Density Gradient Centrifugation of Bacteria and Nonspecific Bacteriophage in Silica Sol

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In density gradient centrifugation, selection of the optimal gradient-forming substance is of great importance, particularly when living cells have to be separated and recovered in viable form. Ludox was reported by G. M. Mateyko and M. J. Kopac (Ann. N. Y. Acad. Sci. 105:219, 1963) as the most successful medium for isopycnic cushioning of living cells in density gradient centrifugation.

Our search for an optimal density gradient medium was prompted by the need to concentrate viable bacteria in a sharp band, to allow their separation from other particulate matter and debris in a relatively short period of time. In this case, nonspecific viable bacteriophage was the contaminating particulate matter. The primary concern was to recover the unharmed bacteria free from phage. The experiments were based on the density difference between bacteria and bacteriophage, and the medium was designed to cover the density range of both and to provide adequate support to both.

Ludox is a colloidal silica sol manufactured by E. I. du Pont de Nemours and Co., Wilmington, Del. For our work we selected Ludox AM, which is a modification by alumina particles on the surface of the silica spheres. According to DuPont Data Sheet A-31957, the stability of Ludox AM is far superior to the other forms of Ludox, especially in the pH range of 4 to 8. The original concentration of the silica sol, according to the manufacturer’s literature, is approximately 31% silica, the density is 1.220 g/cc, the viscosity is 10 centipoises at 25°C, the particle diameter is approximately 14 to 15 μm, and the pH is 9.1.

Because preliminary centrifugation of the Ludox AM showed sedimentation of the silica and a possible gradient formation, no attempts were made to preform a gradient.

The pH of the Ludox AM was adjusted with Sorenson’s phosphate buffer to pH 7.45, changing the density to 1.118 g/cc, and the sol was autoclaved without impairing stability.

Escherichia coli O26B, obtained from the Chemical and Biological Division, Space-General Corp., was cultured and subsequently serially diluted in Hershey broth (M. Chase and A. H. Doerrmann, Genetics 43:332, 1958) to a final concentration of 2 × 10⁶ bacteria per milliliter. T4D bacteriophage (nonadsorbent to E. coli O26B) was diluted in Hershey broth (M. Chase and A. H. Doerrmann, Genetics 43:332, 1958) to a final concentration of 2.8 × 10⁸ particles per milliliter. These suspensions were combined in equal volumes for the experiments.

A Beckman Spinco (model L-2) preparative ultracentrifuge was used with swinging bucket rotor SW 39L, furnished with 5-ml heat-resistant polyallomer tubes. After the tubes were autoclaved, 0.2 ml of sample was layered on top of 3.8 ml of adjusted Ludox AM in each tube, and the tubes were centrifuged for 60 min at 115,000 × g at 20°C. The centrifuge was allowed to coast to a stop, and fractions were removed with a Spinco sample micropipette for assays by standard methods. A solid pellet remained at the bottom of the tube.

A total of 16 fractions (approximately 1.69 mm tube height each) were obtained. Colony counts of E. coli indicated that 89% of the bacterial cells were banded in a single zone, no. 12. An additional 3% were found in the fraction directly beneath this band, with the remaining 8% scattered through the other fractions in concentrations too low for accurate quantitation. The phage titer indicated essentially complete absence of phage in the gradient. Altogether, only six T4D plaques were counted from all 16 fractions above the pellet.

Sedimentation of the silica particles created a density gradient during centrifugation (indicated by increasing viscosity of the removed fractions), with a solidified mass at the bottom of the tube. The phage particles, sedimenting at a slightly faster rate, were imbedded in this silica pellet, whereas the bacteria remained in the supernatant fraction. Presence of a gradient was particularly evident from the location of the bacterial zone, well supported about 6 mm above rather than
directly on top of the pellet. The sharpness of the zone indicated that the gradient was steep. It is presumed that this is a transient stage of the sedimenting silica and that, after an extended period of centrifugation, the gradient would disappear.

The use of Ludox AM in density gradient centrifugation provides an effective medium for rapid banding of bacteria. In addition, the technique of trapping the phage beneath the surface of solidified Ludox AM in a pellet at the bottom of the tube appears to be a very effective way of removing phage or other particulate matter.

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