1 We thank Drs E.A. Bernays, P. Feeny, J.B. Harborne, J.L. Nation and L.M. Schoonhoven for improvements of the manuscript, and the suppliers of pure compounds[16] for their generous gifts.
2 Chapman, R.F., Bull. ent. Res. *64* (1974) 339; Rosenthal, G.A., and Janzen, D.H., eds, Herbivores, their interaction with secondary metabolites. Academic Press, New York 1979.
3 Swain, T., A. Rev. Pl. Physiol. *28* (1977) 479; Harborne, J.B., Introduction to Ecological Biochemistry. Academic Press, London 1982.
4 Finch, S., Appl. Biol. *5* (1980) 67; Schoonhoven, L.M., in: Semiochemicals: Their Role in Pest Control, p.31. Eds D.A. Nordlund, R.L. Jones and W.J. Lewis. John Wiley, New York 1981.
5 Städler, E., in: Animal and Environmental Fitness, p.223. Ed. R. Gilles. Pergamon Press, London 1980.
6 Jermy, T., Butt, B.A., McDonough, L., Dreyer, D.L., and Rose, A.F., Insect Sci. appl. *1* (1981) 237.
7 Bohlen, E., Z. angew. Ent. *59* (1967) 325; Städler, E., Z. angew. Ent. *69* (1971) 425; *70* (1972) 29.
8 Städler, E., Coll. Int. CNRS Comportement des insectes et milieu trophique *265* (1977) 357.
9 Martin, J.T., and Juniper, B.E., The Cuticle of Plants. Edward Arnold, Edinburgh 1970.
10 The 1st column (220 × 20 mm, silicagel 70–230 mesh, Merck) was eluted successively with 200-ml portions of 0, 3, 8, 20% ET_2O in pentane, 100% Et_2O, 20% MeOH in Et_2O and 100% MeOH. The 2nd column (75 × 10 mm) was eluted successively with 15-ml portions of 0, 10, 20, 40% Et_2O in pentane, 100% Et_2O, 100% Et_2O and MeOH.
11 Gas chromatography-mass spectrometry (GC-MS): 25 m SE 54 glass capillary column, 0.31 mm; column temperature 80°C (2 min), 20°C/min to 180°C, 5°C/min to 280°C, vaporizer temp. 240°C, interface temp. 220°C; Finnigan 4000 quadrupole MS operating in electron-impact mode (EI, 70 eV, 240°C) or chemical ionization mode (CI, iso-butane, 0.35 Torr, 180°C), cyclic scanning m/z 35–435 (EI), m/z 85–485 (CI).
12 MS and GC data for the isolated and identified compounds. Most important ions observed are listed from EI data, for compound **6** also from CI data; base peak in italic type: **1**, m/z *178* (M^+), 163, 147, 135, 115, 107, elution temp. 178°C; **2**, m/z *208* (M^+), 193, 165, 163, 150, 147, elution temp. 188°C; **3**, m/z *244* (M^+), 229, 213, 211, 201, 189, elution temp. 216°C; **4**, m/z *216* (M^+), 201, 188, 173, 145, elution temp. 211.5°C; **5**, m/z *216* (M^+), 201, 188, 173, 145, elution temp. 210°C; **6**, m/z (260) (M^+), 242, 171, 157, *129*, 128, 115; CI, m/z 261, 243, 187, *155*, elution temp. 220°C.
13 Berüter, J., and Städler, E., Z. Naturf. *26b* (1971) 339.
14 Guerin, P., Städler, E., and Buser, H.R., J. chem. Ecol. *9* (1983) 843.
15 High-performance liquid chromatography (HPLC): 250 × 9 mm, silica gel SI 60, 5 µm, Merck; 80% pentane – 20% Et_2O, 1% gradient 2.5 ml/min; significant biological activity was found in fractions 6, 7 and 8.
16 **1** and **2** K&K ICN Pharmaceutical Inc., Plainview, N.Y. (USA); **3** E. Lemmich, Danmarks Pharmaceutiske Højskole, Kobenhavn; **4** G. Caporale, Università di Padova, Italy; W. Ivie, College Station, TX (USA); **5** W. Ivie, College Station, TX (USA); Carl Roth, Karlsruhe (FRG); **6** B. Garrod, University of East Anglia, Norwich, England. E. Lemmich, Danmarks Farmaceutiske Højskole, Kobenhavn, K. Munakata, Nagoya University, Japan.
17 Bohlmann, F., Fortschr. Chem. org. NatStoffe *25* (1967) 1.
18 Heywood, V.K., ed., The Biology and Chemistry of the Umbelliferae. Academic Press, London 1971; Hegnauer, R., Chemotaxonomie der Pflanzen. Birkhäuser, Basel 1973.
19 Murray, R.D.H., Méndez, J., and Brown, S.A., The natural Coumarins. John Wiley, Chichester 1982; Kagan, J., and Chan, G., Experientia *39* (1983) 402.
20 Saxena, B.P., Koul, O., Tikku, K., and Atal, C.K., Nature *270* (1977) 512.
21 Berenbaum, M., Ecol. Ent. *6* (1981) 345.
22 Garrod, B., Lewis, B.G., and Coxon, D.T., Physiol. Pl. Path. *13* (1978) 241; Garrod, B., and Lewis, B.G., Trans. Br. mycol. Soc. *75* (1980) 166; Kemp, M.S., Phytochemistry *17* (1978) 1002; de Wit, P.J.G.M., and Kodde, E., Physiol. Pl. Path. *18* (1981) 143.
23 Crosby, D.G., and Aharonson, N., Tetrahedron *23* (1972) 465.
24 Munakata, K., in: Natural Products for Innovative Pest Management, p.299. Eds D.L. Whitehead and W.S. Bowers. Pergamon Press, London 1983.
25 Bernays, E.A., in: Proc. 5th int. Symp. Insect-Plant Relationships, p.3. Eds J.H. Visser and A.K. Minks. Pudoc Wageningen 1982.
26 Fraenkel, G., Ent. exp. appl. *12* (1969) 473; Feeny, P., Rec. Adv. Phytochem. *10* (1976) 1.
27 Prokopy, R.J., and Owens, E.D., A. Rev. ent. *28* (1983) 337.

