"GERMINATION TUBE" GROWTH IN ESCHERICHIA COLI MICROCULTURES

HEINER HOFFMAN AND MICHAEL E. FRANK

Department of Microbiology, New York University, College of Dentistry, New York, New York

Received for publication 1 June 1964

ABSTRACT

Hoffman, Heiner (New York University, New York, N.Y.), and Michael E. Frank. "Germination tube" growth in Escherichia coli microcultures. J. Bacteriol. 88: 1151-1154. 1964.—Analysis of extensive time-lapse photomicrographic records of Escherichia coli microcultures uncovered two cases in which there occurred aberrant cell growth resembling a germination tube. Although previously observed by a number of investigators in gram-positive bacteria, the present observations appear to constitute the first time-lapse photomicrographic record concerning a gram-negative form. In the first case, with photographs taken at 15-sec intervals, the cell initially exhibited a clublike deformation, and the "germ tube" then issued from the club head. The "tube" had developed into a separate cell by the time the photographic record was concluded. In the second case, with the photographs taken at 1-min intervals over a longer period of time, the "germ tube" cell assumed a plastic dumbbell-like form after separation from its sister. It is suggested that the phenomenon results from a disturbance in cell-wall synthesis, while cytoplasmic growth continues unabated.

A number of reports (e.g., Gillespie and Retger, 1939; Kudoh, 1956; Richardson and Schmidt, 1959; Gilmour, 1961; Starr and Kuhn, 1962) have noted among the gram-positive bacteria a form of aberrant growth suggesting the germination tube of higher fungi. The frequency with which this mode of growth occurs varies from the case of tuberele bacilli, where it is rarely seen (Kudoh, 1956), to the case of Bacterionema matruchotii, where it is so prominent both in frequency of occurrence and in the great length of these puzzling structures (see Gilmour, 1961), that Bisset (cited by Davis and Baird-Parker, 1959) was led to suggest that they may represent the relic of an ancestral mycelium. Close examination of our extensive time-lapse photomicrographic records of developing clonal microcolonies of Escherichia coli has uncovered two instances of the bacterial "germination tube," thus extending the phenomenon to the gram-negative bacteria. Analysis of the records indicated that a disturbance in cell-wall synthesis may be the mechanism involved (Chan, 1964).

MATERIALS AND METHODS

The organism used was rough-phase E. coli ATCC 8677. The techniques for the preparation of the cover-slip smear microculture, and for temperature control during incubation, have already been given (Hoffman and Frank, 1963), but the microscope optical and photomicrographic systems have not previously been described in detail.

The microscope used was a Carl Zeiss WL stand with dark-phase optics and a revolving and centering gliding stage, which allows movement in all horizontal directions. The microscope was mounted on a Fisher Vibradamp base (Fisher Scientific Co., New York, N.Y.) to minimize vibration. Two sets of optics were used during a cultivation. The first couple a selected 100× Neofluar oil immersion objective lens (NA, 1.30) with an 8× KPL eyepiece. When the microcolony was at the point of overgrowing the field, increased field size and increased depth of focus were obtained by changing over to a selected 40× apochromat oil immersion objective lens (NA, 1.00) and a 12.5× KPL eyepiece. An achromatic, aplanatic, spherically and chromatically corrected oil immersion condenser of high numerical aperture (1.40) completed the lens system of the microscope.

Illumination for photography was supplied by a built-in Zeiss tungsten-filament lamp (6 v, 15 w). The light filters used consisted of a KG1 heat-absorbing filter (Zeiss), and a Schott OG4 filter, 3 mm thick (Fish-Schurman Corp., New Rochelle, N.Y.), which has 95% transmission at 550 nm and 1% transmission at 500 nm. During photomicrography, light exposures were 5 to 6 sec.
at the higher magnification, and 1 to 2 sec at the lower magnification.

For photomicrography, the Zeiss basic body I, with focusing eyepiece, was mounted on a separate stand and suspended over the microscope. Coupled to the basic body I was a Praktina FX35-mm camera with an accessory 50-ft (15.2 m) film back, which allows exposure of 420 frames without reloading. The camera was driven by a solenoid motor under the control of a modified Samenco intervalometer (National Cine Equipment Co., New York, N.Y.). This arrangement gave a completely automated system, since the following functions could be programmed without further attention being required: single frame advance of the film, frame counting, exposure time, time interval between exposures, and illumination. The film used was Kodak High Contrast Copy 35 mm in 50-ft (15.2 m) rolls. The film was developed for 6 min with Kodak Dektol developer diluted 1:1.

