thesis in asynchronous culture of *Saccharomyces cerevisiae* exposed to F_3CAzU indicates its selective inhibitory effect on cell division (fig. 1). Figure 2a represents an experiment in which F_3CAzU was added to synchronously dividing cells at various times between the first and second synchronized division. According to results obtained, F_3CAzU is effective until 15 min before the onset of the second synchronized division. In responsive cells the inhibitory effect of F_3CAzU is completely reversed by thymine, thymidine or uracil if they are added before, simultaneously with or after F_3CAzU (fig. 2b). Microscopic observation of the cells exposed to F_3CAzU disclosed no inhibition of elongating cell growth or the segregation of nuclear material; however, cell division is blocked and filament (pseudomycelium) formation occurs (fig. 3 and 4). In summary, F_3CAzU was found to be a fungistatic agent which affects the division cycle of yeast cell as one would expect of a specific inhibitor of cell division. In other words, a direct interaction of F_3CAzU with a cell component or structure results in immediate inhibition of a certain biochemical event essential for cell division. Our results with synchronized cultures suggest that this biochemical event coincides probably with a late event in the cell division cycle which is intimately involved in cytokinesis or cell separation. The capacity of pyrimidine bases to reverse the effect of F_3CAzU suggests their effect at the uptake level or competitive displacement of the inhibitor from an active participation in the processes mentioned; however, in relation to the fact that *Saccharomyces cerevisiae* cells are unable to use exogenous pyrimidines[10] a more detailed study of this phenomenon is necessary. In this context, F_3CAzU may have useful applications in studies of the division process of single-cell eukaryotes.

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Bacterial endosymbionts 'theta' of the heterotrich ciliate *Climacostomum virens*[1]

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Summary. Light microscopy using several fluorescent stains, and electron microscopy, reveal that the ciliate *Climacostomum virens* contains numerous bacteria in its cytoplasm. Their number depends on whether or not the host also harbors *Zoochlorella*. The bacteria, called theta, resemble ordinary gram-negative bacteria in their ultrastructure. They are 1.5–5 µm long and 0.4 µm wide, contain nucleoids, and are not enclosed in a vacuole.
Key words. Bacterial endosymbiont; ciliate; *Climacostomum virens;* heterotrich.

As suggested by Roux[2] it was well established at the beginning of the century that intracellular symbionts could develop in protozoa. The first symbionts which were described were the 'bright green chlorophyll bodies'[3,4], later recognized as the green algae *Zoochlorella* sp. Not until nearly a century later did workers discover bacteria-like symbionts in the protozoan *Paramecium*[5]. Only a few host species or genera have now been studied comprehensively (for recent reviews see Soldo[6], Preer[7] on *Paramecium*, Heckmann[8] on *Euplotes* and Jeon[9] on *Amoeba*). Some of these bacteria are endonuclear and are situated either in the micro- or the macronucleus[10]. All others live in the cytoplasm. The present article describes bacteria in the cytoplasm of the heterotrich ciliate *Climacostomum virens*. Successively Peshkowskaya[11], Repak[12], Fischer-Defoy[13], Peck[14] and Hufschmid[15,16] have shown the presence of endosymbiotic algae. They can be called *Zoochlorellae*. It was Peck et al.[14] who first identified the rods as bacteria. Here a short description of these theta bacteria studied by light and electron microscopy is given, and they are compared to the omikron bacteria of the hypotrichous ciliate *Euplotes*[8], and to the different bacteria of the *Paramecium* group[6,7].

Materials and methods. Culture methods have been described elsewhere[16]. We have made our observations on stocks from Geneva with and without the endosymbiotic algae *Zoochlorella* (G 2, G 2cf)[15] and on stocks from Tübingen with and without micronuclei (T and T amcf). The latter were kindly provided by Dr D. Ammermann, University of Tübingen. For light microscopic observations we used the following different staining procedures to visualize the bacteria: the fluorochrome PIC (N,N'-diethylpseudoisocyanin chloride)[17] and the fluorochrome BAO (Bis-(4-amino phenyl)-1,3,4,-oxadiazol))[18] modified by a hydrolysis step of 1 h in 5 N-HCl at room temperature. We also used DAPI (4'-6-diamidino-2-phenyl-indole)[19] at a final concentration of 5 µg/ml, ethidium bromide at a final concentation of 0.5–10 µg/ml and Hoechst 33258[20] at a final concentration of 5 µg/ml. With the latter three fluorochromes cells were incubated after fixation and before staining with a solution containing 0.5–1 mg/ml of RNase (free of DNase) for 1 h at 37 °C. For electron microscopy cells were prepared as described by Pelvat[21]. For antibiotic treatment cells were incubated successively with tetracycline (Achromycin: Lederle Laboratories Division Pear River N.Y., 50 µg/ml), with Penicillin (200 units/ml) and Streptomycin (200 µg/ml) and transferred into antibiotic-free medium.

