THE GROWTH OF DICTYOSTELIUM DISCOIDEUM
UPON PATHOGENIC BACTERIA

KENNETH B. RAPER AND NATHAN R. SMITH
Division of Soil Microbiology, Bureau of Plant Industry, United States Department of Agriculture

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INTRODUCTION

Species of the Dictyosteliaceae, a group of pseudoplasmodium-forming slime molds, are not uncommon in nature and can be readily isolated from soils, from decaying vegetation and from the dung of various animals. In contrast to the Myxogastrellae which possess true plasmodia, the Dictyosteliaceae are characterized by myxamoebae which retain their identity throughout the whole life cycle of the organisms. During the vegetative stage these myxamoebae are free-living and feed by the ingestion and digestion of bacterial cells. With the exhaustion of the available food supply, the myxamoebae collect into aggregates termed pseudoplasmodia, and collectively build fruiting structures, or sorocarps. In these structures some myxamoebae become transformed into sterile, supportive cells to form a stalk, or sorophore, while others become differentiated into fertile spores forming a spore head, or sorus.

The ubiquitous species Dictyostelium mucoroides Bref. has been more widely studied than other members of the group and from the work of Vuillemin (1902), Potts (1902), Pinoy (1903 and 1907), and Schuckmann (1924) a limited bacterial "host range" embracing twelve different species has been reported for it.

1 Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, No. 171. A portion of the results here reported were included in a thesis submitted by the senior author to the faculty of Harvard University, June 1936, in partial fulfillment of the requirements for the degree of doctor of philosophy in biology.
Recently *Dictyostelium discoideum* Raper, has been cultivated in pure-mixed culture with thirty-one different species of saprophytic bacteria representing widely separated groups (1937). This study enlarged by almost threefold the number of bacteria with which a species of the Dictyostelaceae is known to be able to grow.

In the present investigation the writers have studied the growth of *D. discoideum* in association with certain bacteria that are pathogenic to animals or to plants. Such a study seemed particularly desirable in view of the limited attention earlier investigators had given to the cultivation of related slime molds upon bacteria pathogenic to animals. The present work reports for the first time the cultivation of a species of the Dictyosteliaceae with phytopathogenic bacteria.

**MATERIALS AND METHODS**

*D. discoideum* was chosen for this study above other species of the Dictyosteliaceae because of two significant characteristics. First, due to the peculiar migrating habit (Pl. 1, fig. 6b) of its pseudoplasmodia (Raper, 1935) spores entirely free from bacteria are regularly borne on sorocarps that develop 0.5 cm. or more distant from the margin of the bacterial colony (Pl. 2, fig. 1c) in which the myxamoebae grew and in which the pseudoplasmodia formed (Raper, 1937). The importance of this character is at once apparent as it permits the investigator to inoculate this slime mold in pure-mixed culture with any selected bacterial culture at will by selecting isolated sorocarps as spore sources. Second, one is able to compare quantitatively the growth of this slime mold upon different bacterial species with a degree of accuracy that is impossible with other species of the group. This is true because the mature fructifications of *D. discoideum* are regularly erect and can be counted (Pl. 2, fig. 1b) whereas those of other species are commonly decumbent, long, and tangled to such an extent as to preclude their enumeration (Pl. 2, fig. 5a). Furthermore, in *D. discoideum* the proportion of stalk mass to spore mass remains fairly constant in all normal sorocarps irrespective of their size, thus enabling one to express conveniently
the growth represented by sorocarps of different size in terms of common units, such as medium sorocarps or "medium-sorocarp equivalents",² by computing the volumes of sori of different sized sorocarps.

Dictyostelium discoideum was originally isolated from leaf mould (Raper, 1935), and other species of the Dictyosteliaceae such as Dictyostelium mucoroides, Dictyostelium purpureum Olive and Polysphondylium violaceum Bref. regularly occur in decaying vegetation and in soil (Raper and Thom, 1932). Accordingly for this study there have been selected chiefly species of pathogenic bacteria which under certain conditions may occur in soil.

Hay infusion agar (Raper, 1937) was employed for all cultures where quantitative measurements and comparisons of growth with different bacterial species were contemplated. It was chosen for two reasons: First, it had already been successfully used for cultivating D. discoideum in association with a large number of saprophytic bacteria (Raper, 1937); and second, its use in the present study made possible a quantitative comparison of the growth of this slime mold upon pathogenic bacteria with its earlier growth upon non-pathogenic bacteria.