0014-4754/84/101157-03$1.50 + 0.20/0

Inhibition of *Saccharomyces cerevisiae* division by 5-trifluoro-methyl-6-azauracil

V. Jirků, D. Petříková and J. Škoda

Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology, 166 28 Prague 6, and Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6 (Czechoslovakia), 22 February 1982

Summary. Cell division, as studied in asynchronous cultures of yeast cells, is sensitive to 5-trifluoromethyl-6-azauracil (F_3CAzU). Under defined conditions (10 mmoles l^{-1} F_3CAzU) this compound blocks immediately and completely the process of cell division. Using synchronized cells, the time-point at which division process of yeast cell can be inhibited by F_3CAzU has been determined. The inhibitory effect of this compound is completely reversed by thymine, thymidine and uracil.
Key words: Saccharomyces cerevisiae; 5-trifluoromethyl-6-azauracil; yeast cell cultures; cell division, inhibition of.

5-Trifluoromethyl-6-azauracil, prepared by Mertes et al.[1,2], Shen et al.[3], and Dipple and Heidelberger[4], was shown[2] to cause 50% inhibition of thymidylate synthetase *(Escherichia coli)* at a concentration of 4 mmoles l^{-1}. However, the in vitro studies carried out with other enzyme preparations[2] as well as in vivo tests in the case of transformed eukaryotic cells[1,2,4] revealed no inhibitory activity or significant cytotoxicity of this compound. In connection with our study of 5-substituted 6-azauracil derivatives as potential fungicides[5] we investigated the effect of F_3CAzU in asynchronous or physiologically synchronized cultures of yeast cells as well as the antagonistic relationship between this compound and preformed pyrimidines.

Material and methods. F_3CAzU was kindly provided by Professor M.P. Mertes of the School of Pharmacy, University of Kansas, Lawrence, Kansas, USA. Thymine, thymidine and uracil were obtained from Calbiochem and hydroxyurea from Serva; lomofungin was kindly provided by Dr. G.B. Whitfield, Upjohn Co., USA. *Saccharomyces cerevisiae* U 92 was from the culture collection of the Prague Institute of Chemical Technology. Difco yeast nitrogen base (B 391) with the addition of 1% glucose was used exclusively as the cultivation medium. The above medium was solidified where necessary with 2% Oxoid agar No.3. Cultivation in liquid medium was carried out under intensive aeration in 10 ml vols at 28°C. The medium was inoculated to a concentration corresponding to an

