CYTOKINESIS IN GAFFKYA TETRAGENA¹

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There is little agreement as to the manner of division of the cytoplasm of the bacterial cell. This may be due to the use of different stains, techniques, and organisms. Also, most of the workers did not have the advantages provided by the presence of a stained nucleus to correlate with septum formation. At least one exception was DeLamater (1952) who not only reported a nucleus in a Micrococcus but also published a photograph showing a "cell plate". He failed, however, to indicate its origin. Knaysi (1951) states that "the cytoplasm is divided by inward (centripetal) growth of the cytoplasmic membrane forming a septum or plate across the cytoplasm." Subsequently, each sister cell deposits its own cross wall in the middle of the cross plate. Bisset (1950) reports that there is a difference in the mechanism of cellular division as regards the rough and smooth types of cells. For the smooth type, he states that "when the cell is about to divide, a transverse septum, derived from the membrane, develops across the middle of the cell. The cell wall is then secreted at the edges of the new septum and grows inwards dividing the cell." In the division of the rough type of cell, it is stated also that the septum originates from the cell membrane. These are the more recent views on cytokinesis in the bacteria, and a review of the older literature will be omitted.

MATERIALS AND METHODS

Gaffkya tetragena was grown on slide cultures using glucose agar and incubated at 30 C. A suspension of the organism was prepared in sterile water, and smears were made on sterile slides. As soon as the smears were air dry, they were covered with a sheet of glucose agar cut from a poured plate. The cultures were placed in a moist chamber and incubated 12 hours or longer before the agar was peeled from the slide. The slide was

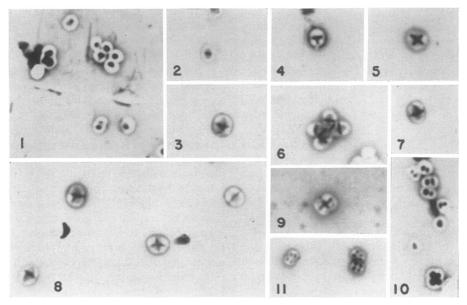
¹ This investigation was supported in part by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract no. DA-49-007-MD-319. allowed to air dry and the smear stained by the crystal violet method reported by Chance (1952).

RESULTS AND DISCUSSION

Since the growth of Gaffkya tetragena is apparently from the single to the four-celled state, an opportunity is provided to observe cellular division in a sequential series. The staining as carried out usually does not stain the cell wall. However, under certain conditions the wall and especially the cell plate first dividing the cytoplasm are stained. During the early stages of cell plate development and for a short time thereafter there apparently is little differentiation between the nuclear and plate substance as both are stained. After decolorizing, the plate is darker in color and can be distinguished readily from the nuclear material. As development continues, the plate stains less deeply and finally may not stain at all.

A mature cell apparently possesses a single nucleus as is true of each of the four cells as the quartet approaches maturity. Prior to the first division, the nucleus in some cells appears to increase in size and becomes more fibrous in appearance. The fibers are not distinct as discrete structures; yet the stained material suggests a closely woven fibrous network. When the nucleus reaches this stage, it may be seen to be divided by a more deeply stained line. This differentially stained material apparently originates in the nucleus, extends through its center, and later projects on each side until the outer wall is reached. Various stages in the extension of this material from the nucleus to the outer wall may be observed. It is believed that this differentially stained material constitutes the cell plate. In some cases the nucleus is scarcely, if at all, visible by the time the cell plate reaches the outer wall.

When the end of the plate is coexistent with the periphery of the nucleus on each side, the whole configuration is much like that of a metaphase as observed in higher plants. Spindle-like fibers may be observed extending from the plate and converging to points on each side of it. At this stage or a little earlier a metaphase may exist, but present observations are insufficient to establish it. While the earlier behavior of the nucleus may simulate that of higher plants, subsequent stages in the process were not observed. Likewise, the The cell plates of the second division are initiated in the nucleus adjacent to the first plate, and initiation may take place almost simultaneously with that of the first division. Both plates may appear at the same time or one earlier than the other; or only one may develop. The nucleus may or may not be visible during or after the formation of these plates. If both plates appear before



Figures 1-11

Figure 1. Cells showing discrete nuclei; one cell shows unequal division of nucleus.

Figures 2-3. Early stages in cell plate formation, metaphase-like structure.

- Figures 4-5. Shows one second division cell plate and two second division cell plates.
- Figure 6. Tetrad showing cell plates.
- Figure 7. Cell plate contacting outer wall.
- Figure 8. Various stages in cell plate development.
- Figure 9. First cellular division complete; 2nd division still in process.
- Figure 10. Nuclei in various stages of division; cell plates not visible.

Figure 11. Early tetrad, showing 4 nuclei. Cell plates, if present, not staining.

All cells \times 2,400.

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nuclear divisions in some cells appear to be amitotic because the daughter nuclei appear unequal in size in some stained preparations. In other cells there is a clear space between the daughter nuclei, which may be due to the deposition of wall material upon the cross plate. Accurate description of the internal behavior of the nuclear material before and during division is difficult to follow since its size is near the limits of microscopic resolution.

the plate of the first division reaches the outer wall, the appearance is that of a cross within the cell. In case all plates have reached the outer wall or if only one of the second division plates develops, then the original cell appears to be divided into four and three parts, respectively.

When the second division of the cell is delayed, the first division may be complete or almost complete prior to the second nuclear division. After the first cell plate is formed, an indentation of the wall may appear on each side of the cell and at the point of juncture of the cell plate with the outer wall. The indications are that cellular division may be complete or almost complete before the initiation of the second division. The second cell plates usually appear opposite each other and usually at right angles to that of the first division. The deposition of the wall material is a matter of further study, but since the formation of the cell plate in this organism is similar to that of higher plants, it may be that the deposition of the wall substance likewise resembles that of higher plants.

SUMMARY

A method has been outlined for the observation of cytokinesis in *Gaffkya tetragena*. It was found that a cell plate is formed in the nucleus and extended so as to intercept the walls and divide the cell into two portions. The process is comparable in many respects to that found in higher plants.

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