

cup plate assay and bioautography as described elsewhere⁸. The results are given in Table 1.

The *E. coli* activity obtained separated upon bioautography into four spots. One of them could be identified as cyanocobalamin and another one as factor B whereas the remaining two were slow moving spots similar to the spots corresponding to factors Z⁹ or GDP-factor B^{8,10}. Approximately 20–30 % of the activity was due to cyanocobalamin. The addition of DMB increased the cyanocobalamin content to about 50 % of the total *E. coli* activity but had a markedly inhibiting effect upon the total activity (*cf.* Table 1). The addition of corn steep liquor seemed to decrease somewhat the *E. coli* activity formed but had no effect on the distribution of this activity between the different factors.

The fermentation liquors contained, in addition, a factor with a growth inhibiting activity for *L. leichmannii* 313. This factor had a mobility similar to that of factor B upon electrophoresis at pH 2.5 (2 M HAc). The presence of similar inhibitors in cultures of vitamin B₁₂-producing organisms has been reported earlier from this laboratory and by other authors^{11,12}.

A second series of experiments was performed in 24 test tubes, each containing 20 ml medium. Two of the tubes contained the complete semi-synthetic medium while the remaining 22 tubes contained instead either different fractions of fresh and treated sewage or milk whey, yeast autolyzate or supernatant from cultures of a blue-green alga (*Anabaena*). Each of these substrates was used both without any further supplementation and also supplemented with the constituents of the synthetic medium apart from the casein hydrolyzates. The pH of all media was adjusted to 6.6 and their *E. coli* activity determined. No re-adjustments of the glucose content or pH were made and the fermentations were interrupted after 5 days. The results can be seen in Table 2.

It seems from Table 2 that the sewage fraction 4 supplemented as described above may provide a good substitute for casein hydrolyzates in the semi-synthetic medium. While, under the conditions described, a five days fermentation in the semi-synthetic medium gave only 0.5 μg *E. coli* activity per ml, a corresponding fermentation using the sewage fraction 4 gave 2.4 $\mu\text{g}/\text{ml}$. Bioautographic determinations did not reveal any significant differences in the distribution of the *E. coli* activity between different factors. It seems also that the semi-continuous method and repeated adjustments of pH and glucose content are of great importance for the yield of *E. coli* activity (*cf.* Tables 1 and 2).

Further experiments devoted to these problems are in progress and the results will be reported within the near future.

1. Leviton, A. and Hargrove, R. E. *Ind. Eng. Chem.* **44** (1952) 2651.
2. Janicki, J. and Pawelkiewicz, J. *Acta Biochim. Polon.* **1** (1954) 307.
3. Pawelkiewicz, J. *Acta Biochim. Polon.* **1** (1954) 313.
4. Janicki, J. and Pawelkiewicz, J. *Acta Biochim. Polon.* **2** (1955) 329.
5. Pawelkiewicz, J. and Nowakowska, K. *Acta Biochim. Polon.* **2** (1955) 259.
6. Pawelkiewicz, J. *Acta Biochim. Polon.* **2** (1955) 321.
7. Janicki, J. and Pedziwilk, Fr. *Acta Biochim. Polon.* **5** (1958) 299.
8. Neujahr, H. Y. *Acta Chem. Scand.* **10** (1956) 917.
9. DiMarco, A., Boretti, G., Migliacci, P. J. and Minghetti, A. *Boll. soc. ital. biol. sper.* **33** (1957) 1513.
10. Pawelkiewicz, J., Walerych, W. and Bartosinski, B. *Abstr. III Internat. Symp. Vit.* Poznan, Poland, p. 224.
11. Neujahr, H. Y. *Acta Chem. Scand.* **13** (1959) 1960.
12. Juillard, M. *Zentr. Bakteriolog. Parasitenk. Abt. II* **110** (1957) 701.

Received December 7, 1959.

Inhibition of *Pediococcus cerevisiae* ATCC 8081 by Deoxyuridine

ARNE E. BOLINDER and WOLFGANG G. KURZ

Division of Food Chemistry, Royal Institute
of Technology, Stockholm 70, Sweden

Plate assays for folic acid factors and thymidine as well as the bioautography of these growth factors have been carried out in this laboratory using *Pediococcus cerevisiae* (*Leuconostoc citrovorum*) ATCC 8081 as the test organism¹⁻³. This paper deals with the growth inhibitory effect of deoxyuridine on *P. cerevisiae* in cup plate and tube assays generally used for folic acid* and thymidine determinations.