OD of 0.05 and the resulting culture was taken to be exponentially growing when attaining OD 0.2. The total cell number was determined using a Bürker chamber. Formaldehyde (0.4%) was included in the diluent. Viable counts were made by the plate technique. Dilutions were made in the starvation medium. Synchronized cultures were obtained by a method which employs temporary inhibition of DNA synthesis by hydroxyurea[6]. DNA content was determined by a modified diphenylamine method[7]. Protein was determined by the method of Lowry et al.[8]. The nuclear material was stained by the lomofungin procedure[9]. Specimens for scanning electron microscopy were coated with gold and observed on a JEOL SEM. Observations by phase-contrast microscopy were made with a Zeiss (Jena) NU 2 microscope.

Results and discussion. The dissociation between the total cell count or viable count and the rates of DNA and protein syn-

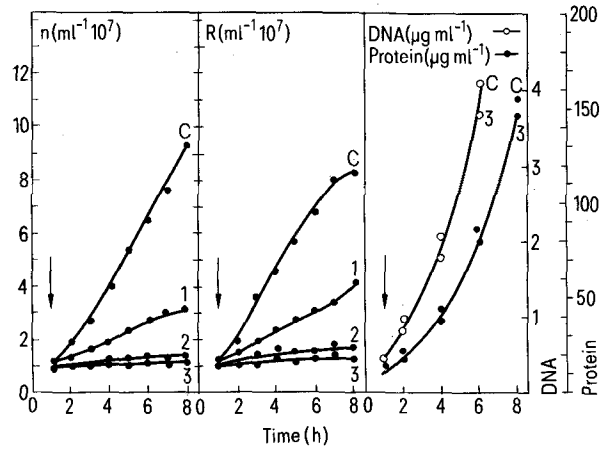

Figure 1. Growth and cell division inhibition by F_3CAzU. The medium was supplemented (↓) with F_3CAzU to final concentration of 0.5 mmoles l^{-1} (1), 1 mmoles l^{-1} (2) and 10 mmoles l^{-1} (3). C, control growth without F_3CAzU; n, total cell count; R, colony-forming ability.

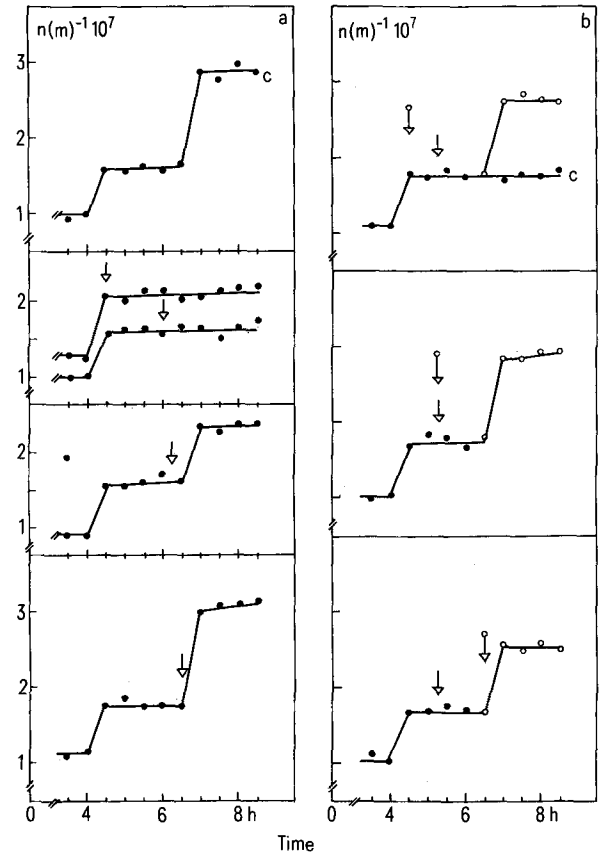

Figure 2. *a* Effect of F_3CAzU in synchronized cultures. F_3CAzU was added (↓) to a final concentration of 1 mmole l^{-1}. C, control synchronized culture without F_3CAzU; n, total cell count; *b* Effect of thymine in reversing synchronized division inhibited by F_3CAzU. A synchronized culture was supplemented (↓) with F_3CAzU to a final concentration of 1 mmole l^{-1}. Thymine was added (↓) to the same final concentration. C, control synchronized culture supplemented (↓) with F_3CAzU; n, total cell count. Because of the identical results the reversion by thymidine or uracil is not presented.

