PRODUCTION OF FILTERABLE PARTICLES BY CELLVIBRIO GILVUS

J. D. TUCKETT AND W. E. C. MOORE

Departments of Biochemistry and Nutrition, and of Biology, Virginia Agricultural Experiment Station, Virginia Polytechnic Institute, Blacksburg, Virginia

Received for publication August 25, 1958

Reports of viable filterable particles in bacterial cultures have often been attributed to artifacts from the media, contaminants, and, in the case of filtration experiments, to laboratory errors (Frobisher, 1928). Since Kleineberger's (1935) observations on L forms, they have received considerable attention, but their significance and function remain obscure. It has been suggested that they are part of a complex life cycle serving reproductive or genetic recombination functions (Kleineberger-Nobel, 1951b). It has been hypothesized that the L forms are aberrant cells or involution forms (Heilman, 1941).

The failure of many filtration experiments to yield positive data on repetition has presented serious problems in determining the physiology of filterable forms, and has also raised doubt concerning the existence of a filterable stage in bacteria. In addition, many reported filterable forms required subculture through several serial transfers before cells reappeared. These manipulations increased the possibility of filtrate growth from contamination rather than from particles (Kleineberger-Nobel, 1951a).

The present report concerns viable, filterable particles which appear to be produced regularly and which regenerate the normal, parent type cell without subculture of the filtrates.

METHODS

A culture of Cellvibrio gilvus (Hulcher and King, 1958) was observed to produce involution forms, particles, and swollen cells even in 16- to 24-hr cultures. Cell suspensions of C. gilvus were grown in 50 ml of cellobiose broth (Hulcher and King, 1958), harvested by centrifugation, and mixed with 50 ml of fresh medium. Escherichia coli cells were harvested from 50 ml of 24-hr cultures and mixed with the suspension to be certain that the filters were impervious to normal cellular forms. The mixed suspensions were filtered on Morton ultrafine bacteriological fritted glass filters to determine the presence of filterable particles of C. gilvus. Filtration was carried out by suction from aspirator pumps applied through a 5-in cotton plug. The assembled filters and plugs were sterilized as a unit at 121°C for 15 min.

After the first appearance of growth, the filtrates were tested for the presence of E. coli by inoculation into lactose broth. Negative tests indicated that E. coli had been unable to penetrate the filters. Positive controls in which E. coli was added to the filtrate demonstrated that E. coli could have been recovered had it penetrated the filter. The filters remained sealed and closed to the atmosphere until the first appearance of growth in the filtrate.

As an arbitrary measure of the size of the particles, a series of filtration experiments was done with Selas porcelain filters of graded pore sizes. These were prepared and used in a similar manner and with similar cell suspensions as the fritted glass type. The E. coli test was carried out in these experiments also. The only difference in procedure was that the cultures were not centrifuged previous to filtration. Plate counts were made from filtrate samples immediately after filtration to determine the number of filterable particles per ml of 48-hr culture of C. gilvus.

Observations of the living organisms were made by use of dark field microscopy. Photographs were taken of certain stages of development of the organism.

RESULTS

Of 10 fritted glass filters, 2 were found to be impenetrable to C. gilvus, 3 passed C. gilvus in every experiment, and the remaining 5 gave variable results. Upon inoculation of filtrate samples into lactose broth, negative tests indicated that all of the filters were impervious to E. coli. The time in which growth occurred varied, but was
Figure 1. Photomicrograph of a large body in a culture of Cellulibrio gilvus under dark field. The body measures approximately 9 μ across its largest dimension (estimated within the limits of dark field observation). The 2 larger light spots represent normal cells. Motility is evident in the normal cell in the lower right corner from the light track made during exposure. The particle above this cell appears similar in size to the average particle observed.

generally within 48 hr. Morphological examination and cultural tests of the growth in the filtrates revealed pure cultures of C. gilvus. The cellular morphology in the filtrate cultures was more uniform than that of the parent culture but after subculture involution forms and swollen cells reappeared. The number of viable particles found in the filtrate by plate count did not vary when the organism was cultured at pH 7, 8, 9, or 10, when the culture was incubated at temperatures of 25, 30, or 37 C, or when the age of the culture was 24, 48, or 72 hr at the time of filtration.

