SHORT COMMUNICATION

Activated Charcoal and Orientation Behaviour by *Dictyostelium discoideum* Slugs

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(Received 1 May 1981; revised 24 July 1981)

Incorporation of activated charcoal into the agar enhanced the accuracy of phototaxis by *Dictyostelium discoideum* slugs two- or threefold. This was due to absorption of stray light rather than adsorption of a phototaxis-impairing chemical. The presence of charcoal had no effect on thermotaxis, the critical cell density at which phototaxis and thermotaxis become impaired, Slug Turning Factor (STF) interference with phototaxis, or chemotaxis by slugs in STF gradients. Activated charcoal in the agar had other effects on *D. discoideum* which may be chemical in nature.

INTRODUCTION

Amoebae of the cellular slime mould *Dictyostelium discoideum* aggregate when starved to form a multicellular migratory organism – the slug (Raper, 1940). After migration each slug is transformed into a fruiting body (Raper, 1940). Like slugs, culminating fruiting bodies orient towards light (Bonner & Whitfield, 1965) and in temperature gradients (unpublished). In addition, they release a gaseous repellent which is adsorbed by activated charcoal (Bonner & Dodd, 1962). In view of the effect of activated charcoal on fruiting body orientation (Bonner & Dodd, 1962), we examined whether incorporation of charcoal into the agar affected slug orientation behaviour. Here we show that the accuracy of *D. discoideum* slug phototaxis is two or three times greater when the agar contains activated charcoal (0.5%, w/v). The increase is due to absorption of stray light by the charcoal rather than adsorption of a chemical that impairs phototaxis.

METHODS

Dictyostelium discoideum strain X22 (Williams & Newell, 1976) was used in all experiments as the orientation behaviour of slugs of this strain has been well characterized (Fisher *et al.*, 1981).

Orientation experiments were performed as described previously (Fisher *et al.*, 1981). Washed vegetative amoebae grown at 21 ± 1 °C were inoculated at known cell density over a 1×1 cm origin at the centre of each plate of water agar (Stenhouse & Williams, 1977) or charcoal agar [water agar plus 0.5% (w/v) activated charcoal (Ajax Chemicals, Sydney, Australia)]. Slugs, spores and slime trails were transferred after incubation to clear PVC discs and stained with Coomassie blue (Fisher *et al.*, 1981). From the discs the direction of travel of each individual slug was measured using a Summagraphics digitizing tablet. Statistical analysis, based on the Mises or circular normal distribution (Mardia, 1972), yielded estimates of the accuracy of orientation (κ) (Fisher *et al.*, 1981).

The relative amounts of stray light for the various media *in situ* in our opaque PVC boxes were measured as follows. Light from a tungsten microscope lamp was shone through a scattering filter (lens tissue) into a PVC box containing a test agar plate. Light emerging from a 1 cm diameter hole drilled in the lid of the box above the centre

of the test plate was then measured with an International IL600 radiometer and silicon photodetector (spectral sensitivity range: 400–1100 nm).

Slug Turning Factor (STF) exudates were prepared and assayed as described previously (Fisher *et al.*, 1981) by lyophilizing the liquid from a bed of water-saturated glass beads supporting high density *D. discoideum* amoebae on Whatman no. 50 filter paper.

RESULTS

Activated charcoal enhances phototaxis. The accuracy of phototaxis at low cell density was greater on charcoal agar than on water agar (Fig. 1*a*). At high cell density this charcoal enhancement of phototaxis was not apparent (Fig. 1*a*). This was because, at high density, orientation improves as slugs migrate further from the origin (Fisher *et al.*, 1981), and slugs cease migration and fruit earlier on charcoal agar than on water agar (unpublished). Thus, the charcoal enhancement of phototaxis was offset at high cell density by the reduction in the measured accuracy of orientation caused by shorter migration distances on charcoal agar.