* Leucovorin (synthetic N⁵-formyl tetrahydropteroylglutamic acid) was used as a source of folic acid throughout these studies.

For the cup plate tests and the bioautography large plates were used, each containing 150 ml of agar medium inoculated with *P. cerevisiae*¹. Three types of media were used; unsupplemented media and media supplemented with either 15 μg of leucovorin or 30 μg of thymidine per plate. The plates with supplemented media were used in order to obtain a good background growth and thereby give a more pronounced appearance of growth inhibition. For the cup plate tests, 0.03 ml aliquots of the test solutions were pipetted into cups, 6 mm in diameter, and the plates were incubated over night at 37°C. Tube tests were made using a final volume of 10 ml per tube. After suitable periods of incubation at 37°C, cell growth was measured turbidimetrically. For making the plates and the tube assays, washed and optically standardized inocula were used. A detailed report of these assays will be published later in this journal.

The growth inhibitory effect of deoxyuridine was discovered while investigating the contamination of a deoxyuridine preparation by thymidine. When checking the results obtained bioautographically with *Lactobacillus leichmannii* 313 ATCC 7830 as the test organism² (which responds to vitamin B₁₂-factors, deoxyribonucleosides, and deoxyribonucleotides) against bioautography with *P. cerevisiae*, we found that deoxyuridine produced a spot of nearly complete growth inhibition in the background growth of the *P. cerevisiae* plates made with unsupplemented media or with media containing leucovorin*. Bioautography using four different solvent systems showed that the UV-absorbing spot of deoxyuridine always corresponded to the growth inhibition spot in the *P. cerevisiae* plates and to a dense growth spot in the *Lb. leichmannii* 313 plates. In the *P. cerevisiae* plates, 3–4 μg of deoxyuridine could be detected bioautographically, which was the amount required for detection by inspection in UV-light.

In the cup plate tests, deoxyuridine formed inhibition zones only when tested at concentrations of 100 $\mu\text{g}/\text{ml}$ and above. In typical cup tests made in *P. cerevisiae* plates using unsupplemented media or media supplemented with leucovorin, the almost completely clear inhibition zones with fairly well defined zone edges pro-

duced by deoxyuridine (100 and 1 000 $\mu\text{g}/\text{ml}$) measured 17 mm and 27 mm, respectively. The growth inhibition produced by deoxyuridine was reversed by leucovorin or by thymidine, if either of the latter compounds were present in sufficient concentration.

In the *P. cerevisiae* plates with unsupplemented media leucovorin produced zones of heavy growth, whereas thymidine produced somewhat fainter growth zones.

In a series of cup plate experiments several nucleic acid compounds were tested for growth stimulation or growth inhibition using the three types of *P. cerevisiae* media. However, of all the compounds tested, deoxyuridine was the only one that showed strong growth inhibition. Very faint and diffuse inhibition zones were formed by uridine, cytidine, adenosine, and guanosine when these compounds were tested at concentrations of 1 mg/ml. These inhibitions were also reversed by leucovorin or thymidine.

Small zones of growth stimulation were formed by 5-methyldeoxycytidine (500 $\mu\text{g}/\text{ml}$) which was confirmed bioautographically. This finding is under further investigation.

No inhibition zones could be detected in the plates when the following compounds were tested separately at concentrations of 1 mg/ml (if not otherwise stated in brackets): (a) uracil (500 $\mu\text{g}/\text{ml}$), cytosine, 5-methylcytosine, thymine (500 $\mu\text{g}/\text{ml}$), orotic acid (500 $\mu\text{g}/\text{ml}$), dihydrouracil, and dihydrothymine, (b) adenine (500 $\mu\text{g}/\text{ml}$), guanine (200 $\mu\text{g}/\text{ml}$), hypoxanthine (500 $\mu\text{g}/\text{ml}$), and xanthine (200 $\mu\text{g}/\text{ml}$), (c) deoxycytidine, deoxyadenosine, deoxyguanosine, and deoxyinosine, (d) inosine and xanthosine (500 $\mu\text{g}/\text{ml}$), (e) the 5'-monophosphates of thymidine, deoxyuridine, deoxycytidine, 5-methyldeoxycytidine (500 $\mu\text{g}/\text{ml}$), deoxyadenosine, and deoxyguanosine, (f) the 5'-monophosphates of uridine, cytidine, adenosine, guanosine, and inosine, (g) uridylic, cytidylic, adenylic, and guanylic acids (mixtures of the 2'- and 3'-monophosphates), (h) the cyclic 2',3'-monophosphates of uridine, cytidine, and adenosine, and (i) the 5'-di- and -tri-phosphates of adenosine and inosine. Each of the compounds uracil, adenine, guanine, and xanthine were present in the basal medium at a concentration of 10 $\mu\text{g}/\text{ml}$.

