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Trans-N-deoxyribosylase activity in dairy cultures of Lactobacillus helveticus

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An excellent communication dealing with the enzymatic preparation of deoxyribonucleosides labelled with ^{14}C in the base, using a crude enzyme extract of *Lactobacillus helveticus* has been published in this Journal [1] quite recently by Cardinaud and Viswanthan. The most important result was a high degree of conversion of pyrimidine bases to the corresponding deoxyribosides.

In connection with this work we wish to present some observations from the same field made in our Laboratory [2]. It was the purpose of our work to isolate the enzyme trans-N-deoxyribosylase from a *Lactobacillus helveticus* culture grown just in sterile milk without any additives, to test the enzymatic activity of the isolated enzyme and its suitability for rapid enzymatic synthesis of deoxyribonucleosides labelled with radiocarbon ^{14}C.

The culture of *Lactobacillus helveticus*, var. pragensis BMSM — 5/8, strain TX was inoculated into sterile full-fat milk (fat content 3.6-3.8%), so that a 0.5-1.0% solution of this culture was obtained. After inoculation the culture was incubated for 14-16 hours at 40° C (all these procedures were carried out in the Institute for Dairy Cultures in Prague). About 100 ml of the grown suspension was centrifuged at 4,000 r.p.m. and 0° C for 20 minutes. The clear supernatant and the fat layer were removed, the centrifuged cells were suspended in 100 ml of 0.05 M citrate buffer of pH 6 and again centrifuged. The wet weight of cells was 55.0 grams. The washed cells were suspended in 120 ml of 0.1 M Tris-buffer of pH 7.2 and disrupted in the MSE ultrasonic desintegrator (input 60 Watts, frequency 20.10^3 cycles/sec) at 0° C for 5 minutes, then the suspension was centrifuged, the cell debris removed and the enzyme present in the supernatant fluid was purified according to the procedure of Roush and Betz [3]. In 7.4 ml of the final enzyme solution, 31.1 mg of protein was found by the method of Lowry *et al.* [4]. The enzyme was then frozen and stored at −15° C.

The deoxyribosyl transfer to the purine bases was studied using adenine-^{14}C(U) of spec. act. 28.6 mCi/mMole, guanine-^{14}C(U) of spec. act. 25.0 mCi/mMole (both made in this laboratory) and non-radioactive thymidine as the "donor" of the deoxyribosyl group. The pyrimidine bases used were uracil-2-^{14}C and cytosine-2-^{14}C of spec. act. 5.0 mCi/mMole resp. 4.1 mCi/mMole (both commercial products of this Institute). In this case non-radioactive deoxyadenosine was applied as the "donor" of deoxyribose. The ratio of radioactive base : "donor" was 1 : 5 (Mole/Mole). For 1 μMole of radioactive substrate 0.125 ml (0.525 mg of protein) of enzyme was used. Such mixtures were then incubated in 0.1 M phosphate buffer of pH 6 at 37° C under aseptic conditions. During incubation samples of the reaction mixture were taken. After heating in a water bath for a short time, the samples were deprived of denaturated protein and the clear supernatants were analysed by paper chromatography in different solvent systems (see Table I). The standards of the nucleic acids components were detected on the chromatographic

TABLE 1. Paper chromatographic analyses. R_F values of bases and deoxyribosides.

Nucleic acid components	Solvent systems [a]		
	A	B	C
Adenine	0.64	0.36	0.11
Deoxyadenosine	0.80	0.37	0.09
Guanine	0.39	0.20	0.00
Deoxyguanosine	0.54	0.20	0.00
Cytosine	0.63	0.42	0.00
Deoxycytidine	0.76	0.55	0.00
Uracil	0.60	0.70	0.27
Deoxyuridine	0.58	0.80	0.14
Thymine	0.76	0.82	0.55
Thymidine	0.74	0.87	0.27

[a] A n-butanol : n-propanol : ammonia : water (7 : 5 : 7 : 2). Developed 2 × 18 hours.
B Iso-propanol : HCl (37%) : water (170 : 41 : 39).
C Ethylacetate saturated with water. Developed 18 hours.

For analyses Whatman No. 3 was used. The temperature of developing was 18-22° C. The adenine-thymidine and guanine-thymidine reactions were analysed in system *A*, the cytosine-deoxyadenosine reaction in system *B*, the uracil-deoxyadenosine reaction in system *C*.