Results. We decided to call the bacterial rods living in the cytoplasm of *C. virens* by the greek letter theta, as is customary for cytoplasmic hereditary units and symbionts of unclear taxonomic affiliation[5]. All stocks examined by DAPI staining harbor these bacteria, including a stock isolated years ago from Geneva (G 2), a recently isolated stock from Geneva (G 10), a stock without *Zoochlorella* (G 2cf) and an amicronucleate stock from Tübingen (T amcf). From BAO or DAPI stained

Figure 1. *C. virens* stock G 10 stained with BAO. Whole cell containing numerous *Zoochlorella* symbionts (C) and very few theta bacteria, Bt = buccal tube, Ma = macronucleus, Mi = micronuclei. Fluorescence light microscopy. × 150. Figure 2. Magnification of a part of figure 1 showing very few theta bacteria (arrow) in the cytoplasm of *C. virens*. × 320. Figure 3. *C. virens* stock G 2cf (*Chlorella*-free) stained with BAO. Detail of the cytoplasm containing numerous theta bacterial rods (arrows). Dv = digestive vacuoles, Ma = macronucleus. Fluorescence light microscopy. × 300. Figure 4. *C. virens* stock G 2cf (*Chlorella*-free) stained with PIC reveals the DNA specific yellow fluorescence of the nucleoids in the theta bacteria (arrows). Mi = micronuclei. Fluorescence light microscopy. × 240. Figure 5. Electron micrograph of a longitudinal section of a theta particule from stock G 2cf (*Chlorella*-free) showing an ultrastructure which resembles that of gram-negative bacteria. In the cytoplasm of theta there are 5–6 lightly stained chromatin regions called the nucleoids (N). × 30,000. Figure 6. Detail of a longitudinal section of a theta bacterium from stock G 2cf (*Chlorella*-free). Note that no vacuole or vesicle surrounds the bacterium but a space largely free of granules and in some regions the cytoplasm is in contact with the bacterial outer membrane (OM), and in some regions an inner membrane (IM) is observed. R = ribosomes, N = nucleoids. × 60,000.

preparations we estimate the number of these bacteria to be from a few to several hundred for stocks with symbiotic algae and at least 1000 per cell for stocks free of *Zoochlorella* (figs 1, 2, 3). The DAPI, ethidium bromide and Hoechst 33258 stained preparations confirm the presence of the bacteria, but these fluorochromes give pictures which are more difficult to interpret. The theta bacteria are randomly distributed throughout the cytoplasm, in contrast to *Zoochlorellae,* which are localized predominantly in the cytoplasm near the cortex of the cell (fig. 1). The theta bacteria have a length of 1.5–5 μm, with a mean of 2.5 μm, and a width of 0.4 μm[22]. When stained by the fluorochrome PIC, each bacterial rod shows aligned bright yellow, fluorescent dots (fig. 4). These dots represent the nucleoids as revealed also by electron microscopy (figs 5, 6). The ultrastructure of the theta particles resembles that of gram-negative bacteria with an outer thin cell wall and an inner membrane visible only in some regions (fig. 6). The bacteria are not enclosed in a vacuole or vesicle, but we can generally see a space around the bacteria free of granules and ribosomes (figs 5, 6). The cytoplasm of the bacteria contains five to six clear, chromatin-containing regions called the nucleoids (figs 5, 6). Preliminary experiments to obtain viable *C. virens* free of bacteria by treatment with antibiotics have failed so far. After the antibiotic treatment cells transferred to fresh medium undergo one or two divisions, then stop multiplying, become smaller, do not feed and die after a week or so.

Discussion. The presence of the symbiotic bacteria theta forces us to correct the designation of our stocks: the term endosymbiont-free (ef, Hufschmid[15,16]) must be replaced by chlorella-free (cf). We choose to keep the name *Chlorella,* partly because of the morphology of these cells, but mostly by analogy with the terms employed for the endosymbionts in *Euplotes*[8] and *P. bursaria*[23] even though no clear proof of identification as *Chlorella* was presented. The theta bacteria of *C. virens* can be compared to the symbiontic bacteria lambda of *Paramecium*[6,7] and to the omikron of *Euplotes*[8]. Like lambda and omikron the theta are rod-shaped, gram-negative and live in the cytoplasm. Their number is about the same as for lambda and omikron, and as in *Euplotes*[8] their number diminishes if their host is invaded by *Zoochlorella.* This could mean that the algae may, to some extent only, replace the bacteria.

