OBSERVATIONS ON THE CELL DIVISION OF SOME YEASTS AND BACTERIA

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A review of the literature on the cell division of yeasts and bacteria brings out the fact that exact knowledge of the process is, at the present, lacking. The descriptions are sometimes too general, sometimes incomplete, and often the structures involved are confused.

According to Guilliermond (1912), the cell division of the yeast *Schizosaccharomyces octosporus* takes place as follows: The cells elongate, then, after having acquired their maximum length, they form in their median region a transversal partition. The latter splits and the two daughter cells round up and rotate, generally around a point of the partition where they remain attached. *Schizosaccharomyces pombe* and *Schizosaccharomyces mellacei* divide in a similar way.

The methods of cell division described for bacteria were classified by the author (Knaysi, 1938) under three headings: (1) *plate formation*, centrifugal or centripetal; (2) *cytoplasmic retraction* and (3) *division by constriction.*

Centrifugal plate formation was described by Schaudinn (1902) for *Bacillus bütschli.* His description may be summarized as follows: The first evidence of division is the appearance of a highly refractive granule in what later becomes the plane of division. This bright granule, which is always in the long axis of the cell, gradually broadens into a disk perpendicular to the long axis of the cell and continues thus until it reaches the cell membrane. At the same time it grows in thickness. Then appears, in the middle of the plate, a "Spaltraum" which gradually spreads to the periphery and finally splits the membrane.

Centripetal plate formation was described by several investigators for a number of bacteria. According to Migula (1897), the cell of *Bacillus oxalaticus* divides as follows: The cell stretches. A cell-sap vacuole appears in the middle of the cell. The cytoplasm becomes a dense peripheral layer which arches inwards at the middle forming a ring. This ring continues its inward advance until it divides the vacuole into two. A certain time after the formation of the plasmatic disc, the membrane seems to arch inwards in the disc in a ring-like fashion, and one observes at both sides a strongly refracting, light ring which advances slowly toward the middle and finally forms a fine, light line in the middle of the plasmatic disc. This line is the young membrane which at the beginning stains with difficulty. Gradually, this membrane becomes thicker and more definite, and then a light constriction is seen at both sides which indicates the division of the rod. Guilliermond (1908), studied cell division in a number of spore forming aerobes including *Bacillus radicosus*, *B. mycoides*, *B. megatherium*, *B. subtilis*, *B. limosus*, *B. alvei*, and *B. asterosporus*. The process is the same in all of these organisms and is described as follows: At first, two stainable granules appear at opposite sides of the cell at the side of the membrane. These send slender extensions toward the center which fuse with one another forming a biconcave disc which separates the cell into two daughter cells. Then there is observed a thickening of the median part of the newly formed partition. Soon this partition splits into two bands by the formation, in its middle, of a hyaline zone. The division takes place often in an oblique plane. A similar description was given independently by Knaysi (1929 *b*, 1930) for *Bacillus subtilis* and *Proteus vulgaris*. More recently, Bisset (1939) described the cell division of *Bacillus mycoides* as follows: The first sign that division is about to take place is the sudden appearance of an extremely fine line across the organism. This is followed in a few minutes by the appearance of a slight curvature of the dividing line, producing a concavity on one side and a corresponding convexity on the other. Gradually, the concave portion becomes convex also, until a progressive indentation appears at the end of the dividing line, thus completing the division.

Division by plate formation has been observed also by a number of other investigators. However, the pictures given make it hard to classify the process under any of the above headings. In particular we like to mention the work of Ellis (1902–03, 1922, 1932) who did not go much into detail, but concluded that rod-like and spherical bacteria divide by plate formation which subsequently splits centripetally. He also considers the clear zone often seen between daughter cells as slime.

Division by cytoplasmic retraction was described by Knaysi (1929, *a* and *b*) for *Mycobacterium tuberculosis*: When the cell is ready to divide, a clear zone is formed by the drawing back of the cytoplasm into two dense, deeply staining masses, on either side of the clear zone, from which they become separated by membranes similar to that of the mother cell. Finally, the longitudinal membrane which is still continuous between the daughter cells withers away. This description is somewhat similar to that given more recently by Ellis (1932) for the sulphur spirilla, although he earlier (1903, 1922) held that helicoidal cells divided by constriction.

Division by constriction was claimed by Vahle (1909) for *Myxococcus ruber* and other bacteria, by Dobell (1911) for *Bacillus flexilis*, by Guilliermond (1908) and Swellengrebel (1909) for *Spirillum volutans*, and by Ellis (1902–03, 1922) for all helicoidal bacteria, although Ellis later (1932) seems to have abandoned that view.