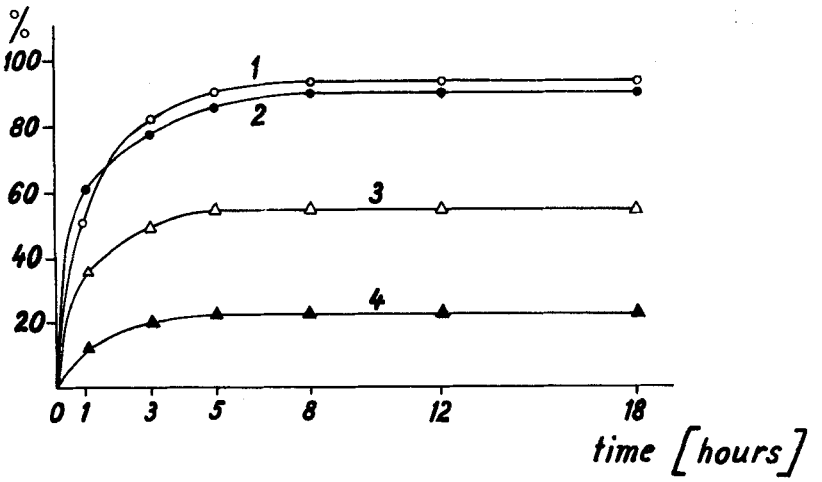

FIG. 1. The time-course of the deoxyribosyl transfer to nucleic acid-^{14}C bases catalysed by trans-N-deoxyribosylase from a dairy culture of Lactobacillus helveticus.

Horizontal coordinate : Time of enzymatic reaction.

Vertical coordinate : Percentage of deoxyriboside-^{14}C formed.

Composition of the incubation mixture as shown in the text.

The course of deoxyribosidation is demonstrated for guanine-^{14}C, 1 (●——●), adenine-^{14}C, 2 (○——○), cytosine-^{14}C, 3(△——△) and uracil-^{14}C, 4 (▲——▲).

sheets in the UV light of a Chromatolite lamp, while the radioactive spots were autoradiographed on Agfa-Texo RTG films. The radioactivity of these spots was measured with a 2π counter. The course of deoxyribosyl transfer evaluated in this manner is shown in figure 1. It is evident that the equilibrium states in all enzymatic reactions were reached after 8 hours of the reaction under the described conditions. At this moment 92-93% of adenine and guanine, 55% of cytosine and 25% of uracil used as the radioactive starting material had been converted to the corresponding deoxyribosides.

From the experimental results obtained the following conclusions may be drawn. The trans-N-deoxyribosylase activity in dairy cultures of *Lactobacillus helveticus* cultivated in sterile milk was determined. No ingredients have to be present in milk in order to obtain an enzyme preparation of sufficient enzymatic activity. The equilibrium constants for the purine deoxyribosides are the same, but those for pyrimidine deoxyribosides are higher in comparison with the results obtained under similar conditions, i.s. where deoxyadenosine as a "donor" of a deoxyribosyl group is used [1, 5].

The described procedure is suitable as a rapid method for the preparation of labelled deoxyribosides with regard to the fact that grown dairy cultures of

Lactobacillus helveticus are commercially available. Besides the satisfactory yields of purine and pyrimidine deoxyribosides another advantage of the described method is the application of non-buffered chromatographic solvents. This makes it possible to obtain deoxyribosides without significant amounts of salts.

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Isotopenaustausch des Sauerstoffheteroatoms in Pyryliumsalzen

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EINLEITUNG

In früheren Arbeiten [1] wurde der Isotopenaustausch von Ringen und Seitenketten Wasserstoffatomen der Pyryliumsalze eingehend studiert. Auch ^{14}C-markierten Pyryliumsalzen wurden synthetisiert [2].

Die Literatur enthält keine experimentelle Hinweise auf einen Sauerstoff — Isotopenaustausch des Ringen Sauerstoffatoms der Pyryliumsalze, obwohl die Vermutung eines solchen Austausches aus theoretischen Gründen ausgesprochen wurde [3]. Deshalb beschlossen wir, den Isotopenaustausch zwischen dem 2,4,6-Trimethylpyryliumperchlorat (I) und $H_2{}^{18}O$ zu untersuchen. Es wurde bewiesen [4] dass das Perchlorat-Ion in unseren Versuchsbedingungen keinen Isotopenaustausch mit $H^{218}O$ nachweist.