SHORT COMMUNICATIONS

Advances in Determination of Cell Viability

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Estimations of cell viability can be direct or indirect. Staining techniques are direct while membrane-filter or slide-culture techniques are indirect (Röcken & Staruss, 1976). The latter are time-consuming because the necessary cell cultivation takes several hours. In contrast, cell viability can be determined immediately when staining methods are employed. Since the introduction of vital staining with 'Anilin'-dyes by Ehrlich (1885), various dyes and fluorochromes have been used to determine the proportions of living and dead mammalian, plant and microbial cells. The pioneer work of Strugger (1949) suggests the use of fluorochromes in viability estimations.

In contrast to single cell counting under the microscope, flow cytometry offers a more practicable and rapid assay for the differentiation of stained and non-stained cells in liquid suspensions (Melamed & Kametsky, 1969; Wilson, Dardano & Rothschild, 1973; Penttilä, McDowell & Trump, 1977). The aim of this contribution is to demonstrate the advantages of flow cytometry for the demonstration of cell viability in microbiological samples.

The viability of a yeast population was tested after staining in a solution of erythrosine B (Serva, 21235) prepared by dissolving 4 mg of the fluorochrome in 1 ml phosphate buffered saline (PBS) pH 7.6. *Saccharomyces cerevisiae* 211 was grown in yeast extract peptone broth [1% (w/v) yeast extract (Difco, 0127-01), 2% (w/v) Bacto-peptone (Difco, 0118-01), 2% (w/v) glucose] for 24 h at 28 °C in a shaking water bath. The yeast cells were then centrifuged (3000g), washed twice in 5 ml Tris/HCl buffer pH 7.5 and resuspended in 10 ml PBS. One part of the suspension was fixed in 10 ml 70% (v/v) ethanol for 1 h. The ethanol was removed by centrifugation, and the cells were washed twice in Tris/HCl buffer and resuspended in PBS. Three samples were prepared for flow cytometric measurement; one contained only fixed cells (*a*), another living cells (*c*) and the third, inactivated fixed cells mixed with viable cells in a ratio of 1:1 (*b*). Two minutes before the measurement, 50 µl of the erythrosine B solution was added to 1 ml volumes of each of the three samples. The fluorochrome erythrosine B is excluded from intact cells but damaged cells take up the dye and are stained red (Gurr, 1971; Protocol No. 19, Ortho Instruments, 1976).

In the cytograms (Fig. 1), one dot represents each measured cell. The dot coordinates are determined by the light scatter signal from the cells (*y*-axis) and by their intensity of red fluorescence (*x*-axis). The scattered light from the unstained living cells is stronger than that from the stained cells due to the strong absorption of the 488 nm laser light by erythrosine B. The emission maximum of this dye is at about 600 nm so an OG590 barrier filter was used for the fluorescence emission light path.

Fig. 1. *Saccharomyces cerevisiae* 211 cells stained with erythrosine B. The light-scatter signals from the cells are registered vertically and the amount of red fluorescence (dead cells) horizontally: (a) inactivated cells only; (b) inactivated and viable cells mixed in a ratio of 1:1; (c) untreated cells.

A total of 5000 cells were measured for each of the cytograms. In Fig. 1(a) no unstained cells were detected on the vertical axis because of inactivation by the ethanol treatment. In Fig. 1(b), 51.3% of the cells took up erythrosine B. The percentage of stained damaged cells in the untreated cell suspension was 2.9% (Fig. 1c).

In contrast to indirect methods to examine cell viability our direct method offers direct discrimination between injured and intact cells in large populations. It allows the precise assay of 10^4 to 10^5 cells per minute and thus is of special advantage where hundreds of samples per day have to be tested in cytotoxic assays. The method may also be applicable to monitoring industrial fermentation processes.

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