The above review is sufficient to show how confusing the situation is, especially when different authors attribute different modes of division to the same organism. Such is the case of *Spirillum volutans* which divides by plate formation according to Migula (1904) and by constriction according to Guilliermond (1908), Swellengrebel (1909), and the earlier views of Ellis (1902–03, 1922). We have also the statement of Henrici (1934) that bacteria divide by constriction when young and by plate formation when old.

PRESENT INVESTIGATION

In the present investigation a study was made of the behavior, during cell division, of the cytoplasm and of the membranes

surrounding the protoplasm of bacteria. These membranes have been previously discussed by the author (see Knaysi, 1938). It has been pointed out that we have a modified outer layer of the cytoplasm, the *cytoplasmic membrane*, conspicuous in dark-field and readily stainable with dilute basic dyes, the *cell wall*, invisible in *dark-field* and in ordinary smears stained in the usual way with methylene blue, and the *slime-layer* which, like the cell-wall, is invisible in dark-field or in ordinary smears stained with methylene blue.

It is not generally realized that, when one examines smears of bacteria prepared in the usual manner, one sees merely the dried-up and shrunken cytoplasm with its cytoplasmic membrane. In figures 1 and 2 we have cells taken from the same, few-hour old culture of *Bacillus cereus*. Figure 1 represents the cells stained for one minute with Meyer's methylene blue, while in figure 2, the cells were especially treated to bring out the cell-wall. Figures 3 and 4 represent homologous preparations from a very young culture of *Schizosaccharomyces pombe*. It is evident that what we see in preparations stained with methylene blue corresponds to the dried-up cytoplasm and its cytoplasmic membrane. The form of the cell is obliterated and its size is greatly reduced.

Procedure. The author had previously observed and illustrated the behavior during cell division, of the cytoplasmic membrane as observed in bright field (see Knaysi, 1930). In the present investigation the behavior of the cytoplasmic membrane was studied by means of the dark-field, which has the advantage of a greater contrast and the elimination of interfering structures. The organisms were grown on a slide as described by the author (Knaysi, 1940), and serial photographs were taken at the proper time intervals with a Zeiss combination including a cardioid condenser, a special objective [60, N.A. 1.00 (X)] and a Contax camera.

To study the behavior of the cell-wall and slime-layer, a method had to be developed. It consists in preparing a smear from a young culture (usually a few hours old), fixing over the flame, mordanting for ten minutes with a mixture of tannic acid and potassium alum prepared by mixing 70 ml. of a saturated aqueous

solution of potassium alum with 30 ml. of a 20 per cent solution of tannic acid, and staining with a drop of Ziehl-Neelsen's carbol fuchsin under a cover glass. If the preparation is sealed with vaspar, a mixture of 50 per cent paraffine and 50 per cent vaseline, the preparation can be kept for considerable time. The mordant mixture should be renewed every two weeks. By this method, the cytoplasm appears dark red, the cytoplasmic membrane still darker, but the cell-wall stains blue and the slime-layer bright red. The photographs were taken with a Zeiss apochromatic objective, N.A. 1.40 and a Contax camera. Various Wratten filters were used alone or in pairs, especially filter E, although on account of the difference in color of the various structures, good results were obtained without light filters.

Organisms studied. The dark-field observations were made on a number of bacteria including *Bacillus cereus*, *Bacillus subtilis*, *Bacillus mesentericus*, *Bacillus megatherium*, *Bacillus aterrimus*, *Bacillus niger*, *Aerobacter aerogenes*, *Proteus vulgaris*, *Streptococcus fecalis*, *Mycobacterium tuberculosis* and the yeast, *Schizosaccharomyces pombe*. Bright field studies were made on *Bacillus cereus*, *Aerobacter aerogenes*, *Proteus vulgaris*, *Streptococcus fecalis* and *Schizosaccharomyces pombe*.

OBSERVATIONS

The dark-field picture. (a) *Schizosaccharomyces pombe:* Pictures 5–8 are serial photographs of growing yeast. Pictures 5 and 6 are consecutive and so are 7 and 8, the time interval in each case being 30 minutes. Observations were also made within that interval in order to be sure that nothing was overlooked. It is clear from these pictures that there are no dark-field indications of the formation of a membrane across the yeast cell. One is not aware of the division until it is completed.