Thermotaxis is unaltered by the presence of activated charcoal. Having observed that activated charcoal enhances phototaxis, we examined whether the presence of charcoal in the agar also affected thermotaxis. At low cell density neither the accuracy of thermotaxis (Fig. 1*b*), nor the temperature of transition from negative to positive thermotaxis, was altered by the presence of activated charcoal. At high cell density the measured accuracy of positive thermotaxis was lower on charcoal agar than on water agar (Fig. 1*b*), because the slugs cease migration sooner on charcoal agar (see above). When only slugs that had migrated less than 2 cm (net distance) were considered, the measured accuracy of thermotaxis was the same on water agar and charcoal agar for slugs formed at high cell density. These results indicate that charcoal enhancement of orientation is restricted to phototaxis.

The critical cell density is unaltered by the presence of activated charcoal. Both phototaxis (Fig. 1*a*) and positive thermotaxis (Fig. 1*b*) became impaired at the same critical cell density and this critical density was unaltered by the presence of activated charcoal (Fig. 1). The metabolite(s) responsible for impairment of orientation at high cell density (Fisher *et al.*, 1981) therefore appear neither to affect only phototaxis nor to be adsorbed strongly by activated charcoal in the agar.

Activated charcoal does not affect slug chemotaxis in STF gradients or STF interference with orientation. To test more rigorously for charcoal adsorption of putative phototaxis-impairing metabolite(s) in the agar, we prepared crude metabolite (STF) exudates as described previously (Fisher *et al.*, 1981). Slugs of *D. discoideum* are repelled by crude STF exudates in the dark and their phototaxis is impaired by high uniform STF concentrations in the agar (Fisher *et al.*, 1981).

Slug chemotaxis in STF gradients was the same whether or not activated charcoal was present in the agar. Pooling data over the total range of STF concentration tested gave an accuracy (κ) of 0.51 ± 0.06 for negative chemotaxis on charcoal agar and 0.46 ± 0.05 on water agar. Sample sizes were 2047 slugs from 64 charcoal agar plates and 2897 slugs from the same number of water agar plates.

STF interfered with phototaxis whether or not activated charcoal was incorporated into the agar. Addition of 1800 Phototaxis Interference Units (see Fisher *et al.*, 1981) of STF per 30 ml plate roughly halved the accuracy of phototaxis on both water and charcoal agar. STF also impairs thermotaxis in the presence of activated charcoal (Fisher *et al.*, 1981). There is therefore no evidence that activated charcoal enhances phototaxis by adsorbing STF or any other phototaxis-impairing metabolite in the agar.

The charcoal effect on phototaxis is due to absorption of stray light. Because activated charcoal in the agar did not alter the critical cell density, thermotaxis, chemotaxis in STF gradients, or STF interference with phototaxis, we decided to test whether charcoal enhancement of phototaxis was due to absorption of stray light rather than adsorption of a

Fig. 1. Effect of activated charcoal on phototaxis (a) and positive thermotaxis (b) by *D. discoideum* X22 slugs over a range of cell densities. Bars represent 90% confidence intervals. The thermal gradient in thermotaxis was $0.2\text{ }^{\circ}\text{C cm}^{-1}$ and the temperature at the centre of the plate was $19.7 \pm 0.2\text{ }^{\circ}\text{C}$. Temperatures were measured at the agar surface by a thermocouple.

phototaxis-impairing chemical. To do this we prepared triple-layered agar plates with either a black or a white filter paper, or a clear impermeable PVC disc inserted between two agar layers. The upper layer of agar (with or without charcoal) was about 1.5 mm deep and the lower layer (with or without charcoal) was about 3.5 mm deep. We then measured the accuracy of phototaxis by X22 slugs formed at low cell density on plates containing charcoal and/or water agar, and a black (Whatman no. 29) or white (Whatman no. 50) filter paper or PVC disc insert in all possible combinations. We also measured the relative amounts of stray light with each combination (see Methods).

