The Isolation from Soil of Macrocyst-forming Strains of the Cellular Slime Mould *Dictyostelium mucoroides*

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The cellular slime mould *Dictyostelium mucoroides* is cultured on a glucose-peptone agar medium with *Escherichia coli* as a food supply (Bonner, 1967). The spores of *Dictyostelium* germinate to release amoebae that grow by feeding on the bacteria. Once the bacterial food supply is exhausted the amoebae aggregate to form numerous slug-shaped multicellular pseudoplasmodia. These undergo morphogenesis and each one differentiates into a sorocarp consisting of a cellular stalk bearing at its tip a spherical mass of spores. Some strains of *D. mucoroides* have an alternative mode of development in which some of the aggregates formed on an agar surface differentiate into a multicellular encystment structure, the macrocyst, instead of the usual sorocarp. In such strains the proportion of macrocysts to sorocarps varies and some cultures are without macrocysts. However, when vegetative amoebae of macrocyst-forming strains are removed from an agar surface and placed under water, they form aggregates which always give rise to macrocysts.

Blaskovics & Raper (1957) claim that the ability to form macrocysts is possessed only by occasional strains of *Dictyostelium mucoroides*, having isolated 'not more than a half-dozen among the hundreds of strains of *D. mucoroides* examined'. Because of the variation in macrocyst formation on an agar surface it is possible that macrocyst-forming strains went undetected. We therefore investigated the occurrence of such strains in nature by testing each isolate under water for its ability to form macrocysts.

Soil samples were collected from the spring of 1970 until the autumn of 1971, mainly in Ontario and York counties in Ontario. If the samples were not used immediately they were stored in plastic bags at 2 °C. Ten g of a soil sample were thoroughly shaken with 90 ml of sterile distilled water. One half of a millilitre of the soil suspension together with 0.5 ml of a dense suspension of *Escherichia coli* were spread on the surface of alfalfa extract agar prepared as follows: 10 g of dried ground alfalfa was suspended in 1 l distilled water, boiled for 30 min and filtered through cotton. Twenty g of agar were added and the filtrate autoclaved for 20 min and poured into plastic Petri dishes which were kept at room temperature for 5 days before using. After spreading, the plates were incubated at room temperature and examined daily for clones of *Dictyostelium mucoroides*, which usually formed sorocarps in 4 to 6 days. For each soil sample, spores from each of four clones were used to start separate subcultures on glucose-peptone agar. Before a subculture showed signs of aggregation, a loopful of vegetative amoebae was removed and dispersed in 4 ml of distilled water in a small plastic Petri dish (35 × 10 ml). These dishes were kept at room temperature and examined for macrocysts about 36 h later.

Eighty-one soil samples were examined. Macrocyst-forming strains were isolated from 35 of the 70 soil samples which yielded *Dictyostelium mucoroides*. The high proportion (50 %)

of macrocyst-forming strains contrasts with the results of Blaskovics & Raper (1957). This may be due to differences in ecological conditions between the soils sampled, but it is more likely that the screening procedure used is responsible. On an agar surface macrocyst formation is highly variable, but amoebae capable of producing macrocysts will do so when submerged in water. Only three of the 35 macrocyst-forming strains isolated produced macrocysts on the agar media used during isolation. The abundance of macrocyst-forming strains in nature raises the question of the ecological conditions that favour selection of such strains and of the adaptive significance of the macrocyst.

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