(b) *The bacteria:* Pictures 9–19 were taken serially of growing *Bacillus cereus* at time intervals of 20 minutes each. All other bacteria investigated gave similar pictures. If the principal, longitudinal optical section of the cell be considered, the division here is indicated by a thinning of the cytoplasmic membrane at the place where the division is to take place, accompanied by

what seems to be a thickening of a contiguous point of the membrane (cell *a*, pictures 16 and 17). This is followed by a sharp break at the thinned part (picture 18, cell *a*), and a subsequent partial or complete separation of the cytoplasms of the sister cells (picture 19*a*). This separation is accomplished centripetally and, since the cytoplasm and its surrounding membrane are the only structures usually seen, this is probably responsible for the general belief that bacteria divide by fission.

The bright-field picture. (a) *Schizosaccharomyces pombe:* The first step, as seen in bright-field, is illustrated in figure 35, cell *a*. We observe there a light, faintly stained line across the middle of the cell. This, of course, represents a disk poor in stainable material. It can be seen that the outline of the cytoplasm still bounds that light line laterally indicating that the continuity of the cytoplasm is not yet fully broken. The line probably corresponds to the migration of chromatic material from that part of the cell. The next step is shown in figure 21, cell *b* and figure 22, cell *a*, where we observe the beginning of deposition of cell-wall material. Two cell walls are deposited, one on each side of the disc. It can be seen from the above figure that the boundary of the cytoplasm can still be discerned even while cell-wall material is being deposited. In later stages (figure 21, cell *a*) it can be seen that the cytoplasm of the dividing cell has been completely severed in the median part of the cell, and the cross-walls continuously reach from one side of the cell to the other. Finally, the portion of the cell-wall that binds the two daughter cells gradually gets thinner and may finally disintegrate, liberating the two cells; or, as happens most often in the case of the present organism, that portion of the wall can no more withstand the pressure of growth and splits on one side, resulting in a rotary movement of the daughter-cells which now lie at an angle to each other (figs. 5 and 6, 7 and 8).

(b) *The bacteria:* Pictures 23 and 24 represent cells of *Bacillus cereus*, and pictures 25 to 34 represent cells of *Streptococcus fecalis*. In picture 23, it is clear that cells *a* and *b* already have separate cytoplasms, although no deposition of cell-wall material between the two cells is yet noticeable. The same is true of

picture 24. Picture 23, *e* and *d* shows an intermediate stage. Sometimes the cytoplasm is still attached at a small area at the center of the cell (pictures 24, and 34, *a* and *b*, *c* and *d*), forming the so-called plasmodesma. However, in no case does the cytoplasmic membrane remain intact. It is broken long before any evidence of the deposition of cell-wall material is seen. As in the case of yeasts, here also two cell-walls are deposited (picture 23, *b* and *c*, *d* and *e*, and many examples in pictures 25 to 34). Finally it may be seen that the portion of the mother-cell wall corresponding to the place of division becomes less and less stainable and looks more and more reddish, instead of blue, until it withers away liberating the two cells. It also often happens that the pressure of growth hastens the separation of daughter-cells.

DISCUSSION

We have given in the preceding paragraphs a faithful, although incomplete description of the behavior of the various structures involved in the cell division of yeasts and bacteria, and we believe have demonstrated a fundamental difference in the process between the two groups of microorganisms. In the yeasts, the cell-wall material is deposited at both bases of a cylindrical portion, in the middle of the cell, from which chromatic material disappeared. The relatively thin cytoplasmic membrane of the yeasts remains continuous for a time while such material is being deposited. In bacteria, the cytoplasmic membrane breaks long before evidences of cell-wall deposition are seen. This difference is most impressive if the process is observed in dark-field. In the bacteria one observes a gradual, centripetal break up of the cytoplasm, beginning with a sharp break in the cytoplasmic membrane. The division of cell *a* of pictures 10 to 19 was completed only in picture 19, but the place of division was indicated as early as picture 15, when the process must have really started. With the yeasts, no such gradual process is observed. The cytoplasmic membrane remains intact throughout most of the process, and, during that time, optical continuity is maintained in the median part of the cell. We have, therefore, no dark-field evidences of the coming division until it is completed.

Judging from this and from our observations in bright field, we come to the conclusion that, in yeasts, cell-wall material is initially deposited in the midst of the cytoplasm or around a milieu of the same optical density as the cytoplasm, while in bacteria, the contiguous cytoplasmic surfaces of the sister cells become optically distinct before any evidence of cell-wall deposition is seen.

