Growth of Single Plant Cells

Abstract. *Suspension of plant cells from tissue cultures have been plated in nutrient agar medium so that practically all of the individual cells yielded colonies.*

Single plant cells have been grown by three methods. The first was developed by Muir, Hildebrandt and Riker (*1*), who used growing callus tissue to nurture single cells through filter paper. About 50 percent of the cells grew. Braun (*2*) also used this method. Jones, Hildebrandt, Riker and Wu (*3*) reported the use of micro chambers in which 40 to 50 percent of the single cells divided. Bergmann (*4*) reported that suspensions of single plant cells could be obtained easily by filtration of shake cultures. When the cells of filtered suspensions were immobilized in nutrient agar plates and incubated, visible colonies appeared from 13 percent of the cells. Bergmann's method was used by Torrey and Reinert (*5*), but they did not report their yield of colonies. Blakely and Steward (*6*) indicated that with Bergmann's method they had obtained visible colonies from 12 percent of the cells which were plated.

Bergmann's method has two major advantages: the single cell suspensions and the plates are easy to prepare, and a large number of cells can be handled conveniently at one time. The major disadvantages are the difficulty of observation of the cells and the low yield of colonies which is obtained. In many experiments visual observation of the cells during growth is not necessary so that Bergmann's method is satisfactory. With respect to the low yield, our studies show that it is possible to grow a colony from each cell put into a petri dish.

The cells in these experiments were from cultures grown on Hildebrandt's "D" medium (*7*) supplemented with 0.1 percent Difco yeast extract.

The tissue was obtained from the stem pith of *Nicotiana tabacum* var. Hickory Prior and the culture was established in June, 1961. To obtain cell suspensions, pieces of the tissue were shaken for seven days on a rotary platform shaker in the D medium plus 0.1 percent yeast extract, agar being omitted. The suspensions were filtered through silk bolting cloth such as is used in the flour milling industry. The grade of the bolting cloth was chosen by trial so that it would permit single cells to pass through but would retain large clumps. In all these filtrates there were small groups of cells of the same size as the larger single cells. In these small groups, two to four cells were joined to one another; we therefore regarded each of the cells in a group as being derived from the same original cell. The cells and groups of cells in the filtrates were counted by placing a known volume (about 20 μl) of filtrate on a slide, covering the drop with a coverslip 12 mm in diameter and viewing this preparation with a "Nikon" Shadowgraph using a × 20 objective. Plasmolysed or burst cells were not counted. Cells and groups were counted separately. Never less than five of such preparations were counted for each filtrate.

The results in Table 1 show the percentages of cells, plus groups of cells, in each plate which yielded visible colonies. This constitutes the "plating efficiency." Because of some difficulties with recounting due to infection of plates and collapse of agar, some of the plates could not be counted for the second time, after 17 days. In these instances, the table was completed by including the values obtained at the first counting after 8 to 10 days of incubation.

In all instances the plating efficiency was in excess of 13 percent. Furthermore, in 15 plates out of 68 prepared, the plating efficiency was 90 percent or greater. The plating efficiency in four plates exceeded 100 percent. This is undoubtedly due to sampling errors. Values greater than 100 percent are required in any experiment in which the plating efficiency closely approaches 100 percent. The data in this experiment can not be subjected to a thorough statistical analysis because of the different times on which the plates were counted. However, these results show that with single plant cells it is possible to achieve plating efficiencies very close to 100 percent. In continuing our studies of this method we hope to show how various factors influence the plating efficiency.

Table 1. The plating efficiency of cells derived from *N. tabacum* var Hickory Prior. The cells were plated in Hildebrandt's "D" medium supplemented with 0.1 percent Difco yeast extract from which the 2:4-dichlorophenoxyacetic acid was omitted entirely and the napthalene acetic acid was reduced to 0.05 mg per liter. Filtrates were prepared from four different shake flasks; 1.0-ml samples of filtrate were pipetted into each petri dish (3½ in. diameter) and mixed with 10 ml of nutrient agar. Before use, the 10-ml portions of nutrient agar were kept in plugged test tubes in water baths maintained at the desired temperatures. Ca and G represent, respectively, the average number of cells and number of groups of cells per plate. Results are expressed as percentages. Values in italics are counts made after 8 to 10 days of incubation. The other values were obtained after 17 days of incubation.

Temperature of agar on pouring (°C)	Amount of agar in nutrient medium (%)	Flask 1 C: 375 G: 225	Flask 2 C: 730 G: 390	Flask 3 C: 390 G: 245	Flask 4 C: 710 G: 440
		Incubated at 30°C.			
40	0.5	65	*88*	95	92
	.4	73	*88*	*78*	*97*
35	.5	56	*70*	83	87
	.4	76	*80*	*83*	*84*
	.0	71		98	88
30	.5	52	57	89	*88*
	.4	*78*	*96*	85	94
	.0	77	91	*104*	111
		Incubated at 25°C.			
40	.5	70	88	86	104
	.4	73	*72*	78	*61*
35	.5	60	65	89	94
	.4	71	84	*81*	89
	.0		*79*	97	94
30	.5	65	*35*	63	76
	.4	64	89	71	96
	.0	90	103	82	

JOY L. GIBBS
D. K. DOUGALL*
University of Sydney, Department of Biochemistry, New South Wales, Australia

References and Notes

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* Present address: Department of Agricultural Biochemistry, Ohio Agricultural Experiment Station, Wooster, Ohio.

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