As in the earlier study (Raper, 1937), each of the selected bacterial cultures was inoculated in triplicate upon hay-infusion agar plates in the following manner: Six colonies were established at regular intervals near the periphery of a plate and a single colony at its center. For each colony the inoculum was spread over an area of approximately 1 cm². The cultures of plant pathogens were incubated at 22 to 24°C. throughout the experiments, whereas the cultures of animal and human pathogens were incubated at 26 to 28°C. prior to inoculation with Dictyostelium and at 20 to 22°C. after the introduction of the slime mold. In all cases the spores of D. discoideum were planted after the bacteria had grown for 2 to 3 days. The spores used for inoculation

² This unit was first introduced for quantitatively comparing the growth of D. discoideum in association with different saprophytic bacteria (Raper, 1937): a detailed account of the procedure employed in effecting the necessary conversions was given at that time.
were invariably obtained from sorocarps that had developed 0.5 cm. or more beyond any evidence of bacterial growth (Pl. 2, fig. 1c), the purity of the inoculum being repeatedly verified by streaking spores from similarly located sorocarps upon nutrient agar. Spores were planted in each of the colonies in two plates and in six of the seven in the remaining plate.

The subsequent growth of D. discoideum was determined by counting the number of sorocarps of large, medium and small size in each colony; and from these counts the average growth per colony was obtained for each bacterial culture. To facilitate comparison of the growth of the slime mold with the different bacteria, the average number of large, medium, and small sorocarps per colony was computed in terms of medium sorocarps (Raper, 1937) and expressed as medium-sorocarp equivalents (fig. 1).

EXPERIMENTAL

The results of this study can best be considered by discussing separately the growth of D. discoideum in association with bacteria that are pathogenic to plants, to animals, and to man.

With the bacteria pathogenic to plants the slime mold grew well and produced fruiting structures of normal pattern (Pl. 2, fig. 1) in cultures of Erwinia carotovora, Erwinia amylovora, Phytomonas phaseoli, and Phytomonas flaccumfaciens. In each case the bacterial colonies were completely consumed by the feeding myxamoebae of the slime mold. Somewhat less growth of the Dictyostelium occurred in cultures with Phytomonas malvacearum, Phytomonas medicagenis, Phytomonas syringae, Phytomonas campestris, and Phytomonas phaseoli var. fuscus (fig. 1). In the first three of these cultures the colonies were completely consumed, in the remaining two only partially. Thus the reduced growth of the slime mold in the former cases can be attributed directly to a food shortage; whereas in the latter cases it must be ascribed to the inability of the myxamoebae to feed effectively upon the bacteria present. Such a condition is illustrated even better perhaps by the cultures with Phytomonas tumefaciens where good growth of D. discoideum occurred in
association with a strain, no. 176, isolated from “crown gall” of peach, while only fair growth of the slime mold took place with a second strain, no. 185, isolated from “hairy-root” of nursery stock (fig. 1). No explanation for this difference in growth of

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Growth</th>
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<tbody>
<tr>
<td>Erwinia amylovora</td>
<td>Strong</td>
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<tr>
<td>Erwinia carotovora</td>
<td>Strong</td>
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<tr>
<td>Phytomonas tumefaciens, 176</td>
<td>Moderate</td>
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<tr>
<td>Phytomonas tumefaciens, 185</td>
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<td>Phytomonas campesiris</td>
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<td>Phytomonas flaccumfaciens</td>
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<td>Phytomonas medicaginis</td>
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<tr>
<td>Phytomonas phaseoli</td>
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<tr>
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<tr>
<td>Salmonella suipestifer</td>
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<tr>
<td>Salmonella enteritidis</td>
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<td>Pseudomonas aeruginosa</td>
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<td>Salmonella paratyphi</td>
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<tr>
<td>Salmonella schottmuelleri</td>
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<td>Staphylococcus citreus</td>
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FIG. 1. COMPARATIVE GROWTH UPON HAY INFUSION AGAR OF DICTYOSTELIUM DISCOIDEUM IN ASSOCIATION WITH DIFFERENT BACTERIA PATHOGENIC TO PLANTS, TO ANIMALS AND TO MAN

the slime mold is at hand, for the bacterial growth in the two cases was essentially alike in amount and seemingly in character.

