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Short Communication

Turbidimetric Measurement of Plant Cell Culture Growth[1]

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ZINMAY RENEE SUNG
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

ABSTRACT

Turbidimetric measurement of cultures grown in sidearm flasks was used to measure the growth of plant cells. The turbidity was shown to vary proportionally with cell number and dry weight over time. The effect of different freezing conditions on the growth of the culture was presented to demonstrate the application of the sidearm-turbidity method.

Growth of plant cells in liquid culture is usually determined by cell counting, packed cell volume, cell mass, or cellular protein content (6). These techniques involve tedious preparation and sacrificing of the cells. Withdrawal of samples risks contamination and may result in an unrepresentative sample. Monitoring growth by measurement of turbidity of liquid cultures has been reported (1-3, 8). I report here a method of measuring the growth of cultures grown in sidearm flasks (Fig. 1) by the turbidity, which is determined through the sidearm with a Klett Summerson photoelectric colorimeter. Once turbidity has been calibrated against cell number, this technique allows growth to be measured easily and routinely in batch cultures. In this paper, I report experiments with carrot and soybean that demonstrate the method and present an example of its application.

MATERIALS AND METHODS

All the cultures were grown in Linsmaier and Skoog (4) medium on a rotary shaker at 28 C. Soybean culture initiated from soybean stems (*Glycine max* (L.) Merr. cv. Kanrich) in this laboratory, was grown in medium supplemented with 3 mg/l NAA[2] and 0.5 mg/l of 2-IP as the hormones. Wild carrot (*Daucus carota* L.) culture was obtained from H. E. Street, University of Leicester, England, and was grown in 0.1 mg/l 2,4-D.

For growth determination, cultures were routinely grown in sidearm flasks (Medical Research Corp., Boston, Mass. 02121, nipped neck, 100 × 14 mm sidearm tube). The nipped neck allows incubation of large volumes of culture; 500-ml flasks will contain 200-ml culture; 250-ml flasks, 100-ml culture; and 125-ml flasks, 50-ml culture. Sidewise tilting of the flask fills the sidearm, so that turbidity can be read directly through the sidearm in a Klett Summerson colorimeter with a blue filter at 400 to 465 nm.

For cell number determination, soybean suspension cultures were digested with $Cr_2O_3 \cdot 5H_2O$, 2.5%, at 60 C for 0.5 hr and 2.5% HCl for 1 hr at 60 C, to dissociate clumps. Carrot cultures were treated with the same acids for 10 min each. Clumps were then dispersed with hypodermic needles of gauge numbers 20, 22, 26 and counted under the microscope using a cell counter of 0.2 mm in depth.

Dry weight was determined by slowly pipetting 2 ml of cells onto a preweighed filter paper (Whatman no. 906, 21 mm diameter), placed on top of a deck of paper towels to absorb most of the liquid that passed through the filter paper. The cells and the filter paper were then dried overnight at room temperature and weighed.

The size of aggregates was estimated by filtering cell suspensions through Nitex filters made of nylon sheet (Paul O. Abbe Inc., Little Falls, N. J. 07424) of different porosity.

For the freezing experiment, cultures at Klett 500 were frozen in growth medium containing 5% DMSO at a rate of 2 C/min down to −196 C. The thawing rate was 120 C/min (5).

RESULTS

Carrot. Carrot cells grew in clumps up to 0.2 mm in diameter. Figure 2 shows growth of a culture as measured by determination of dry weight, cell number, and turbidity. Turbidity readings were recorded from a single culture grown in sidearm flask and each time duplicate samples were taken from the flask for dry weight and cell number determination. In Figure 2, the turbidity and the dry weight shared the same curve which is parallel to the curve of cell number with less variability. Increase in turbidity with time was directly proportional to the increase in cell number and dry weight. Therefore, growth could be measured as increase in turbidity, expressed in arbitrary Klett units.

Soybean. Soybean cells grew in large clumps up to 1 mm in diameter. Since the large clumps settled fast in the sidearm, the cultures needed to be shaken well and the readings taken quickly, and repeated a couple of times. Figure 3 is a calibration curve of Klett units against cell number. In this experiment, soybean cultures of varying turbidity were diluted to 100 Klett units, and then digested and scored for cell number. Therefore, the standard deviation in Figure 3 was calculated to indicate variation in both dilution and counting.