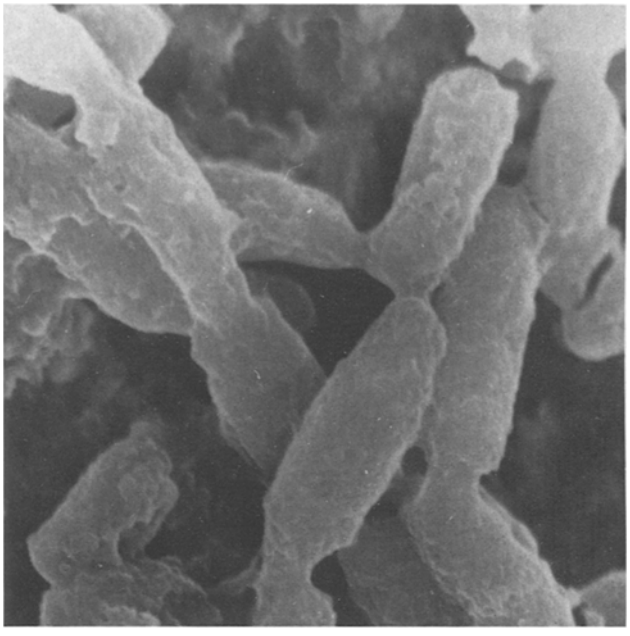

Figure 3. Scanning electron micrograph of *Saccharomyces cerevisiae* cells grown for 4 h in medium supplemented with F_3CAzU to final concentration of 1 mmole l^{-1}. × 3000.

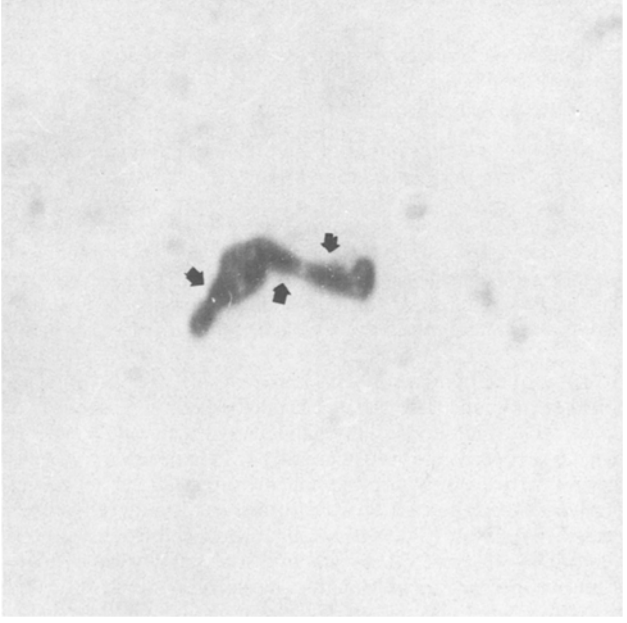

Figure 4. Detection of nuclear material in included filamentous form of *Saccharomyces cerevisiae*. Growth conditions were as described for figure 3. × 1500.

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.

1 Mertes, M.P., and Saheb, S.E., J. heterocycl. Chem. *2* (1965) 491.
2 Mertes, M.P., Saheb, S.E., and Miller, D., J. med. Chem. *9* (1966) 876.
3 Shen, T.Y., Ruyle, W.V., and Bugianesi, R.L., J. heterocycl. Chem. *2* (1965) 495.
4 Dipple, A., and Heidelberger, C., J. med. Chem. *9* (1966) 715.
5 Jirků, V., Vorlová, V., and Škoda, J., Experientia *37* (1981) 36.
6 Jirků, V., Hašek, J. and Páca, J., Sci. Papers Prague Inst. chem. Technol. E *51* (1981) 123.
7 Giles, K.W., and Myers, A., Nature *206* (1965) 93.
8 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
9 Kopecká, M., and Gabriel, M., Archs Microbiol. *119* (1978) 305.
10 Grenson, M., Eur. J. Biochem. *11* (1969) 249.

0014-4754/84/101159-03$1.50 + 0.20/0

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

J. D. Hufschmid

Laboratory of Protistology, Department of Animal Biology, University of Geneva, CH-1211 Geneva 4 (Switzerland),
21 December 1983

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