SHORT COMMUNICATIONS

Preparation of Synchronous Cultures of *Escherichia coli* by Continuous-flow Size Selection

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Methods of producing synchronous cultures in eukaryotic and prokaryotic organisms by both induction and selection synchrony have been described in detail by Mitchison (1971).

Induction synchrony of bacteria by heat shock (Smith & Pardee, 1970) and by starvation (Cutler & Evans, 1966; Inouye & Pardee, 1970) have produced synchronous divisions through at least two cell cycles. The methods of induction synchrony are likely to produce disturbed metabolism, so that where possible synchronous cultures obtained by selection procedures are preferable. Selection synchrony of bacteria by sucrose density centrifugation (Mitchison & Vincent, 1965) and by membrane elution (Helmstetter & Cummings, 1964) produce synchronous cultures with little metabolic disturbance. The major disadvantage of the density gradient centrifugation procedure is that it takes a considerable time, which would probably exceed a single cell cycle in experiments involving short generation times. The membrane elution technique is applicable only to a narrow range of strains of *Escherichia coli* (Cummings, 1970).

The method of selection synchrony described here provides a rapid method for the selection of metabolically undisturbed, large-scale synchronous cultures of *E. coli*.

METHODS

Maintenance and growth conditions of the organism. *Escherichia coli* W1485 was maintained on slopes of tryptone soya agar (Oxoid) and grown in a basic minimal medium containing (per litre distilled water): 0.3 % (w/v) DL-alanine as carbon source; 2 g (NH₄)₂SO₄; 100 ml 1.0 M-phosphate buffer pH 7.2; 5 ml mineral salts solution (10 g MgSO₄·7H₂O, 1 g MnCl₂·4H₂O, 0.05 g FeSO₄ and 0.1 g CaCl₂/l distilled water).

Cell counts. Culture samples (0.9 ml) were added to 0.1 ml of 40 % (w/v) formaldehyde, agitated with a Whirlimix (Hook & Tucker Instruments Ltd., Croydon, Surrey) for 30 s, and the number of cells counted in a Helber bacterial counting chamber (Gallenkamp & Co., London) within 8 h.

Synchronization of exponentially-growing cells. Starter cultures were grown from tryptone soya agar slopes in a shaking waterbath at 37 °C and used to inoculate a larger volume of prewarmed medium at a 1/200 dilution. The culture was force-aerated at 1 l air/min/l at 37 °C for 17 to 18 h when cells were in the late-exponential phase of growth at between 5×10^8 and 10^9 cells/ml. The procedure for continuous-flow size selection synchrony has been given in detail by Lloyd *et al.* (1975).

The MSE continuous flow rotor and all attached tubing was autoclaved (103 kN m⁻² for 20 min) before use and left overnight at 37 °C. The thermostat on the MSE 18 high speed

Fig. 1. Cell numbers and oxygen uptake rates in synchronous and exponential cultures of *E. coli*. F_1 and F_2 denote synchrony indices of the first and second doublings in cell numbers (A) respectively. Oxygen uptake measurements (B) were made on samples removed at 10 min intervals from the synchronous culture. Exponential growth of *E. coli* (C) and oxygen uptake rates (D) are shown over a mean generation time.

centrifuge was pre-set to its maximum setting of 30 °C and both the culture and the collection vessel were continuously aerated at 37 °C.

A flow rate of 200 ml/min and a rotor speed of 10500 rev./min produced an effluent containing approximately 10 % of the smallest cells in the culture. The number of cells per millilitre of effluent was always checked before collection in a sterile glass culture vessel (Quickfit, Jobling Laboratory Division, Stone, Staffordshire). A port, near the base of the culture vessel, covered with a Teflon membrane made rapid, aseptic sampling possible through a sterile needle attached to a three-way tap and syringe. The culture vessel was maintained at 37 °C, magnetically stirred (400 rev./min) and aerated at 1 l air/min/l culture.

