Agar Culture Medium modified to approximate Soil Conditions

SMALL soil or mud surface plants frequently fail to develop their characteristic morphology when grown on an ordinary agar medium. In particular this applies to the parts penetrating the substrate. Inspection frequently suggests that what is wrong is that agar, unlike soil, is a medium which does not exclude light as a formative factor. We have devised a simple corrective system with good results.

The plants tested were the green alga *Fritschiella tuberosa* (inoculum a small agar disc cut from a stock culture, derived from material from Allahabad, India, sent by Dr A. K. Mitra) and the moss *Funaria hygrometrica* (inoculum four spores per dish, from a local weed population). The medium consisted of 125 ml. of mineral agar (10 g/l. any proven formulation will do; we used that cited in ref. 1) per 9 cm diameter, 5 cm high Pyrex crystallizing dish with Petri dish lid. After inoculation the agar surface was overlaid with a sterilized heavy aqueous suspension of powdered charcoal applied with a pipette, to yield a settled charcoal layer 1–2 mm deep. Illumination was restricted to the surface layer by attaching black paper to the side and bottom of the dish (*A*). Two kinds of controls were cultured concurrently, one with charcoal but no black paper (*B*), the other with unobscured agar (*C*). Incubation was at 17° C with 40 W cool white fluorescent bulbs providing 325 foot candles as illumination; the photoperiod was 16 h day and 8 h night.

Development of *Fritschiella* was most rapid in the unobscured control dishes (*C*), forming prostrate and tufted erect green filaments, and abundant others which grew down into the agar. The latter were mostly also fully green, some were pale and unsharply rhizoidal. In all the green filaments there was a random and messy alternation of unseriate and parenchymatous areas, the parenchymatization starting at the surface of the agar. Typical *Fritschiella* differentiation was not achieved. In the test cultures proper (*A*) erect *Stigeoclonium*-type filaments developed and narrow colourless rhizoids penetrated the agar. Parenchyma soon began to form, but only in the upper part of the carbon film, yielding a stratum of almost colourless tubers. The resultant thalli had the diagnostic characters of *Fritschiella*, and closely matched field material grown on mud. Only in old cultures did additional parenchyma form in the subaerial filaments. In the controls with carbon but no black paper (*B*) there was, even at the centre, a more limited production of tubers, and, instead of only colourless rhizoids, a heavy growth of ultimately largely parenchymatous green filaments down into the agar.

In the test culture of *Funaria* (*A*), there was a reasonably sharp caulonema (developed at or just below the agar surface)—chloronema differentiation, and a lush development of totally colourless and richly branched rhizoids penetrating the agar, as in material grown in the field. The rhizoids from the resultant gametophores were also free of chlorophyll; the only brown wall colour in rhizoids was in gametophoric ones above the carbon only. The unobscured control (*C*) had a lesser degree of differentiation; the scarce and sparsely branched rhizoids had small green plastids and there was a heavy development of green filaments into the agar, yielding gametophores even below the agar surface. Rhizoidal differentiation in the control with carbon but no black paper (*B*) was better in the centre of the dishes, but again all the agar contained green filaments. (A newly observed culture artefact is that here, and in the low light intensity of the centre of the dish only, the coarse basal gametophoric rhizoids, once below the carbon, not only turned green but continued to grow as green filaments. By contrast, in control (*C*), the equivalent structures, apart from their downward growth, started essentially like caulonema but at greater depth lost their green colour.)

Clearly agar must be totally obscured in this type of culture. Only then is differentiation fully normal and growth in the agar restricted to colourless rhizoids. The carbon film method is messy in the sense that for examination the material has to be slaked in ample water. Making the entire agar medium black may possibly suffice in the case of *Funaria*. But to find out which environmental factors induced "normal" differentiation will require much further experimentation. While it seems that for certain of the phenomena light exclusion alone may be responsible, there is a strong suggestion that for others the texture of the coating (gas exchange ?, adsorption ?) may also be involved. Thus *Fritschiella* tubers are formed in the upper portion of the carbon film only, and apparently not in utter darkness; it seems that they are initially practically colourless, but gradually become faintly green. At full maturity they also contain some carotenoid pigments, but far less than the exposed parts of the plant, which turn bright orange.

There is an extensive literature about the differentiation of moss protonema, and many of the results are doubtful and inconclusive. Much of the work requires repetition, using a dark substrate so that differentiation is normal.

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1 Proskauer, J., *Phytomorphology*, 19, 53 (1969).

Algae as Nutrient Material for studying Ca-Sr Relationships in Heterotrophic Organisms

A RECENT review of the mineral nutrition of algae[1] summarizes the inorganic requirements and substitution elements for a number of green algal forms. Among those presented, it was especially noted that algae which can grow with an Sr replacement for Ca, such as *Chlorella*[2] or *Protosiphon*[3], constitute a potential tool for investigating Ca nutrition in heterotrophic organisms. They may be particularly useful for comparing the effects of an adequate Ca supply with the effects of making Ca deficient and replacing it with Sr.

The feasibility of such a use for the alga, however, depends on several suppositions. The first is that the alga will provide all the organic nutrients needed by the heterotroph; second, that the alga in Sr, except for its lack of Ca, would be as suitable a source of nutrients for the heterotroph as the same alga grown in adequate Ca; third, the alga must be able to grow in Sr with little enough Ca to make it Ca-deficient in relation to the heterotroph. In order to test these suppositions, autoclaved extracts of *Protosiphon botryoides*, a unicellular green alga, were used as nutrient material for the heterotrophic protozoan *Tetrahymena pyriformis*. The selection of these two organisms seemed ideal for studying the interrelationships of Ca and Sr; *Protosiphon* can synthesize organic materials starting with CO_2, water and inorganic salts using[3] either Ca or Sr, and *Tetrahymena* has, for the most part, defined organic and inorganic requirements that simulate those of higher heterotrophs[4].

In our experiments *Tetrahymena* used for inoculum were removed from stock medium, washed to remove Ca and other nutrients, and placed in test media prepared from algal material. Basically, two types of test medium were prepared. 1 g (dry weight) of *Protosiphon* grown in mineral nutrient medium[5] containing Ca was autoclaved in fresh mineral medium. The extract obtained from this