water where it can circulate. It has been found that very young cultures do not produce very many large myxosporangia which break through the swarm slime. Older casitone-grown cultures of 4 to 6 days do produce larger myxosporangia which break through. The method of circulating water is diagramed in Fig. 4. The results are shown in Fig. 5A and B.

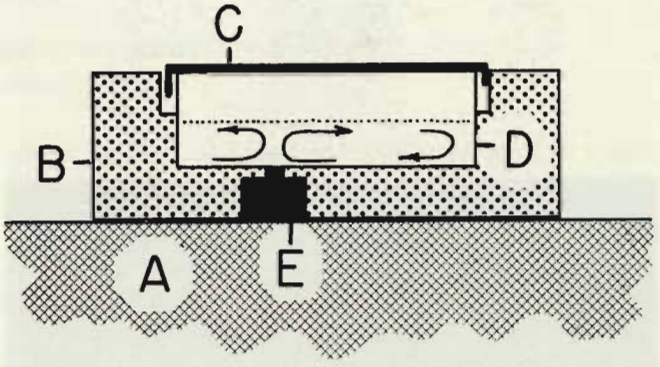

FIGURE 4. Method for circulating inducer solution in petri dish.

A. mild heat source (flat laboratory fluorescent lamp)
B. foam plastic insulation.
C. porous porcelain cover which aided in evaporation and cooling of the petri dish "D".
E. brass plug which delivered heat to one spot in dish. Arrows in dish show path of water current.

Figure 1 shows a fruiting zone around the myxosporangium. Figure 5A also shows this. In the circulation experiments, this zone arises because cells enmeshed in the slime are not affected by the circulating inducer. However, once the formed myxosporangium breaks through the slime mat, or once there is sufficient chemotactic substance available from the developing myxosporangium, other cells will respond (Fig. 5A). Hence, there is a clear zone ahead of the myxosporangium. When the responder cells are all collected behind the original myxosporangium, they aggregate into individual fruits (Fig. 5B).

Cellophane technique. McVittie and Zahler's (1962) cellophane technique requires dispersedly-grown cells. It is difficult to acquire such mutants from stock cultures, and there is no record of such a mutant from *M. fulvus.* My cellophane method is as follows:

The bottom of a small jar was cut off with a diamond wheel. (Thanks are expressed to Dr. Heller of the Geology Dept.) A large hole was made in the bakelite cover, and a piece of cellophane was stretched over the mouth of the jar; then the cover was put on tightly. The assembly was submerged, cover side up, in a 90 × 50 mm Pyrex dish with casitone medium and sterilized. Regular petri dishes and the cellophane were inoculated. A swarm grew on the upper surface of the cellophane (Fig. 6A).

