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Citation	Journal of the Faculty of Science, Hokkaido University. Series 5, Botany, 10(3), 177-180
Issue Date	1976
Doc URL	<a href="https://hdl.handle.net/2115/26342">https://hdl.handle.net/2115/26342</a>
Type	departmental bulletin paper
File Information	10(3)_P177-180.pdf



## A membraneous ground mat excreted by the cellular slime mold *Dictyostelium discoideum*

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It has long been recognized that the cellular slime molds are organisms of particular interest for developmental biology (BONNER, 1958; RAPER, 1960). The cellular slime molds feed on bacteria *e.g.* *E. coli* by phagocytosis and proliferate by binary fission. After growing myxamoebae have exhausted a food supply, they begin to aggregate into multicellular organisms to form a mass of cells known as a pseudoplasmodium. At the beginning of aggregation a chemotactic substance, Acrasin, is secreted from the center of an amoebae population (BONNER, 1947). Recently Acrasin was identified as cyclic AMP by KONIJN *et al.* (1967).

KONIJN *et al.* (1961, 1966, 1968) observed that ordinarily when myxamoebae are deposited as a small drop on hydrophobic agar, the deposited amoebae remain within the confine of the area covered by the deposited drop; but they will burst from the confine of the circle if a drop of attractant solution is placed near the drop filled with amoebae. Based on these facts they described a quantitative bio-assay method for chemotactic attractants. This confinement impressed on us an idea of the existence of excreted materials spreading under the deposited amoebae and confining the migrating area of amoebae. Although investigators working with this organism have often observed the flotation of mature fruiting bodies when water is poured into Petri dishes containing the amoebae (see, Fig. 1). No one has previously paid particular attention to this phenomenon. YAOI and KANASEKI (1972) also observed that cultured chick embryo cells lay down a thin carpet of microexudate on the surface of Petri dishes.

We observed a membraneous structure under the deposited amoebae after less than 10 hr from the beginning of replacement of food-exhausted amoebae onto non-nutrient agar.

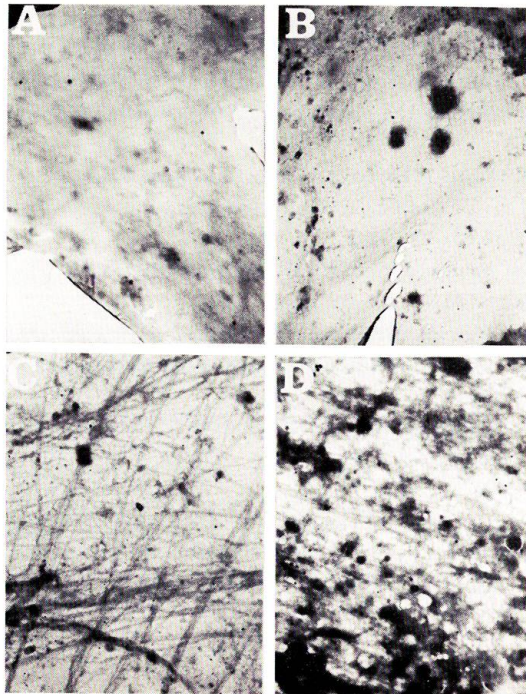
For this study *Dictyostelium discoideum* NC-4 (haploid) grown in association with *E. coli* on an agar plate was used. The logarithmically growing cells were harvested in a 100× diluted Bonner's salt solution



**Fig. 1.** Mature fruiting bodies of *D. discoideum* floated by pouring water onto an agar surface. Myxamoebae were harvested during the exponential phase of growth, suspended in Bonner's salt solution, diluted 100 times, and washed three or four times. The amoebae suspension was placed on an agar surface and allowed to develop until the maturation of fruiting bodies.

(BONNER, 1947) and centrifuged 3 to 4 times to remove excess bacteria. The packed cells were suspended approximately  $2 \times 10^7$  cells per milliliter and 0.1 ml cell suspensions were spread on 14 mM phosphate-buffered agar in flat small Petri dishes (diameter, 6 cm) and incubated at 22°. Under these conditions amoebae began to aggregate after 7–8 hr and aggregation was completed in 14–16 hr. A Petri dish containing replaced amoebae was used for electron microscopic observation, at 2 hr intervals. Distilled water was introduced carefully into the Petri dish and then the area of liquid-air interphase where floating organisms were absent was carefully scooped up onto an electron microscope mesh. Meshes were observed under a Hitachi electron microscope at 80 kV. At a late stage (10 hr or more) of the replacement culture, a membraneous structure was clearly observed (Fig. 2). This latter stage membraneous mat seems to be thicker than the earlier ones judging from the increased resistance of the mat to electron beams. At 20 hr and later stages the fibrous structure in the membraneous mat became very dense (Fig. 2, C and D).

The biological significance of this membraneous structure remains unknown. However, judging from the stage of the appearance of this mat it may be supposed that the migrating area of myxamoebae and slugs is confined to the distributed area of the exudated ground mat because of the necessity for cells to remain within reasonable proximity and to form multicellular organisms. This mat may also be the means for wave propa-



**Fig. 2.** Electron micrographs of the membraneous ground mat excreted by *D. discoideum* cells at various stage of development of the replacement culture. The preparations were performed as described in the text. Photographs show the membraneous ground mat picked up on an electron microscope mesh at 12 hr (A), 14 hr (B), 22 hr (C), and 24 hr (D) after amoebae deposition onto a new agar plate. Magnification: A, B, and D,  $\times 5000$ ; C,  $\times 10000$ .

gation of periodic pulses of the attractant emitted from the aggregation center, cyclic-3', 5'-AMP (SHAFFER, 1957; HASE and OCHIAI, 1973). Major questions regarding the chemical characteristics of this membraneous mat, its role in biological system, and its specificity are left open and await clarification. For understanding of the aggregation and differentiation of the cellular slime mold, this structure must eventually be more thoroughly investigated.

We thank Prof. I. HARADA for his encouragement in the course of this study and for his critical reading of the manuscript. Thanks are also due to M. Sc. T. NIKI for his technical help in the operation of the electron microscope.

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