CULTIVATION OF DICTYOSTELIUM DISCOIDEUM IN AXENIC MEDIUM[1]

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The use of cellular slime molds as objects of developmental study at the molecular level has intensified the need to cultivate them in defined medium. The successful axenic cultivation of Polysphondylium pallidum was reported in recent years (Hohl & Raper, 1963; Sussman, 1963) and the routine use of this species, grown in defined medium, is now possible (Phillips et al., 1964; R. Sussman, 1967). However, D. discoideum has remained the organism of choice for developmental study. Its axenic cultivation was also accomplished in the past (Sussman & Bradley, 1954) but growth rate and cell yield were poor and unimprovable & an ill-defined bacterial fraction was required. We can now report the serial passage of this species in a relatively simple medium at a rate and yield sufficient for routine use.

The medium (CF)[3] has been employed for the growth of the amebo-flagellate Naegleria (Fulton, 1967) and is itself a modification of the medium of Balamuth, (1964). The composition is given below:

I	KH_2PO_4	3.4 g	in 100 ml H_2O	(autoclaved)
	$Na_2HPO_4 \cdot 7\ H_2O$	6.7 g		
II	Glucose	13.5 g	in 50 ml H_2O	(autoclaved)
III	proteose peptone	10 g	in 650 ml H_2O	(autoclaved)
	yeast extract	5 g		

The above are mixed in the ratio 2:2:65

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To 3.5 ml of the mixture are added 1.0 ml of a 5% solution of Wilson's Liver concentrate (NF), autoclaved, cleared by centrifugation and reautoclaved, and 0.5 ml of fetal calf serum (Grand Island Biologicals Co.).

D. discoideum spores were suspended in sterile nutrient broth and incubated overnight to permit germination and demonstrate the absence of bacterial contamination. Aliquots were inoculated into 5 ml volumes of CF medium in 125 ml Erlenmeyer flasks and incubated on a shaker (110 cycles per min.) at 22°C. Initial growth rate and cell yield were very poor (Table 1) but these improved progressively during serial subculture. By the 9th passage the cells grew exponentially without a lag, with a doubling time of ca 12 hrs and a stationary phase yield of over 1×10^7 cells/ml, equivalent to ca 500 mg dry weight/liter, (Fig. 1). The latter value is equal to the yield obtained after growth in broth with living bacteria (Sussman, 1961). Cells harvested from the later passages and dispensed on millipore filters supported by pads saturated with a buffer-salts-streptomycin solution (Sussman, 1966) aggregated promptly and constructed normal fruits with viable spores. Their morphogenetic competence was in all respects identical with that of cells grown on living bacteria.

The absence of contaminants was established by microscopic inspection and by routine platings of culture samples on nutrient agar.

TABLE 1

Record of serial passages on CF medium

Passage	days incubated	Inoculum (No./ml)	Stationary phase yield (No./ml)
1	20	5×10^3	8×10^4
2	20	1×10^4	1.2×10^6
3	13	2×10^4	5×10^6
6	7	2×10^4	9.5×10^6
9	6	1×10^5	1.1×10^7

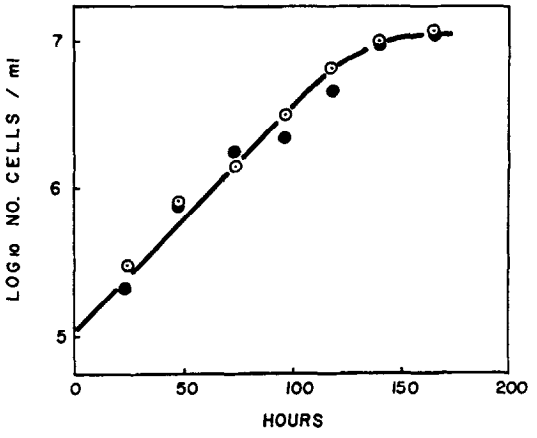

Fig. 1 Growth kinetics during 9th passage on CF medium. Open and closed circles represent data for duplicate cultures determined by hemocytometer counts.

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