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Introduction: The fruiting myxobacteria and the cellular slime molds *(Acrasiales)* have many superficial resemblances with respect to fruiting behavior. Cells in the swarm are triggered by chemical substances to aggregate and form fruiting bodies (Bonner, 1947; Fluegel, 1963a). A chief difference between the two with respect to fruiting behavior, is that the myxoamoeba are free cells whereas the myxobacters are enmeshed in slime threads (Fluegel, 1963b). However, it is tacitly assumed that if parallel studies be undertaken in myxobacteria as has been done with the slime molds, the cells must be grown dispersed. Most isolates of myxobacteria do not grow dispersed; when grown in liquid, they form an adherent swarm on the walls of their container beneath or on the surface of the medium. This swarm can be induced to form fruiting bodies using the methods outlined below, but they do not, as a rule, form submerged fruiting bodies in growing cultures.

To my knowledge there are no reports concerning the inducement of myxobacterial swarms to fruit.

Methods and Results: All growth methods for the test organism are the same as reported previously (Fluegel, 1963a). *Myxococcus fulvus* is found in most soils and can be isolated by a variety of simple techniques. As the bacterial swarm grows over the surface of agar (0.8% w/v non-fat milk) from a spot inoculation, a number of small fruiting bodies develop some distance behind the advancing front of cells. The fruiting bodies are a salmon-pink, hemispherical to spherical mass of microcysts (myxospores). The fruiting bodies measure about 1 mm in diameter or smaller. The myxospores are resting cells and each is derived from a single swarm cell (Voelz and Dworkin, 1962). A spot inoculation or massive myxospore inoculation in static, liquid culture in petri dishes produces no fruiting bodies in the submerged swarm. To induce fruiting in these swarms, it was thought necessary to free the cells from the growth medium. A simple 3-min. wash in water induced fruiting; the fruiting bodies emerged from the swarm in 2 to 3 days. It was discovered that the longer the adherent swarm was washed in water or 0.01 M phosphate buffer at pH7.0 (over 10 min), the poorer the inducement results. Further experiments showed that buffer removed ions necessary for fruiting and could completely halt the process when washing was extended to 30 min. The wash procedure included a 15 to 20 min. soak in buffer.

Ion need. Either Ca^{++}, Mg^{++}, Sr^{++}, K^{+}, or Na^{+}, as 0.01 M chloride in distilled water (10 ml solution per standard dish) served as replacement ions and caused fruiting in water-washed, buffer-treated swarms. Buffer and water controls did not fruit but cells were viable for 5 days. The K+ and Na+ produced fruits of poor quality in 2 to 3 days; the other ions caused fruiting in 24 hr. or less. Calcium as saturated $Ca_3(PO_4)_2$ solution neutralized with H_3PO_4 and filtered, 0.01 M $CaCl_2$, or saturated $CaCO_3$ produced fruiting bodies in 24 hr. or less; usually beginning at 14 hr. Apparently the phospate is not an inhibitor nor is the pH (as the chloride or carbonate) of great importance. Other experiments with $CaCl_2$ showed that CO_2 may be necessary in fruiting. When young swarms were induced with 0.01 M $CaCl_2$, and CO_2 was removed from the air with 15% KOH, they did not fruit but they were viable. Controls fruited in the regular time (14 hr). Hence, the $CaCO_3$ is preferred as a wash and soak solution inducer.

Fruiting process. The original casitone-grown swarm (Fluegel, 1963a) is composed of randomly oriented cells and is several cells thick. Induced submerged fruiting bodies began to appear about 14 hr. after Ca^{++} wash and soak treatment. Irregular fracture patterns separate translucent, cloud-like masses of the slime and cells. The clouds measure roughly between 50μ to 1 mm or more across. Each cloud of cells rounds up or shrinks and becomes hemispherical. Larger clouds may break up into 2 to 3 smaller clouds. As myxospores begin to form from the aggregated cells, at about 30 hr, the masses become opaque. The fruiting bodies may be pink to white depending upon cultural conditions. The in-gathering of cells actually takes place from beneath the slime matrix next to the dish surface. As the fruit increases in size it pushes up the slime, breaks through, and matures. A mature fruiting body is one which has a sharp outline and contains myxospores. The fruiting body remains intact for over 2 weeks in inducer and does not collapse as it does on agar (3 to 5 days).