Application. Freezing of carrot cells to preserve cell lines has been described by Nag and Street (5), who used vital stain as an indication of viability. Figure 4 shows the use of the turbidimetric method to determine the effect of freezing under different conditions and subsequent thawing on a carrot culture. Although cultures frozen under different conditions all recovered after a period of 20 days after thawing, the kinetics of recovery varied with different treatments. There was a longer delay in the onset of growth when DMSO was added at room temperature then at 0 C. In contrast, the control culture showed a smooth, semilogarithmic increase in turbidity. Thus, a growth curve recorded daily revealed detailed and precise information about viability of the culture.

This technique has also been employed successfully to study the mutagenesis in terms of the mutagen effect on the growth and viability of the plant cell culture (7).

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[2] Abbreviations: NAA: naphthaleneacetic acid; 2-IP: 2-isopentenyl adenine; DMSO: dimethylsulfoxide.

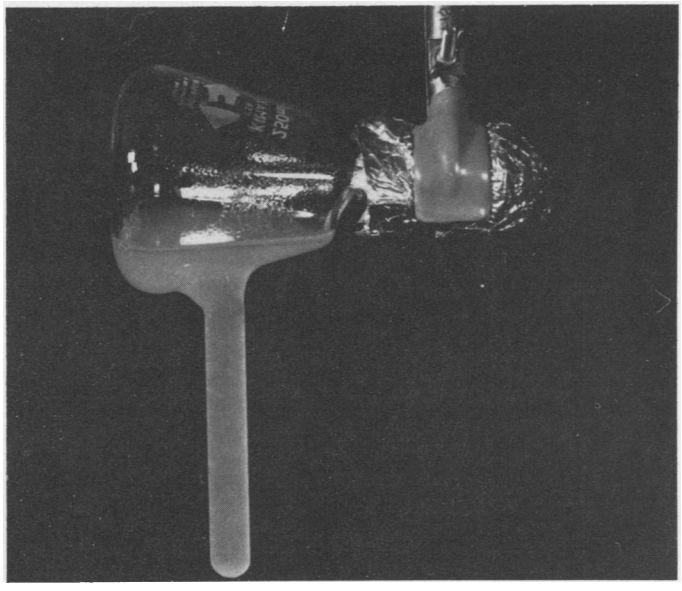

FIG. 1. Sidearm flask containing carrot culture placed at a position as inserted in the Klett Summerson colorimeter.

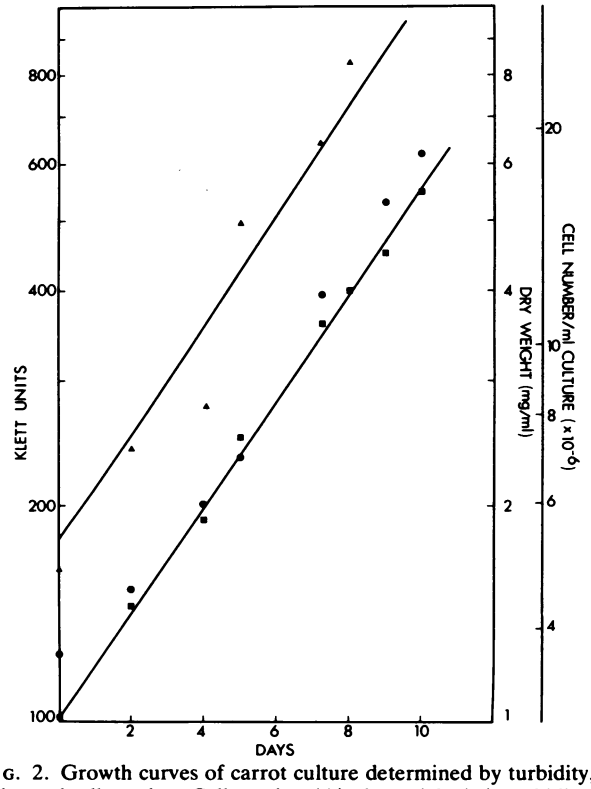

FIG. 2. Growth curves of carrot culture determined by turbidity, dry weight, and cell number. Cell number (▲); dry weight (●); turbidity (■).

DISCUSSION

The turbidimetric measurement of cultures grown in sidearm flasks is a quick way of recording plant cell growth. I have shown that turbidity varies proportionally with cell number and dry weight over time, as one would expect. Turbidity is affected by factors such as cell size, aggregate size, homogeneity of the clump size, the color of the culture, and the amount of cell debris. Therefore, it is always necessary to obtain a standard curve relating turbidity to cell number for a given cell line and growth condition.