As Fig. 2 shows, the accuracy of phototaxis was inversely related to the amount of stray light present, but was not dependent upon the possibility of chemical adsorption by activated charcoal in the agar. Thus, the accuracy of phototaxis remained high even when charcoal was present only in the lower layer of agar separated from the upper layer by a clear impermeable PVC disc. The accuracy of phototaxis was also high when a black filter paper separated two layers of water agar but was low when a white filter paper was present. We conclude that the charcoal effect on phototaxis was due to absorption of stray light rather than adsorption of a phototaxis-impairing chemical.

DISCUSSION

Bonner & Dodd (1962) used the chemical adsorption properties of activated charcoal to obtain evidence for a gaseous repellent affecting orientation by culminating fruiting bodies. The repellent, possibly CO_2 , may be identical with a gas which inhibits aggregation of a

Fig. 2. The accuracy of *D. discoideum* X22 slug phototaxis depends on the amount of stray light and not metabolite adsorption by activated charcoal. Slugs were formed from $5.8 \times 10^5 \pm 0.6 \times 10^5$ amoebae per plate on the central 1×1 cm origin and migrated towards a lateral light source as described previously (Fisher *et al.*, 1981). Sample sizes ranged from 147 to 259 slugs analysed from 10 plates. Bars are 90% confidence intervals but in some cases (to avoid congestion on the graph) only the upper or lower portion of the interval is shown. Groups of three symbols represent each layer of the triple-layered plates: the bottom symbol represents the bottom layer of charcoal or water agar, the middle symbol represents the middle layer of either a black or white filter paper or a clear PVC disc, and the top symbol represents the top layer of either charcoal or water agar. Single symbols represent the single layer of control charcoal or water agar plates. The amount of stray light was measured as described in Methods and expressed as a percentage of the amount measured for a plate with water agar as the top and bottom layers and a white filter paper as the middle layer.

number of slime mould species (but not *D. discoideum*) (Bonner & Hoffman, 1963). For similar reasons, a volatile charcoal-adsorbable substance is thought necessary for macrocyst formation by *D. mucoroides* amoebae (Weinkauff & Filosa, 1965; Filosa, 1979). We have been unable to detect any chemical effect of activated charcoal on orientation behaviour by migrating *D. discoideum* slugs. This may be partly because in our experiments the activated charcoal is incorporated into the agar where it is in direct contact with metabolites released into the aqueous but not the gaseous phase. We have shown that activated charcoal in the agar enhances the accuracy of phototaxis by absorption of stray light, not adsorption of a phototaxis-impairing metabolite.

At high cell densities *D. discoideum* does release a low molecular weight metabolite (Slug Turning Factor, STF) into the medium and this metabolite impairs phototaxis (Fisher *et al.*, 1981). STF interferes with both phototaxis and thermotaxis even if activated charcoal is present in the agar. Similarly, negative chemotaxis by *D. discoideum* slugs in STF gradients is unaltered by the presence of charcoal. The critical cell density at which phototaxis and thermotaxis become impaired (presumably by STF) is the same on charcoal and water agar. We conclude that STF is not strongly adsorbed by the activated charcoal used in these experiments.

While activated charcoal in the agar affects phototaxis by absorbing stray light, it has other effects on *D. discoideum* which may be chemical in nature. Thus, *D. discoideum* slugs migrate faster (unpublished) and fruit earlier on charcoal agar than they do on water agar. Slime trails on charcoal agar are more 'fluid', and stain less intensely with Coomassie blue because they

contain fewer cells left behind by the migrating slug (unpublished). The presence of activated charcoal in the agar also enhances aggregation and fruiting by poorly aggregating strains of *Polysphondylium pallidum* (Williams, 1980) and *D. discoideum* (unpublished). Incorporation of activated charcoal into water agar alters the pH and surface tension of the agar as well as its light-absorbing and chemical-adsorbing properties. Its biological effects should not be too readily ascribed directly to chemical adsorption without testing other alternatives.

We thank R. Smith for technical assistance and D. Welker for comments on the manuscript. P.R.F. was the recipient of Australian Government and Australian National University Postgraduate Research Awards.

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