We wish also to state that in no case were we able to observe a division by simple fission unless the mode of separation of the cytoplasm of bacteria be so-called. Not having studied any representative of helicoidal bacteria, we are unable to confirm or contest the results of Ellis and others. One often finds a slight constriction of the cell-wall at the place of division, but this may be due to osmotic effects, or to drying, or to a partial collapse due to weakness or to lack of adequate support. We observed slight constriction in some fungi. However, this minor constriction can be magnified by the wrong focusing such as we have in picture 33, cells *a* and *b*. Reports of constriction in rods and cocci may have been partially due to that cause, but they probably are due in a large part to inability to see the cell-wall, and to incomplete observation of the behavior of the cytoplasm and its membrane. Picture 20 corresponds to this case. It was photographed with filters A and D and overexposed, while the positive was underexposed. The result is that some of the structures involved are not visible and the cells of *Schizosaccharomyces pombe* appear to divide by simple constriction. When photographed and printed properly, the cells of figure 20 present the same picture as those of figures 21, 22 and 35.

We may add that in no case did we observe formation of a single wall with subsequent splitting.

The observations of Guilliermond (1908) and of the author (Knaysi, 1929*b*, 1930) are partially correct and apply only to the cytoplasmic membrane. The mode of division of *Mycobacterium tuberculosis* as described by the author (Knaysi, 1929, *a* and *b*) is in general agreement with the findings of the present research. Further bright-field investigations of other bacteria by this and other methods are necessary to ascertain whether the formation

of a vacuole, as claimed by Migula (1897) takes place in certain organisms.

SUMMARY

The process of cell division of the yeast *Schizosaccharomyces pombe* and of a number of common bacteria was investigated in dark and bright fields. In the latter case a new way of making the cell-wall visible is given. Our observations indicate a fundamental difference between cell-division of yeasts and bacteria. In yeasts, there are no dark-field indications of cell division until it is completed. In bacteria, a break in the cytoplasmic membrane presages cell division. Bright-field observations show that the deposition of cell-wall material precedes the break-up of the cytoplasm in yeasts and follows it in bacteria. In both groups two walls are deposited from the beginning. In the organisms studied, we found no indication of the formation of a single wall with subsequent splitting, nor of division by simple constriction unless this is applied to the cytoplasm alone.

We are still uncertain whether the slime-layer plays any rôle in cell division.

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PLATE 1

The numbers are those of the pictures

1. Young cells of *B. cereus* stained in the usual way with Meyer's methylene blue for 1 minute.

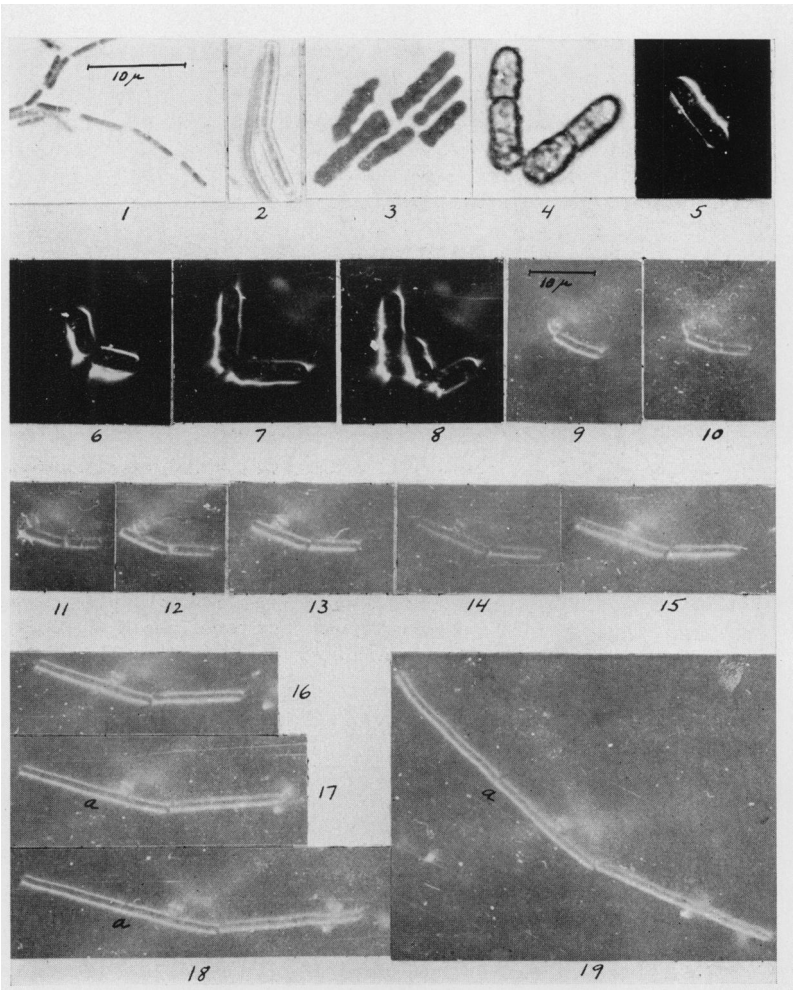
2. Young cells of *B. cereus* stained by the tannin-alum-fuchsin method.