In general, the sidearm-turbidity method can be used to measure growth rate in cultures having clump size up to 1.5 to 2 mm in diameter, determined by the porosity of the Nitex filter through which cultures are filtered. It is suitable for most friable plant cultures such as tobacco, carrot, haplopappus, and sycamore. It would be difficult to apply this technique to cultures of large clump sizes, such as rice, because they settle rapidly.

In freshly initiated cultures before a cell line is established, the average clump size often changes over a period of time. In such

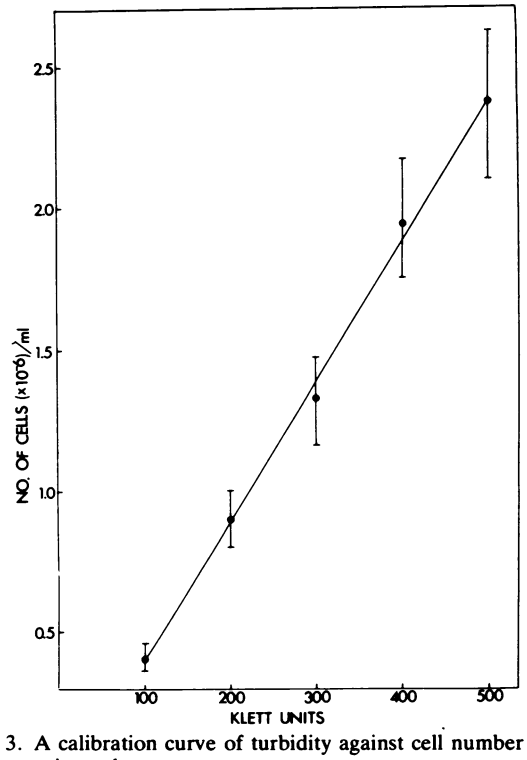

FIG. 3. A calibration curve of turbidity against cell number for soybean suspension culture.

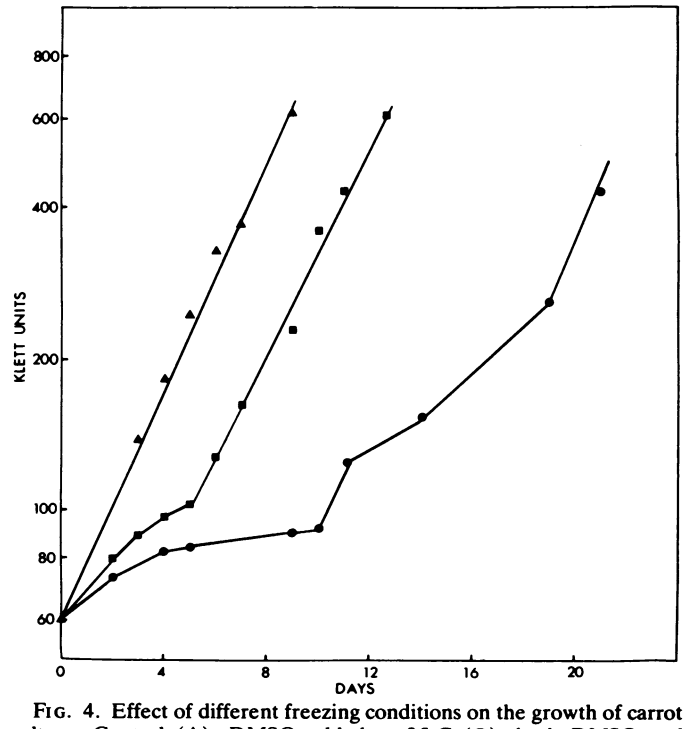

FIG. 4. Effect of different freezing conditions on the growth of carrot culture. Control (▲); DMSO added at 25 C (●); both DMSO and culture were brought to 0 C before DMSO was added (■).

cases, the standard curve has to be determined more frequently, until it is found to stabilize.

The Klett Summerson photoelectric colorimeter covers a relatively narrow range, about 2 logs of growth of a culture. Other colorimeters might cover a wider range and perhaps give a better reading. Above Klett 600, the growth curve starts to level off, even though the culture may still be in the exponential phase of growth. This phenomenon seems to vary with plant species. We usually follow the growth curve up to Klett 500, and transfer the culture at this stage for experimental or routine propagation by inoculating 5 ml of Klett 500 cells into 50- or 100-ml medium. Thus, the growth and propagation of the plant suspension culture can be controlled and described in a simple, precise manner.

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