With the bacteria pathogenic to animals Dictyostelium discoideum grew fairly well in association with Salmonella pullorum, Salmonella suipestifer and Salmonella enteritidis (fig. 1). In
each case the bacterial colonies were completely consumed by the feeding myxamoebae, pseudoplasmodia began forming two days after the introduction of slime mold spores, and sorocarps of normal pattern were subsequently produced. Since the bacterial colonies were completely consumed, it may be noted that the somewhat limited growth of the slime mold with these cultures is attributable to a correspondingly restricted growth of host bacteria. Excellent growth and normal development of D. discoideum occurred in association with Escherichia coli\(^5\) whereas fair growth but normal development of the slime mold took place with Pseudomonas aeruginosa;\(^4\) the bacterial colonies being wholly consumed in the former case but only partially so in the latter.

Moderately good growth of Dictyostelium discoideum occurred with the following human pathogens of the colon-typhoid-dysentery group: Eberthella typhosa, Shigella dysenteriae "Flexner," Shigella dysenteriae "Strong," Salmonella paratyphi, and Salmonella schottmuelleri. In all cases the bacterial colonies were wholly consumed by the feeding myxamoebae, pseudoplasmodia were formed after 2 to 2½ days, and sorocarps of entirely normal pattern were subsequently produced. In association with Staphylococcus citreus fair growth of D. discoideum occurred, the bacterial colonies were largely consumed, and sorocarps of essentially normal pattern were produced.

Realizing that hay infusion agar was at best a poor medium for Eberthella typhosa and related bacteria, some cultures of D. discoideum in association with E. typhosa were subsequently made upon media containing 1 per cent each of peptone and lactose or peptone and glucose. Upon these media occurred a rich growth of bacteria which was wholly consumed by the feeding myxamoebae of the slime mold and fruiting structures were subsequently produced. These, however, were predominantly abnormal in pattern thus indicating the presence in the cultures

\(^4\) In recognition of the fact that these species ordinarily behave as saprophytes they were included in the previous study of the growth of D. discoideum with saprophytic bacteria (Raper, 1937); nonetheless, their occasional pathogenicity warrants their inclusion in this study also.
of a condition mildly toxic to *D. discoideum* (Raper, 1939). A more favorable medium consisted of a carrot infusion enriched with 0.5 per cent peptone and buffered with equimolar quantities of monobasic potassium phosphate and dibasic sodium phosphate. Upon this medium the growth of *E. typhosa* approached that upon peptone-lactose and peptone glucose media, the colonies were completely consumed by the feeding myxamoebae, and the production of normal sorocarps indicated that the cultures were wholly favorable for the slime mold (Pl. 2, figs. 2 and 3).

Studies were made of the growth of *D. discoideum* upon three additional species of bacteria that are pathogenic to man, namely: *Staphylococcus aureus*, *Bacillus anthracis*, and *Corynebacterium diphtheriae*. Cultures with these bacteria were not grown upon hay-infusion agar and accordingly the growth of the slime mold with them cannot be quantitatively compared with those previously considered. However, an evaluation of the growth of *Dictyostelium* in association with each will be given. *D. discoideum* was grown in association with *S. aureus* upon a medium containing 1 per cent glucose and 0.5 per cent peptone. Moderately good growth of bacteria occurred, the colonies were completely consumed by the slime mold and some fruiting structures were produced. *B. anthracis* was grown upon a medium containing 1 per cent glucose and 0.2 per cent peptone. Fair growth of the slime mold occurred and sorocarps of normal pattern were formed, but the bacterial colonies were only partially consumed. *C. diphtheriae* was grown upon horse serum-peptone agar for two days at 35°C. after which time the resultant colonies were transferred *en masse* to the surface of nonnutrient agar tubes and inoculated with pure spores of *D. discoideum*. Incubation was at 20 to 22°C. following the introduction of the slime mold. In these cultures the *Dictyostelium* grew well, completely consumed the masses of transferred bacteria and subsequently formed fruiting structures.

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4 Carrot-peptone agar: 300 grams fresh carrots boiled for 1 hour in liter of tap water; filtered; filtrate made up to 1 liter; 0.5 per cent peptone and 1.5 per cent agar added; solution made M/100 in KH₂PO₄ and in Na₂HPO₄ + 12 H₂O; sterilized.
DISCUSSION

The present study greatly enlarges the list of pathogenic bacteria in association with which a species of the Dictyosteliaceae is known to be capable of growing. Previously Pinoy (1907) cultivated *D. mucoroides* with *Vibrio cholera*, *Vibrio metchnikovi* and *Bacillus friedlanderi* but failed to culture it with *Bacillus anthracis* or *Pseudomonas aeruginosa*. Potts (1902) likewise attempted unsuccessfully to grow *D. mucoroides* with *B. anthracis*, while Vuillemin (1902) failed to cultivate the slime mold with *P. aeruginosa*. In our experiments *D. discoideum* has been grown with each of these bacteria, although it should be noted, with somewhat less success than with many other pathogens (fig. 1). These divergent results are believed to be due more to the different culture media employed than to any fundamental difference between the nutritional requirements of *D. discoideum* and *D. mucoroides*. Parallel cultures of these slime molds indicate the correctness of such an assumption, for in association with the same species of bacteria and upon media of similar composition the growth of the two species of *Dictyostelium* is regularly comparable (Pl. 2, fig. 4).

