SYNCHRONIZATION OF E. COLI K 12 BY MEMBRANE SELECTION

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Summary

E. coli K 12 Hfr and F⁻ strains were synchronized using the membrane selection technique (1,2). The bacteria were deposited onto a Polyvic (Polyvinyl chloride) Membrane and eluted with growth medium. About 20 percent of the Hfr strain and 7 to 9 percent of the F⁻ strain remained bound to the membrane and at division, each bacterial strain yielded a culture of the youngest cells in an exponential population.

Introduction

Helmstetter and Cummings (1) reported that cultures of E. coli B/r could be synchronized using a membrane selection procedure. In this procedure the bacteria were deposited on a millipore filter of pore size smaller than the bacterium; the filter was inverted and the cells eluted with growth medium at 37°C. Within 35 to 40 minutes, about 95 percent of the initial population of the cells were eluted from the filter and discarded (2); 5 percent of the cells remained firmly bound to the membrane and growth of these cells persisted upon the continual addition of eluant. At division, 95 percent of the newly divided cells eluted, thus providing a continuous source of the youngest cells in an exponential population.

The main advantage of this membrane method was that no stress was applied to the exponential population in order to achieve synchrony (1,3). Consequently, investigations on the periodicity of protein synthesis and DNA replication had a direct bearing on understanding the cell cycle (2,4,5,6).

A disadvantage was that only E. coli B and B/r strains were subject to this selection (1,2). Other bacteria, especially E. coli K 12 in which the effects of the sex factor and conjugation on macromolecular synthesis could be studied, did not bind to the membrane in the manner described above. Instead of eluting readily from the membrane, about half the population of E. coli K 12 remained on the filter and eluted sporadically with time (unpublished results), thus preventing the collection of the youngest cells when division occurred on the membrane. We considered the possibility that this difficulty with E. coli K 12 could be eliminated if a different type of membrane were utilized. This communication describes the application of the membrane selection technique to E. coli K 12 in which synchrony was achieved using a polyvinyl chloride membrane.

Materials and Methods

Growth of Bacteria. E. coli K 12 strains AB 253 F^- and AB 259 Hfr were kindly supplied by Dr. A. L. Taylor. As before (1,2) the bacteria were first grown in nutrient broth and then adapted to minimal medium (a modified C-salts medium (2)). A 7-liter culture was inoculated with about 2×10^8 cells and grown for 12 to 14 hours at 37°C. Two liters of the culture were used to inoculate the synchrony apparatus (2) and the remaining 5-liters were filtered through a 0.65μ MF (Millipore Corporation, Bedford, Mass.) and used as the eluant (1,2).

Synchrony. When the cell density of E. coli K 12 Hfr and F^- had reached a titer of 3 to 5 x 10^7/ml and 6 to 10 x 10^7/ml, respectively, the bacteria were deposited on a Polyvic filter (BDWP, 293 mm diameter, 0.6 μ pore size, polyvinyl chloride membrane, Millipore Corporation, Bedford, Mass.) The filter was first wetted with 40 ml of 0.8 percent of Bacto-Tryptone broth. Elution was begun with 2 l of growth medium and the eluant volume was restored to 2 l after 25 min. and 40 min. This yielded a flow rate of 40 to 50 ml/min. at 50 to 55 min. after commencing elution. Within the first 40 minutes, about 80 percent of the Hfr strain and 91 to 93 percent

of F^- strain eluted from the membrane. Samples for analysis of synchrony were collected at 50 to 55 min. which corresponded to the generation times of these organisms. This time of collection has been previously shown (1,2) to yield optimum synchrony.

Counting. Cell number and size distribution were determined using a Coulter Counter Model B, a 35 μ aperture tube and a Model J Automatic Particle Size Distribution Analyzer (1,2,6).