Plate count data from the filtrates of the graded porcelain filters indicated that an average of 250 particles per ml of 48-hr C. gilvus culture passed through the 0.85-μ maximal pore radius filter, an average of 100 passed through the 0.60-μ maximal pore radius filter, an average of 20 particles passed through the 0.45-μ maximal pore radius filter, and 5 through the 0.35-μ maximal pore radius filter. The 0.30-μ maximal pore radius filter evidently stopped all viable particles since no colonies appeared on the plates and no growth appeared in the filtrates. All of the porcelain filters were impervious to E. coli.

Dark field observations of living organisms suggested the existence of a complex life cycle. This cycle consisted of the enlargement of barely visible particles into normal cells, the swelling of normal cells into shapeless, flexible, large bodies, and the appearance of particles and cells within the large bodies. These particles and cells became highly motile and, after 6 to 8 hr, finally were observed to emerge through the flexible wall of the large body. The complete process was estimated to take about 48 hr. Binary fission of the normal cells in the culture was also observed. Although more attention was given to the large bodies, binary fission may have occurred with equal frequency. The high degree of motility displayed by the particles and cells interfered with the photomicrographic studies. Complete photomicrographic series of the process could not be made. Figure 1 shows the irregular large body. The granules within the large body were motile. The light bodies outside the large body are the size of normal cells. What appeared to be granules ranged in size down to the limit of visibility, but because of their size and motility they generally failed to reflect sufficient light to be recorded with the available equipment. The fragile large bodies were disrupted by staining techniques employed for light field observations.

An attempt was made to concentrate the filterable particles for chemical analysis. Centrifugation of 50 ml of the filtrate from the 0.85-μ maximal pore radius filter for one hour at 23,500 × G failed to sediment quantities of filterable particles suitable for chemical analysis. Attempts at different centrifugation of the original culture also did not produce macroscopic quantities of particle sediment.

DISCUSSION

These experiments indicate that C. gilvus produces filterable particles. Since, in the critical experiments, the filters remained sealed throughout the operation, contamination was improbable. The cotton plug between the filter and aspirator remained dry, therefore it is unlikely that contamination could have entered through the side arm of the flask. The presence of E. coli in the cell suspension and its absence in the filtrate indicates that the filters were capable of holding back cellular forms of the bacterium. The variable results given by the sintered glass filters are probably due to their varying pore size. Those
filters which gave both positive and negative results evidently had a pore size on the borderline between the size that admits the smallest particles and that which admits none. In the case of one or two particles admitted to the filtrate, it would be a matter of chance whether they remained viable long enough to regenerate the bacterial form. Since, during filtration with fritted glass and porcelain filters the pores would become clogged, the numbers reported here are probably somewhat less than the actual numbers of filterable particles in the cultures. No evidence concerning recombination of particles was obtained but such a mechanism would further modify interpretation of the number of particles produced. This bacterium seems to give more consistent results and is more convenient to work with than the majority of filterable bacteria.

Particle production is apparently not affected by several changes in environment which still allow growth of the organism.

**SUMMARY**

Experiments with both fritted glass and porcelain bacteriological filters indicated that *Cellvibrio gilvus* regularly produces filterable particles which regenerate normal bacterial cells without subculture. Dark field microscopic observations suggested the presence of a life cycle involving the enlargement of minute particles into normal cells, the swelling of normal cells into irregular large bodies, and the appearance of motile particles and cells which finally emerge from the large bodies.

**REFERENCES**


Heilman, F. R. 1941 A study of *Asterococcus muris* (*Streptobacillus moniliformis*). I. Morphological aspects and nomenclature. J. Infectious Diseases, 69, 32-44.