The average growth of *D. discoideum* in association with the twenty-one cultures of pathogenic bacteria grown upon hay infusion agar and included in this survey was 42 medium-sorocarp equivalents per bacterial colony, or only slightly less than in association with the saprophytic bacteria previously studied where the average was 44 medium-sorocarp equivalents per colony. This meagre difference would seem to substantiate the conclusion earlier set forth (Raper, 1937) that the growth of *D. discoideum* is not particularly favored by any species or group of bacteria as was maintained by Nadson (1899) and Skupienski (1920) who considered *D. mucoroides* symbiotic with *Bacillus fluorescens-liquefaciens*. Any claim of symbiosis between species of the Dictyosteliaceae and the bacteria associated therewith is refuted by the following facts: (1) They will grow equally well in company with a large number of bacterial species possessing widely different morphological and physiological characters. (2) They are associated in nature with a multitude of bacterial
species, a fact that is attested by the diversity of forms accompanying these slime molds when they are isolated in laboratory culture (Raper, 1937, Table 2).

The myxamoebae of these slime molds are considered by the writers to be predators upon the accompanying bacteria. This interpretation is somewhat at variance with that of Pinoy (1907), who considered *D. mucoroides* to be an obligate parasite infecting bacterial colonies and that of Vuillemin (1902), who described the same species as a "bacteriophage" annihilating bacterial colonies. Pinoy's view would seem unjustified since spores will germinate in the total absence of bacteria, and myxamoebae will feed upon dead bacterial cells as well as living ones. Furthermore, one must ascribe to a bacterial colony an unwarranted unity before one is justified in considering it capable of becoming parasitized. Vuillemin's interpretation more nearly represents the true case. He reported the ingestion and digestion of bacterial cells as individuals by the myxamoebae of *D. mucoroides*, but obviously referred to the macroscopic clearance of bacterial colonies when he termed the slime mold an "Acrasiae bacteriophage." And indeed, colonies of bacteria in which myxamoebae are actively feeding do present pictures simulating those of colonies being destroyed by a phage (Pl. 1, figs. 2 and 3). But the agents here responsible for the destruction of bacterial cells are not submicroscopic in size, but easily observable myxamoebae preying upon bacterial cells, engulfing and digesting them.

Sufficient studies have been made to indicate that either *Dictyostelium mucoroides*, *D. purpureum*, or *Polysphondylium violaceum* will grow with as great a number of different bacteria as *D. discoideum*, and with a vigor equalling or exceeding that species. The latter point is fairly well illustrated in Pl. 2, (figs. 4 and 5) where the growth of *D. mucoroides*, *D. purpureum*, and *D. discoideum* in association with *Eberthella typhosa* may be compared.

The question arises, naturally, as to whether any practical significance can rightfully be attributed to the ability of *Dictyostelium discoideum* to feed upon pathogenic bacteria. This slime mold is probably not widely distributed in nature for it has been
isolated only a few times and only in the writers' laboratory. Further, since in so far as known, it can grow only under strict aerobic conditions and cannot grow at a temperature in excess of 28–30°C., its use as a phagocytic agent within the bodies of animals is precluded. On the other hand, because of the ease with which the species can be cultured in the laboratory and because of the ease with which it can at any time be placed in pure-mixed cultures with any selected culture of bacteria, pathogenic and non-pathogenic alike, it offers an excellent tool with which to study many problems pertaining to the nutrition and feeding habits of amoeboïd cells.

While *D. discoideum* is not abundant in nature, other species of the Dictyosteliaceae such as *D. mucoroides*, *D. purpureum* and *Polysphondylium violaceum* are widely distributed and may play a more important rôle in the microbiology of the soil than has hitherto been generally suspected. Although bacteria pathogenic to animals are not commonly encountered in the soil, certain forms may and do occasionally occur there. It is suggested that the fact that they do not long remain may in some measure be due to their removal by the myxamoebae of the Dictyosteliaceae and of the Myxogastreales and the true amoeboïd of the soil. Finally, as one observes the speed and completeness with which *D. discoideum* (or some other species of the group) consumes large colonies of bacteria in a period of two to three days, one can imagine the possibility of employing these organisms to combat certain bacterial diseases of plants where the inhibiting differentials in temperature characteristic of human pathogens do not occur.