The continuous need for Ca^{++}. The continuous needs for Ca^{++} is demonstrated by the following "on-off-on" experiment. A number of young, casitone-grown swarms in petri dishes were induced with $CaCO_3$. Twelve hours later, when no fruiting bodies were evident, two dishes were taken and Ca^{++} was removed by buffer wash and then soaked in buffer. At each hour thereafter, the process was repeated with another pair of dishes for a total of six pairs of swarms. Fruiting in controls began

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about the 14th hour. By the 17th hour, the developing masses were immature, recognized fruiting bodies. Each time the treated swarms and controls were examined, it was obvious that the buffer treatment suspended the fruiting process and maturation. The buffer-treated swarms were kept at 31°C overnight. There was no change 35 hr. after Ca^{++} inducement time, but controls had myxospores in the developed fruiting bodies. At 35 hr. one member of the buffer-treated pair was rewashed for 15 min. in water to remove buffer and given $CaC0_3$. The 12, 13, 14, and 15 hr. swarms developed mature fruiting bodies and could not be distinguished from the first controls. In the 16 and 17 hr. dishes, about one-third to one-half of the structures were ghost fruiting bodies with no myxospores. The removal of calcium at that time evidently crippled fruiting. Buffer controls remained unchanged for 48 more hours.

Age of the swarm seems important. The maximum number of induced fruiting bodies occurs in the upper log phase. As the swarm ages, the number of induced fruiting bodies diminishes and the fruiting in the swarm is patchy. The site "chosen" for fruiting is governed by some unknown process producing fruiting bodies that tend towards an even distribution. Details concerning the distribution, number, and size of the fruiting body will be published elsewhere.

Optimum temperature for induced *M. fulvus* fruiting is between 32° to 34° C (casitone grown). The swarm will not fruit at 36° C, but the cells remain viable. The same swarm will fruit when the temperature is reduced to its optimum. Inducement above 36° C results in death of the swarm. These critical temperatures were determined by the use of a shelf-type gradient incubator Fluegel, 1963c.

Other myxobacteria. The Ca^{++} inducement procedure is successful with a wide variety of isolates which fruit on agar, including many other *Myxococcus* spp., a *Stelangium* sp., and a *Chondrococcus* sp. The latter two produce hard fruiting bodies on agar, but the submerged fruiting bodies are hemispherical, soft masses. A tentatively-identified *Sporocytophaga* (myxospore-producing but a non-fruiter), kindly sent to me by Dr. Peterson (University of Missouri), responded to $CaC0_3$ inducement. However, it had to be grown in a liquid medium consisting of 1 g $CaC1_2$ and 5 g casitone (Difco) in 1000 ml water. This latter treatment was applied successfully to other strains which apparently lost their fruiting capacity when grown on agar medium. Such strains could easily be mistaken for *Sporocytophaga* if freshly isolated.

Not all isolates produced as abundant fruiting bodies as *M. fulvus*. There were "poor" or "good" fruiters depending upon number of fruiting bodies. The isolates which lost their fruiting capacity were generally poor fruiters with calcium medium. Since number of fruiting bodies is dependent upon age of the swarm, the "poor" or "good" designation awaits defining in terms of optimum capacity by the outlined or other conceivable methods.

Discussion. The process of fruiting involves *initiation* and *migration* of cells toward a common center. Conversion of cells to myxospores is a separate event and is not dependent upon the fruiting process (Voelz and Dworkin, 1962) since cells can form myxospores without fruiting body formation. This raises an interesting taxonomic problem. When do we remove all doubts that a myxospore-producing, non-fruiter is truly a *Sporocytophaga?*

Dworkin (1963) showed that *M. xanthus* fruiting is controlled by the amino acid diet. What $Ca++$ (or the other divalent ions) does at the molecular level and how it is instrumental in initiating and maintaining the process remains to be discovered.

Abstract. Various fruiting myxobacteria are grown in static, liquid media as submerged, adherent swarms. They can be induced to form fruiting bodies with the continuous presence of Ca^{++} solutions. The inducement may have morphological and taxonomic value.

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