Results and Discussion

The synchrony achieved for E. coli K 12 Hfr and F^- is illustrated in

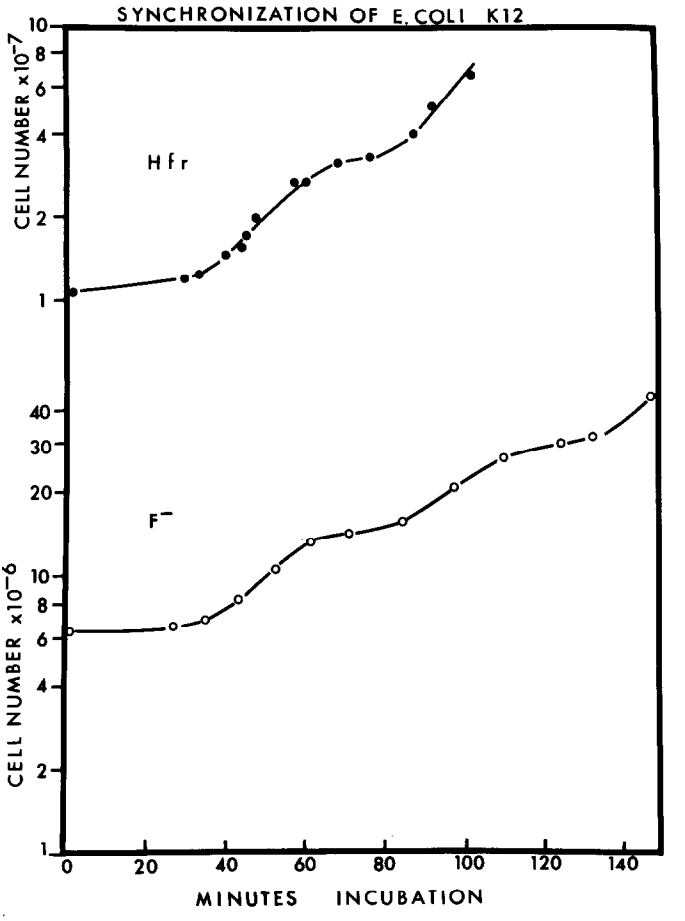

Figure 1. Synchronization of E. coli K 12. For the Hfr strain, 2-1 at 5 x 10^7/ml were deposited on the membrane yielding a synchronous population with a cell density of about 1 x 10^7/ml. For the F^- strain, 2-1 at 1 x 10^8/ml were used yielding a synchronous population with a cell density of 6 x 10^6/ml.

Fig. 1. As expected, for about the first 40 minutes of growth, little increase in cell number occurred; the bacteria then doubled in number during the next 20 minutes and synchrony persisted for at least two generations. In general,

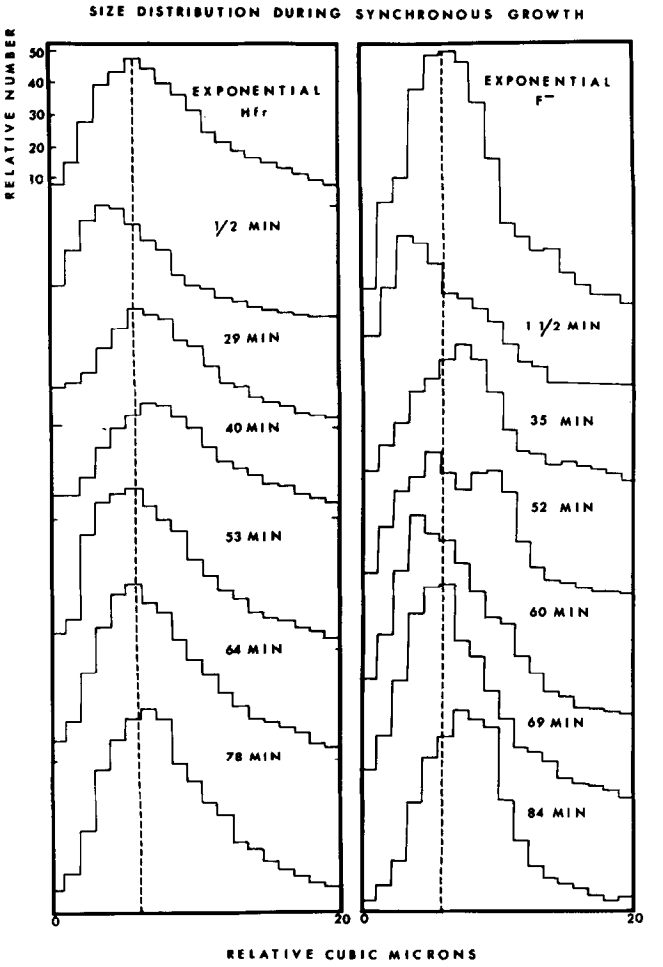

Figure 2. Size distribution of the synchronously dividing populations shown in Fig. 1. Note that at 52 and 53 minutes, both strains displayed a broad size distribution and that the F^- strain size distribution was biphasic. The vertical dotted lines indicate the midpoint of the exponential size distributions.

these results agreed with those obtained previously (1,2) with E. coli B/r. Sampling the Hfr strain was complicated by the fact that due to the interaction of the pili (7), self agglutination occurred at higher titers. This property may also account for the greater number of cells bound to the Polyvic membrane; the Hfr yielded about 20 percent of the cells bound whereas only 7 to 9 percent of the F^- cells remained on the membrane after the first 40 minutes of elution. This is evident in the number of cells in synchrony under the same conditions (Fig. 1) and is an indication that the degree of synchrony achieved is best in the F^- strain.

Periodic increases in cell number is only one criterion for synchrony. This must be accompanied by a periodic change in cell size (2,6). In Fig. 2, it can be noted that just after collection, the selected cells were distributed within the smallest range of the exponential size distribution. The cells increased in size with time and after one generation time, the cells again were smaller, as expected. The size distribution of the E. coli K 12 F^- after one generation time (52 min.) was, in fact, biphasic. About half the cells were in the smallest size range and the other half in the larger range. This could only occur in a synchronously dividing population.

The results presented here indicated that E. coli K 12 can be synchronized by the membrane selection technique. Whether or not F^- strains are intrinsically subject to better synchrony than Hfr strains is not clear. It may be that alteration of the minimal growth medium could yield better synchrony and such alterations are being investigated.

Acknowledgements

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