The growth inhibitory activity of deoxyuridine and some other nucleic acid compounds was also studied by turbidimetric tube tests using *P. cerevisiae*. Read-

* In *P. cerevisiae* plates made with media containing thymidine, only faint zones of growth inhibition were formed by deoxyuridine in cup tests or bioautographic tests.

ings were made after 24, 48, and 68 h of incubation. For these tube tests a batch of deoxyuridine free of contaminating thymidine was prepared by chromatography on Whatman No. 3MM filter paper followed by elution with distilled water. We found that the growth promoting effects of suboptimal amounts of leucovorin or thymidine were strongly inhibited by deoxyuridine and to a much lesser extent by uridine and adenosine. Under the same testing conditions cytidine, guanosine, and inosine were only very slightly growth inhibitory, whereas no growth inhibition was caused by deoxycytidine, deoxyadenosine, deoxyguanosine or xanthosine. These findings are in accordance with the plate tests mentioned above.

The growth promoting effect of suboptimal amounts of leucovorin was inhibited noncompetitively by deoxyuridine, the inhibition index* increased as the amount of leucovorin was increased in the tubes. However, the growth promoting effect of suboptimal amounts of thymidine was competitively inhibited by deoxyuridine. An inhibition index of about 30 was obtained after 48 h of incubation when thymidine was present in the medium in amounts of 0.1 to 3 μg per tube. Larger amounts of thymidine increased the inhibition index. No inhibitions occurred in the tube tests when leucovorin or thymidine were present in amounts sufficient to promote optimal growth of *P. cerevisiae*.

Earlier work on the nutritional requirements of *P. cerevisiae* using the tube assay method, have shown that thymidine was the only deoxyribonucleoside which supported growth when substituted for leucovorin^{4,5}. However, in these investigations only low concentrations of deoxyribonucleo-

sides were tested. Some synthetic nucleosides of thymine (e. g. 1-D-glycosylthymine) have been reported to have a slight inhibitory effect upon the growth of *P. cerevisiae* when cultured in the presence of limiting amounts of thymidine⁶.

Further work is needed before any conclusions can be drawn concerning the inhibition mechanism of *P. cerevisiae* by deoxyuridine. Our findings for *P. cerevisiae* are very similar to those obtained by Lansford *et al.*⁷ using 2,4-diamino-6,7-diphenylpteridine-inhibited *Lactobacillus arabinosus*. This latter is a microbiological testing system in which the biosynthesis of thymidine is inhibited. Thus, these workers found that deoxyuridine was the only deoxyribonucleoside that showed any growth inhibition. Adenosine, inosine, guanosine, cytidine, and uridine were also found to be growth inhibitory, whereas deoxyadenosine, deoxyguanosine, deoxycytidine, and xanthosine showed neither inhibitory nor stimulatory effects upon growth. However, we found that no inhibition of growth was exerted by kinetin (100, 300, and 1 000 $\mu\text{g}/\text{ml}$) in plate tests with *P. cerevisiae*. This is in contrast to the *Lb. arabinosus* testing system of Lansford *et al.*⁷ in which kinetin and related compounds showed growth inhibitory effects.

1. Bolinder, A., Widoff, E. and Ericson, L.-E. *Arkiv Kemi* 6 (1953) 487.
2. Ericson, L.-E. *Arkiv kemi* 6 (1953) 503.
3. Bolinder, A., Kurz, W. and Lundin, H. *J. Inst. Brewing* 62 (1956) 497.
4. Kitay, E., MacNutt, W. S. and Snell, E. E. *J. Biol. Chem.* 177 (1949) 993.
5. MacNutt, W. S. *Biochem. J.* 50 (1951) 384.
6. Sauberlich, H. E. *Arch. Biochem.* 24 (1949) 224.
7. Lansford, E. M., Skinner, C. G. and Shive, W. *Arch. Biochem. Biophys.* 73 (1958) 191.

Received December 10, 1959.

* The inhibition index was calculated as the molar ratio of deoxyuridine to growth factor, present in the medium at 50 % inhibition of growth.