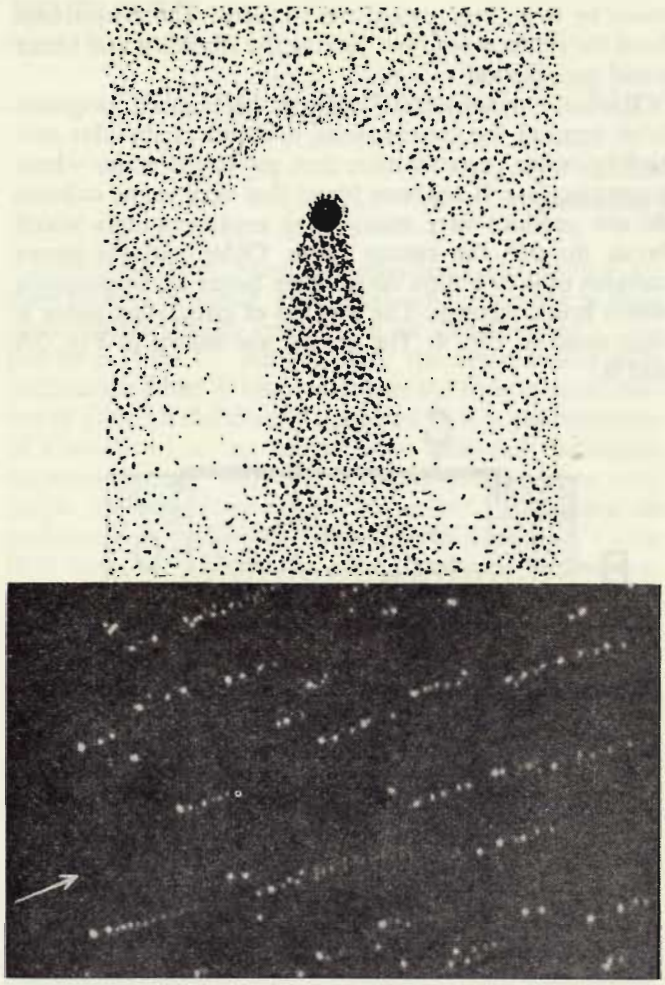

FIGURE 5. Responder cells produce a "comet-tail" effect in the lee of the myxosporangium. (B) When the responders are of sufficient concentration they become myxosporangia. Note that some myxosporangia do not have responders. This is probably caused by the formation not breaking the surface of the slime mat. Arrow indicates directional flow.

Once growth was adequate (3 to 5 days) the assembly was washed in calcium inducer solution and inverted in inducer. The petri dish swarms were also washed in inducer. Swarms (about 1 cm sq) were dislodged from the petri dishes with jets of water from a long, tapered, bulb pipette. These pieces were transferred to the inverted cellophane assembly. The transferred piece was allowed to settle on the bottom on the cellophane. It was oriented by jets of water before water was removed. When water was removed, the swarm adhered to the cellophane. The whole assembly could then be removed from its container, with a swarm on either side of the cellophane (Fig. 6B). Fruiting took place in a moist chamber in less than 24 hr.

If both swarms were induced at the same time, the myxosporangia on the top did not align themselves with those on the underside of the cellophane membrane (Fig. 6C). If the top swarm was allowed to start producing myxosporangia (14 hr in inducer) and then the second swarm was applied, there was good alignment (Fig. 6D). One interpretation is that it takes time for the chemotactic substance to penetrate the cellophane (20μ). When the two swarms are out of phase with respect to induction time, a chemotactic response is demonstrated.

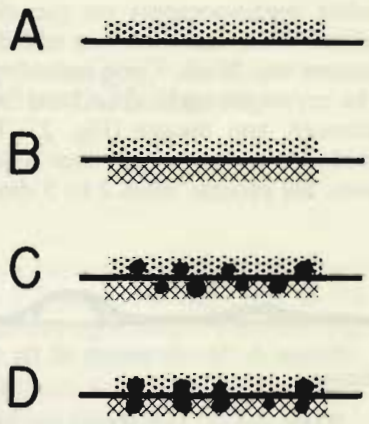

FIGURE 6. Diagram of cellophane method. Text gives details. At D, note that one myxosporangium on the lower surface does not have a companion on top. It sometimes appears that a myxosporangium "belongs" in a space regardless of whether a myxosporangium is "missing" on top. The "missing" one is probably not there because of accidental inadequate growth or damage to the swarm in preparation of the second swarm. The occasional out-of-place myxosporangium on either side is not usual. The alignment of the others is exact and predominates.

DISCUSSION: The present chemotaxis experiments, and those done by others, confirm only one thing; i.e., myxobacterial cells will migrate toward a central supply of diffusing chemical substance(s) from a developing myxosporangium. Fruiting is at least three separate processes: (1) *initiation*, (2) migration leading to *accumulation* of cells, and (3) *conversion* of cells to myxospores. In this paper, only the first two concern the stated problem. Of these, only accumulation has been confirmed as being under chemical control. Initiation now needs to be understood.

ABSTRACT: Fruiting chemotaxis in *Myxococcus fulvus* (myxobacteria). Myxobacterial fruiting chemotaxis is confirmed by a number of methods. The methods make use of the submerged swarm from liquid cultures of *Myxococcus fulvus*. Swarm movement, circulation currents, and cellophane membrane technique demonstrate that the myxobacteria will migrate toward a developing fruiting body (*myxosporangium*). The myxosporangium produces the attractant substance(s), but it is not known what initiates the process.

LITERATURE CITED

BONNER, J. T. 1947. Evidence for the Formation of Cell Aggregates by Chemotaxis in the Development of the Slime Mold. *J. Exptl. Zool.* 106:1-26.

BONNER, J. T. 1952. *Morphogenesis*, pp. 165-173. Princeton University Press, Princeton, N. J.

BONNER, J. T. 1959. *The Cellular Slime Molds*. Princeton University Press, Princeton, N. J.

FLUEGEL, W. 1962. Slime and calcium: factors in myxobacterial fruiting. *Bacteriol. Proc.*, p. 46.