**SUMMARY**

1. *Dictyostelium discoideum* a non-plasmodium-forming slime mold, was grown in pure-mixed culture upon hay-infusion agar with 22 species and strains of bacteria that are pathogenic to plants, to animals, or to man and its growth with these organisms quantitatively compared.

2. Included in this number as bacterial associates were common plant pathogens such as *Erwinia amylovora*, *E. carotovora*, *Phyto-
monas tumefaciens, P. campestris, P. phaseoli, P. syringae, P. malvacearum, P. flaccumfaciens, P. medicaginis and P. phaseoli var. fuscus; animal pathogens such as Salmonella swipestifer, S. pullorum, S. enteritidis, Escherichia coli and Pseudomonas aeruginosa; and human pathogens such as Eberthella typhosa, Shigella dysenteriae “Flexner,” S. dysenteriae “Strong,” Salmonella schottmuelleri, S. paratyphi, and Staphylococcus citreus.

3. In the majority of cases the colonies of associated bacteria were completely consumed by the myxamoebae which constitute the vegetative stage of this and related slime molds; in a few cases the bacterial colonies were only partially consumed.

4. Using other media than hay-infusion agar, D. discoideum was grown upon three additional human pathogens, namely: Staphylococcus aureus, Bacillus anthracis, and Corynebacterium diphtheriae.

5. Other species of the Dictyosteliaceae such as Dictyostelium mucoroides, D. purpureum and Polysphondylium violaceum, which are widely distributed in nature, are likewise capable of feeding upon a variety of pathogenic bacteria.

6. Because of the ease with which they can be identified and maintained in culture, species of the Dictyosteliaceae afford excellent material for experimental studies relating to the nutrition and feeding habits of amoeboid cells.

The authors are indebted to the following investigators for supplying bacterial cultures for this study: Leland W. Parr, A. J. Riker, B. E. Proctor, F. W. Tilley, Wm. S. Gochenour, Charlotte Elliott, Lucia McCulloch, and J. T. Presley. The senior author wishes to express his deep appreciation to Wm. H. Weston, Professor of Cryptogamic Botany at Harvard University, and Charles Thom, Chief of the Division of Soil Microbiology, U. S. Department of Agriculture, for their advice and criticism during this work.

REFERENCES
PLATE 1


Fig. 1, bacterial colony prior to inoculation with *Dictyostelium*.

Fig. 2, 24-hour culture of *D. discoideum* showing central area of bacterial colony consumed by the slime mold.

Fig. 3, 32-hour culture showing increased clearance of bacterial colony.

Fig. 4, 48-hour culture; early fruiting stages of *Dictyostelium* evident.

Fig. 5, 54-hour culture showing increased growth and further development of *D. discoideum*. *a*, forming pseudoplasmodia; *b*, migrating pseudo-plasmodia (early stage).

Fig. 6, 72-hour culture; bacterial colony almost wholly consumed; fruiting structures of *Dictyostelium* abundant: *a*, forming pseudoplasmodia, *b*, migrating pseudoplasmodia (later stage), *c*, mature sorocarps, sori or spore heads not in focus.
(Kenneth B. Raper and Nathan R. Smith: Growth of Dictyostelium discoideum)
PLATE 2

Fig. 1. *Dictyostelium discoideum* growing in association with *Escherichia coli* upon buffered peptone agar: a, uninoculated bacterial colony; b, colony similar to a 10 days after inoculation with *Dictyostelium*; c, isolated sorocarps bearing bacteria-free spores. × 11/2.

Fig. 2. Three-day culture of *D. discoideum* in association with *Eberthella typhosa* upon carrot-peptone agar; colony at left uninoculated with *Dictyostelium*. × 1/2.

Fig. 3. Culture shown in figure 2 ten days after inoculation with *D. discoideum*. Note the completeness with which inoculated colonies have been consumed and the present invasion of the control colony by the slime mold. × 1/2.

Fig. 4. Four-day culture of *D. mucoroides*, a, and *D. discoideum*, b, in association with *E. typhosa* upon carrot-peptone agar. Slime mold spores inoculated at left end of bacterial streaks. × 1/2.

Fig. 5. Five-day culture of *D. mucoroides*, a, and *D. purpureum*, b, in association with *E. typhosa*. × 1/2.
(Kenneth B. Raper and Nathan R. Smith: Growth of Dictyostelium discoideum)