Assessment of synchrony. The degree of synchrony was assessed by the synchrony index of Blumenthal & Zahler (1962):

$$F = (N/N_0) - 2^{t/g},$$

where F is the synchrony index and has a maximum value of 1.0 in a theoretically perfect synchronous culture, N is the final cell count, N_0 the initial cell count, t the time taken for the cell count to increase from N_0 to N , and g is the mean generation time.

Oxygen measurements. Oxygen uptake measurements were made with an oxygen electrode (Lloyd & Brookman, 1967).

RESULTS

The growth of organisms in a typical experiment following collection of 9.9 % of the initial exponentially-growing population from the continuous flow rotor is shown in Fig. 1, curve A. The cell cycle times in the first and second cycles were 95 and 100 min respectively. In the first division 88 % of the cells divided, giving a synchrony index (F_1) of 0.69. A similar

index (F_2) was maintained in the second division. In five experiments, the length of the cell cycle ranged from 90 to 95 min in the first cycle and the synchrony indices were between 0.67 and 0.96. The respiration rate of the culture (Fig. 1, curve B) approximately doubled during a cell cycle, but did so discontinuously, producing an oscillation. The periodicity of the oscillation was similar in both cell cycles and was reproducible in five experiments.

In an exponentially-growing culture (Fig. 1, curve C), oxygen uptake rates increased continuously, so as to double over one generation time (Fig. 1, curve D). The cell cycle time of the synchronous culture was similar to the mean generation time of the exponentially-growing culture.

DISCUSSION

The results indicate that the method of continuous-flow size selection previously used to prepare synchronous cultures of various eukaryotic micro-organisms (Lloyd *et al.* 1975) may also be applied successfully to cultures of *E. coli*. That the resulting cultures have suffered little or no metabolic alteration is shown by the consistency of the length of the cell cycle with the mean generation time of exponential cultures. The reproducibility of the pattern of respiration over the first two cycles provides further evidence that growth has not been substantially altered by the selection synchrony procedure. The theoretical advantages offered by an aseptic method, which maintains the growth temperature, nutrient and oxygen status of the culture throughout and which requires only minutes for completion, are evident. It provides a satisfactory alternative to existing methods for the preparation of small-scale synchronous cultures and is more easily applicable to large culture volumes.

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REFERENCES

- BLUMENTHAL, L. K. & ZAHLER, S. A. (1962). Index for measurement of synchronisation of cell populations. *Science, New York* **135**, 724.
- CUMMINGS, D. J. (1970). Synchronisation of *E. coli* K-12 by membrane elution. *Biochemical and Biophysical Research Communications* **41**, 471-476.
- CUTLER, R. G. & EVANS, J. E. (1966). Synchronisation of bacteria by a stationary phase method. *Journal of Bacteriology* **91**, 469-476.
- HELMSTETTER, C. E. & CUMMINGS, D. J. (1964). A method for the selection of bacterial cells at division. *Biochimica et biophysica acta* **82**, 608-610.
- INOUE, M. & PARDEE, A. B. (1970). Requirement of polyamines for bacterial division. *Journal of Bacteriology* **101**, 770-776.
- LLOYD, D. & BROOKMAN, J. S. G. (1967). An oxygen electrode reaction vessel. *Biotechnology and Bioengineering* **9**, 271-272.
- LLOYD, D., JOHN, L., EDWARDS, C. & CHAGLA, A. H. (1975). Synchronous cultures of microorganisms: large-scale preparation by continuous-flow size selection. *Journal of General Microbiology* **88**, 153-158.
- MITCHISON, J. M. (1971). Synchronous cultures. In *The Biology of the Cell Cycle*, pp. 25-58. Cambridge: Cambridge University Press.
- MITCHISON, J. M. & VINCENT, W. S. (1965). Preparation of synchronous cell cultures by sedimentation. *Nature, London* **205**, 987-989.
- SMITH, H. S. & PARDEE, A. B. (1970). Accumulation of a protein required for division during the cell cycle of *Escherichia coli*. *Journal of Bacteriology* **101**, 901-909.