RESULTS

A microcolony photographed at 15-sec intervals (rapid sequence run) gave rise to an aberrant cell early in the course of cultivation. The cell appeared to be quite normal in appearance initially (Fig. 1), but became progressively clublike, with the club head lying at the periphery of the colony (Fig. 2). Gradually, the cell began to elongate and constrict at the center, giving a slightly dumbbell-like shape (Fig. 3). In the last available photographic frame (Fig. 4), division has occurred, with one of the daughter cells lying toward the colony center and exhibiting normal length and form, and the other daughter cell (referred to as the “germination tube” cell) lying peripherally and exhibiting a somewhat longer and thinned-out appearance. The normal-length daughter cell was the same length as its mother.

A second microcolony, photographed at our conventional frequency of 1-min intervals, gave rise to an initially normal cell in the ninth generation, from which arose a terminal budlike protrusion of smaller transverse diameter than the main body of the cell (Fig. 5). The bud gradually increased in length (Fig. 6), and the elongated cell then divided to yield one daughter of normal appearance and one daughter which seemed to be simply the thin elongated bud or tube (Fig. 7). When the photographic record ended (Fig. 8), the thinned-out cell had constricted at its middle to give a rather plastic dumbbell-like form. At this time, the daughter with normal appearance had increased in length slightly, and its aberrant (“germination tube” cell) sister was slightly longer than it. Although these two cells were soon lost from overgrowth by other cells, the “germination tube” cell was under observation for 8 min after it had separated from its sister. In contrast to this, the “germination tube” cell in the rapid sequence run was lost immediately after it had separated from its sister.

DISCUSSION

The time-lapse photomicrographic observations can be nicely accounted for on the basis of the recent suggestion by Chan (1964) that morphological aberration of Arthrobacter globiformis is caused either (i) by the rate of cell-wall synthesis lagging behind the rate of synthesis of cytoplasmic constituents, or (ii) by structural weaknesses being present in the cell so that a rigid cell morphology could not be maintained. In either case, the biochemical reactions for cell-wall synthesis are involved.

In our first series of time-lapse photomicrographs (Fig. 1 to 4), the cell wall of the mother does not appear to have grown during the period when the “germination tube” was observed, assuming that a measure of the cell wall is obtained from the length of the normally formed cell. In the second series (Fig. 5 to 8), cell-wall growth appears to have developed only very slowly during the period of observation. The only other time-lapse photomicrographic study of “germination tube” growth which we have encountered is that by Gilmour (1961) of B. matruchotii. Measurements made from Fig. 12 to 21 of Gilmour’s report indicate that the bacillus-like body from which the germinating tube arose did not grow in length through the observation period of over 27 hr, although the germination tube itself grew considerably.

The clublike form assumed by the aberrant cell in our first series (Fig. 2) is the first detectable indication of cell-wall disturbance. In this regard, it is of some interest to note that Gilmour, Howell, and Bibby (1961) also found clubbed forms in their cultures of B. matruchotii. Presumably, as cell-wall synthesis lags, the pressure from accumulating cytoplasm stretches the cell wall in its weakest region, and finally the cytoplasmic pressure breaks through this barrier.
FIG. 1 to 4. Microcultures of Escherichia coli; dark-contrast phase microscopy; arrows indicate cell or cell part named in the legend. Rapid-sequence culture, photographed at 15-sec intervals; X3,000. (1) Mother cell of the "germination tube"; time, 0. (2) Club phase of mother cell; time, 9.75 min. (3) "Germination tube" extended, resulting in dumbbell form; time, 18.75 min. (4) "Germination tube" cell (arrow a); separated from sister cell (arrow b); time, 27.75 min.

FIG. 5 to 8. Conventional sequence culture, photographed at 1-min intervals; X4,500. (5) Ninth generation cell with terminal bud or "germination tube"; time, 0. (6) Elongated "germination tube"; time, 13 min. (7) "Germination tube" cell (arrow a); separated from its sister (arrow b); time, 21 min. (8) "Germination tube" cell drawn out into plastic dumbbell form (arrow a); apparently normal sister (arrow b); time, 29 min.
completely. The ensuing tube may still be synthesizing some portion of the cell wall, since it retains the rod form. However, the second series of photomicrographs indicates that considerable plasticity of form of the "germination tube" cell may develop eventually.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grants DE 01462-02 and DE 01462-03 from the National Institute of Dental Research.

LITERATURE CITED