3. Young cells of *Schizosaccharomyces pombe* stained as in 1.

4. Young cells of *Schizosaccharomyces pombe* stained as in 2.

5–8. Dark-field photographs of growing cells of *Schizosaccharomyces pombe*. 5 and 6 are consecutive at 30 minute intervals. The same is true of 7 and 8.

9–19. Serial photographs of growing cells of *B. cereus* taken at 20 minute intervals.



(Georges Knaysi: Cell Division of some Yeasts and Bacteria)

PLATE 2

20. Young cells of *Schizosaccharomyces pombe* stained as in 2, but not photographed properly.

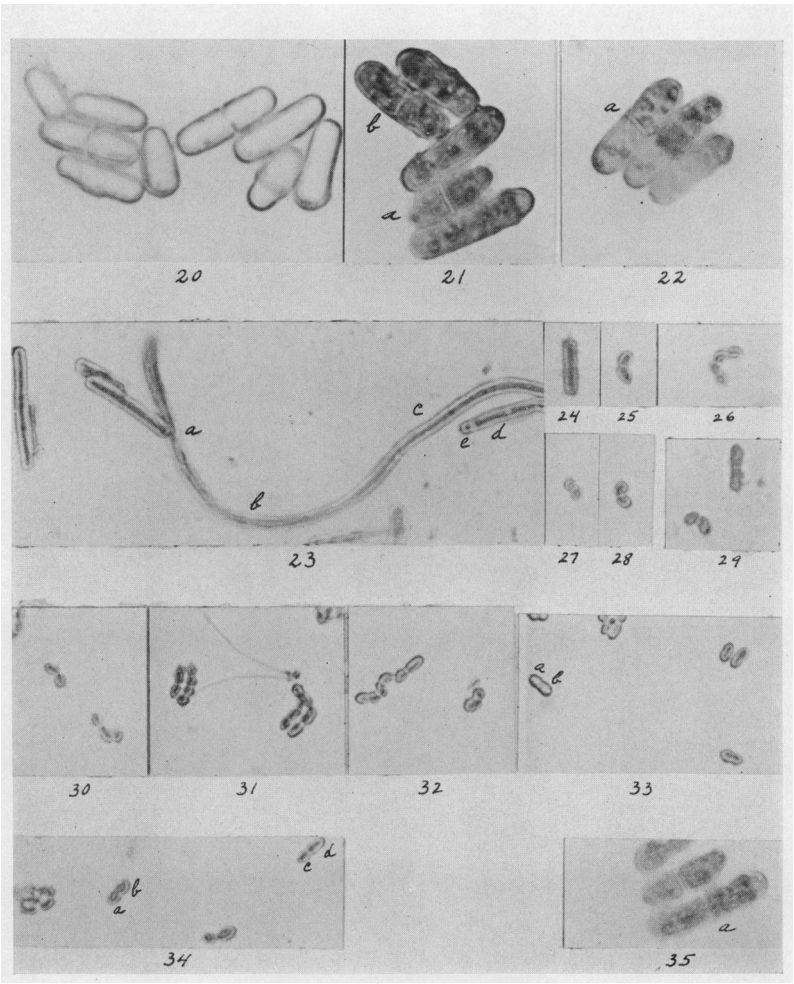
21 and 22. Young cells of *Schizosaccharomyces pombe* stained as in 2 and properly photographed. Various stages of division can be discerned.

23 and 24. Young cells of *B. cereus* stained as in 2.

25–34. Young cells of *Streptococcus fecalis* stained as in 2, and showing various stages in cell division.

35. Young cells of *Schizosaccharomyces pombe* stained as in 2. Cell *a* shows the earliest stage in division.

Scales of magnifications are given for the bright-field pictures on figure 1, and for the dark-field pictures on figure 9.



(Georges Knaysi: Cell Division of some Yeasts and Bacteria)