LEV, M. 1954. Demonstration of a Diffusible Fruiting Factor in Myxobacteria. *Nature* 173:501.

MCVITTIE, A. and S. A. ZAHLER. 1962. Chemotaxis in *Myxococcus*. *Nature* 194:1299-1300.

RUNYON, E. H. 1942. Aggregation of Separate Cells of Dictyostelium to Form a Multicellular Body. *Collecting Net* 17:88.

SMITH, M. M. 1956. *Cultural and Morphogenetic Studies on Chondromyces crocatus*. Unpublished M.S. Thesis, University of Wisconsin, Madison.

SUSSMAN, M., F. LEE, and N. S. KERR. 1956. Fractionation of Acrasin, a Specific Chemotactic Agent for Slime Mold Aggregation. *Science* 123:1171-1172.

Factors Affecting Infection and Oospore Formation of Aphanomyces euteiches Drech. In Excised Root Tip of Pisum sativum [1]

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University of Minnesota, St. Paul

Common root rot of canning peas incited by *Aphanomyces euteiches* Drech. is one of the most serious root diseases of this host. It is the limiting factor in the production of peas in the Midwest at the present time, and little resistance is available in any commercial varieties now in use.

It is suspected that the organism is a free-living saprophyte in the soil and continually cropping an area to peas results in a build-up of the fungus to such a level that severe losses occur. It has been reported to remain dormant in the soil for as long as 10-20 years (14).

Hyphae from zoospores grow through cell walls into the root and the fungus causes a severe cortical rot (10). The fungus produces oospores in the host tissue and it has been suggested that the oospores remaining in plant debris are the principal means of survival. If an adequate and inexpensive means of control of this important disease is to be developed, more information is needed on the factors that affect infection, the sporulation of the organism and the relationships of the host and other micro-organisms in the development of the disease.

The causal organism, *A. euteiches*, was first fully described by Jones and Drechsler in 1925 (10). Their observations established that the fungus enters cortex tissue of roots at the base of stems, where it produces a rapid decay, leaving the vascular elements exposed to attack by other organisms. Thick-walled oospores, considered to be the sexual and dormant stage of the fungus, could be observed in large numbers in the cortical tissue.

Cunningham and Hagedorn (4) exposed pea roots to zoospore suspensions of *A. euteiches* and within 2 hours penetration and infection had occurred. They also observed that germ tubes of zoospores enter through root hairs, and between epidermal cells.

A method for culturing the organism and obtaining sufficient zoospores for use as inoculum was first developed by Schneider and Johnson (15). The fungus was grown on corn meal agar and then transferred to a sterilized decoction of maize kernels in distilled water. After 5-7 days, the decoction was poured off and the mycelium rinsed in three changes of sterilized tap water and allowed to remain in the last rinse. Twelve hours later more than 100,000 zoospores per ml were obtained. In all instances the zoospores were produced asexually and the optimum temperature for production was between 15° and 20° C. Carmen and Lockwood (1, 2) modified this technique slightly and reported maximum production occurred at 24° C, the range being 20° to 28° C.

Various methods have been established for inoculation of peas with zoospores of *A. euteiches* (8, 9, 11, 13). Johnson and Bissonnette (9, 11) inoculated soil in which peas were growing, with a known concentration of zoospores. Lockwood and Ballard (13) followed the same procedure, using silica sand instead of soil. At present the technique followed at the University of Minnesota is that developed by Haglund and King (8). Plants are germinated in sterilized vermiculite for 7 days at 21° C, removed, and the roots washed with running tap water. The plants are then suspended in a zoospore concentration of 100,000 zoospores per plant root for 24 hours at room temperature. After that time they are planted in 5-inch pots containing steamed soil and placed in the greenhouse.

With this method of inoculation, a uniform infection and destruction of seedling roots is obtained with pathogenic isolates of the organism, and is effective in screening varieties and lines of peas for resistance to the disease. The disadvantage of the method is the time required and the greenhouse space necessary.

Very little information is available on the factors affecting infection on the roots of pea plants, the factors that affect oospore formation and germination and the relation of the roots of susceptible and resistant pea plants to sporulation of the fungus. Therefore, studies were made to determine the relationship that exists between the roots of the host, the organism and the factors that influence the host-parasite relationship.

MATERIALS AND METHODS: The four isolates of *A.*

[1] Paper No. 4935, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul 1, Minnesota.

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