Euplotes and *C. virens* can grow without algae but not without bacteria. The theta are shorter than omikron (5 μm[8]) and lambda (3.2 μm[6,7]). They are not toxic for their host as is commonly the case for kappa symbionts of *P. aurelia*[6,7]. The bacteria are not surrounded by a vacuole membrane like lambda[6,7] or by a vesicle like omikron[8], but instead, there is a space free of cytoplasmic granules and ribosomes around theta. This empty space around the bacteria may be an artifact. On the other hand the space and the presence of a region where the host cytoplasm is in direct contact with the bacterial cell wall, may be also due to interactions between the host cell and the bacteria. Theta contain nucleoids like omikron, but no electron-dense area[8]. The presence of many nucleoids, assuming each one represents a genome unit, indicates a certain degree of ploidy. Numerous nucleoids seem to be a particularity of these ciliate endosymbiotic bacteria[8]. It is noticeable that these bacteria are present in all shocks which, moreover, have different origins (Geneva or Tübingen) and are affected, when treated with antibiotics, in exactly the same way as omikron-bearing *Euplotes*[8]. Heckmann[8] observed that antibiotic treatment killed the omikon. The similarities with omikron allow us to postulate that the theta bacteria are, like omikron, essential for their host.

We have no idea at present of what essential nutrient the bacteria provide for *C. virens.* It has been established that the lambda particles provide the folic acid necessary for survival of their host *Paramecium*[24].

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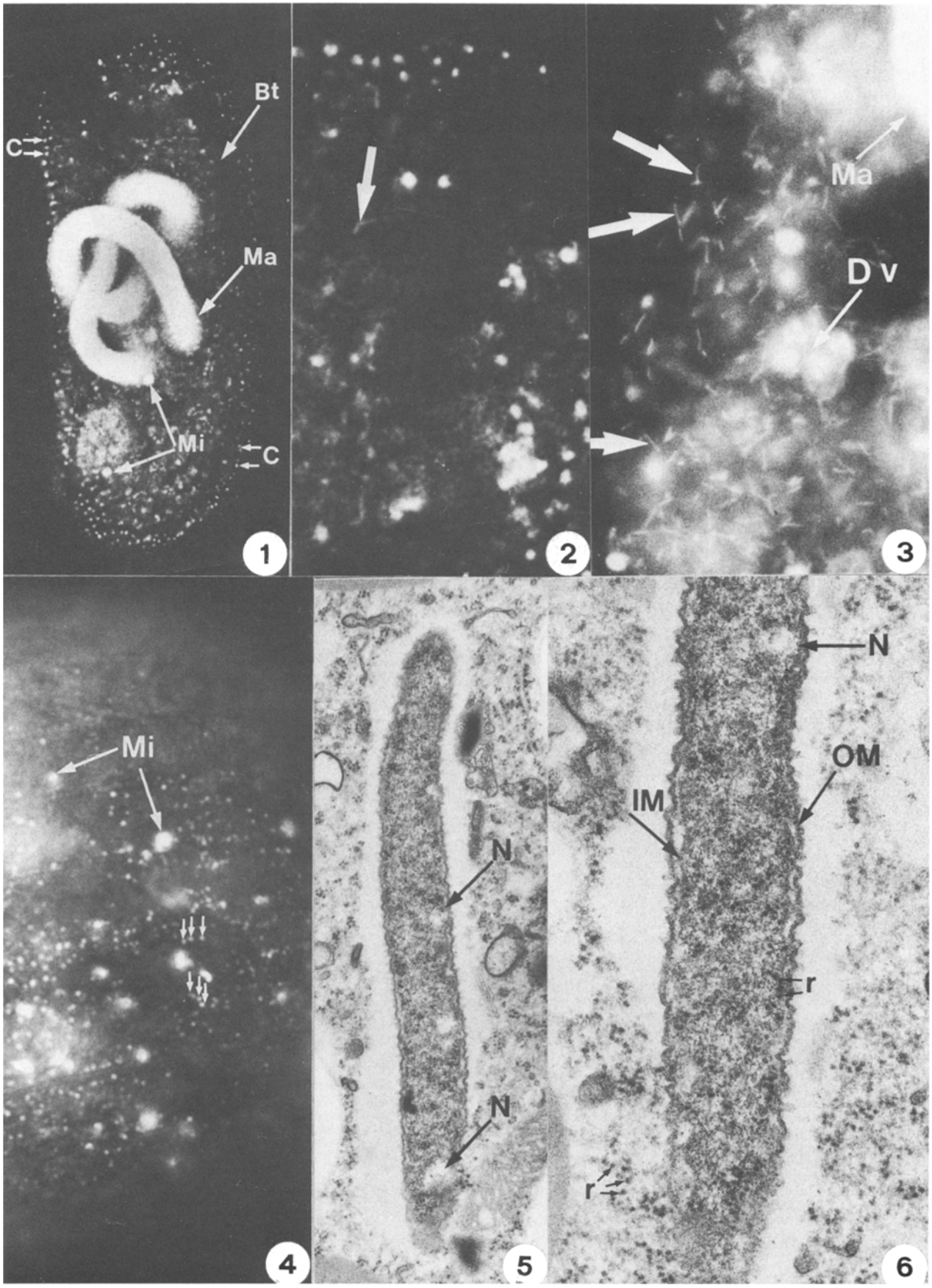
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