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Sewage as a Substitute for Casein Hydrolyzates in Fermentations with *Propionibacterium shermannii*

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The bacteria belonging to the genus *Propionibacterium* are characterized by an outstanding ability to produce vitamin B_{12} as reported by several authors [1,2]. Janicki, Pawelkiewicz *et al.*[3-5] used *Propionibacterium shermannii* in their comprehensive studies on vitamin B_{12} production by propionic acid bacteria and isolated several vitamin B_{12} factors from cultures of this organism. These authors found that the addition of small amounts of Aureomycin or sulfathiazole stimulated the synthesis of cyanocobalamin at the expense of other vitamin B_{12} factors [6,7] as is also the case with the addition of 5,6-dimethyl benzimidazole (DMB)[6]. The semi-synthetic medium used in fermentations with *Propionibacterium shermannii* is composed of casein hydrolyzates, glucose, phosphates, certain mineral salts and the vitamins, calcium pantothenate and biotin. Since the most expensive constituents of the medium are the casein hydrolyzates, it was considered

Table 1. Semi-continuous stationary fermentations with *Propionibacterium shermannii* in 2 l conical flasks.

Expt. No.	1	2	3
Medium	semi-synth.	semi-synth. + DMB 1 μg/ml	semi-synth. + corn steep liquor, **
E. coli activity after 5 days μg/ml *	1.8	0.7	0.9
E. coli activity after 12 days μg/ml *	3.6	0.5	2.6

* calculated as cyanocobalamin in cup plate assay.
** 4 % calculated on the basis of dry product containing 4 % N.

Table 2. Fermentation and vitamin B_{12} production by *Propionibacterium shermannii* grown for 5 days in different media. Suppl.-supplemented with the salts, vitamins and glucose of the semi-synthetic medium.

No.	Medium	Suppl.	pH	Increase in *E. coli* activity μg/ml *
1	Sewage fraction 1	−	4.9	0.1
2	» » 1	+	4.7	0.3
3	» » 2	−	4.7	0.1
4	» » 2	+	4.7	0.3
5	» » 3	−	4.8	0.1
6	» » 3	+	4.6	0.2
7	» » 4	−	6.5	0.0
8	» » 4	+	4.5	2.4
9	» » 5	−	5.1	0.1
10	» » 5	+	5.1	0.1
11	» » 6	−	5.7	0.0
12	» » 6	+	4.8	1.0
13	» » 7	−	5.4	0.0
14	» » 7	+	4.7	0.3
15	» » 8	−	4.8	0.1
16	» » 8	+	4.6	0.2
17	Milk whey	−	4.7	1.0
18	» »	+	4.8	0.5
19	*Anabaena supernat.*	−	4.9	0.1
20	» »	+	4.9	0.1
21	Yeast autolyzate	−	5.2	0.0
22	» »	+	4.3	0.2
23	Semi-synthetic	−	4.3	0.5
24	» »		4.5	0.5

* calculated as cyanocobalamin in plate assay.

to be of interest to find a suitable cheap substitute for this substrate.

A preliminary series of experiments was devoted to finding ways for stimulating the production of vitamin B_{12} in the semi-synthetic medium used by other authors. This series consisted of three fermentations in 2 l conical flasks using the semi-synthetic medium * supplemented as indicated in Table 1. The fermentations lasted over a period of 12 days and were operated on a semi-continuous basis — 100 ml of the fermented medium was replaced every day by fresh medium. The glucose content was adjusted every day to 1 % and the pH to 6.6. The vitamin B_{12} activity was determined every day using *E. coli* 113—3 in

* Composition of the semi-synthetic medium: Casaminoacids Technical (Difco), 14 g; Bactogen (Vitrum), 12.5 g; $NaH_2PO_4 \cdot 2H_2O$, 2.2 g; K_2PO_4, 1.8 g; $MgCl_2 \cdot 6\ H_2O$, 0.4 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; $CoCl_2 \cdot 6H_2O$, 10 p. p.m.; dist. water to 1 000 ml.

cup plate assay and bioautography as described elsewhere [8]. 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 Z[8] or GDP-factor B[9,10]. 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 B_{12}-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 μg/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.

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Inhibition of *Pediococcus cerevisiae* ATCC 8081 by Deoxyuridine

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Plate assays for folinic 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 organism [1-3]. This paper deals with the growth inhibitory effect of deoxyuridine on *P. cerevisiae* in cup plate and tube assays generally used for folinic acid * and thymidine determinations.

* Leucovorin (synthetic N^5-formyl tetrahydropteroylglutamic acid) was used as a source of folinic acid throughout these studies.