# MICROBIOLOGICAL DETERMINATION OF DEOXYRIBONUCLEIC ACID

## Influence of Deoxyribosides on the Standard Curves

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In the microbiological method for determination of deoxyribonucleic acid (DNA) developed by Hoff-Jørgensen (1951, 1954) thymidine is used as a source of deoxyriboside both in the inoculum and in the standard curves. However, in the digested DNA samples used in this assay, the four main deoxyribosides are generally found in almost equal amounts. In order to eliminate a possible source of error arising from this difference between standard curve and unknown sample, we have undertaken a study of the influence of deoxyribosides on the growth of the bacteria used in this method.

### METHOD

The lactic acid bacteria *Thermobacterium acidophilum* (*Lactobacillus acidophilus*) strain R 26 Orla Jensen was grown in the chemically defined medium developed by us (Løvtrup and Roos, 1957). As far as details are concerned the reader may refer to this paper. As described by Hoff-Jørgensen the thymidine stock solution is $10^{-3}$ M in 25 per cent ethanol. At the lowest point on the standard curves the stock solution is diluted 4000 times (final concentration $0.25 \times 10^{-6}$ M), the amount of thymidine is thus 0.121 $\mu$g in a final volume of 2 ml. The other deoxyribosides, deoxyadenosine, deoxyguanosine, and deoxycytidine, were used in the same concentration. When all four were added simultaneously, equal volumes of the four stock solutions were mixed, and this mixture was subsequently diluted as the stock solutions of the individual nucleosides. The stock solutions were also used for the inoculum media but here the final concentration was $10^{-5}$ M. The deoxyribosides were chromatographically homogeneous preparations from Schwarz Laboratories, Inc. For reference in the present work we have used inoculum and standard curve media containing all four nucleosides. The mean values and standard deviations for six standard curves of this type are shown in figure 1. It should be mentioned that in all standard curves each point is determined as the mean of the absorbance from two tubes.

### RESULTS

*Effect of the deoxyriboside composition of the inoculum medium.* Five different media were made, containing either thymidine, deoxycytidine, deoxyadenosine, deoxyguanosine, or all four at the same time. After the normal incubation of 22 hr, standard curve tubes containing all four nucleosides were inoculated from these. The resulting standard curves are shown in figure 2. From this it appears that the differences are only slightly greater than the normal variations observed in the standard curves (figure 1). We must therefore conclude that the deoxyriboside composition of the inoculum medium is of little influence on the standard curves.

*Effect of the deoxyriboside composition of the standard curve medium.* Standard curve media, containing one of the four nucleosides, were inoculated with bacteria from an inoculum containing all four nucleosides. The results of these experiments are shown in figure 3. It is seen that considerable differences are found under these conditions. It was repeatedly found that the standard curve containing thymidine was above, and the one containing deoxyguanosine was below the remaining two curves. These latter are on the other hand very close to the reference standard curve.

Having observed this influence of the standard curve medium we repeated the experiments, this time using the same nucleoside in both inoculum and standard curve medium. The results of these experiments are shown in figure 4. It is here seen that we obtained the same type of variation with the four individual nucleosides as in the preceding experiments, i. e. the thymidine lies above, and the deoxyguanosine curve below the others. This variation is thus apparently an

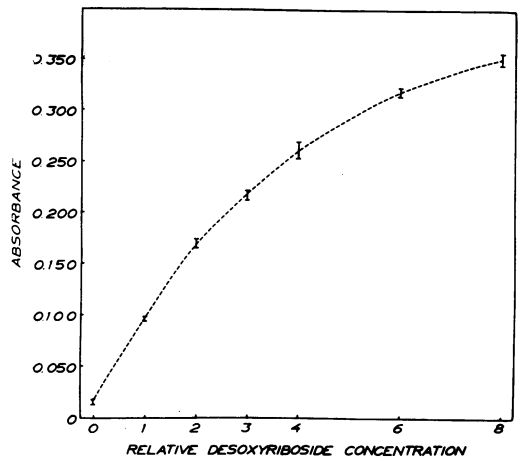


*Figure 1.* Standard curve obtained when both assay medium and inoculum contain all four deoxyribosides. The standard deviations indicated are calculated from six individual standard curves.

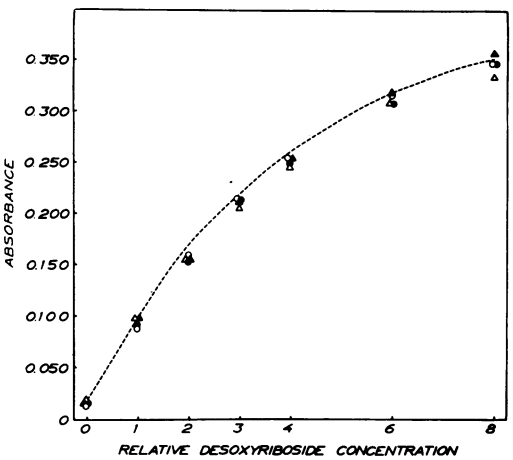


*Figure 2.* Standard curves obtained when the assay medium contains all four deoxyribosides, and the inoculum thymidine, △; deoxycytidine, ●; deoxyadenosine, ▲; or deoxyguanosine, ○. Every point represents the average of two different experiments. The stippled curve is the reference curve from figure 1.

effect of the composition of the standard curve medium. It seems, however, that under these conditions even the composition of the inoculum may have some influence because all four curves with only one nucleoside are seen to coincide with or lie below the reference containing all four nucleosides. In figure 3 this curve is intermediate.

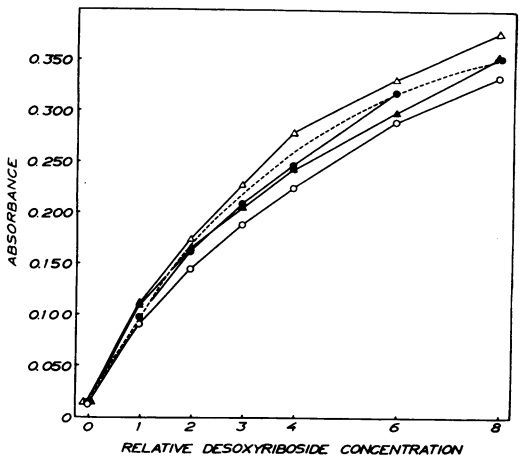


*Figure 3.* Standard curves obtained when the inoculum contains all four deoxyribosides, and the assay medium thymidine, △; deoxycytidine, ●; deoxyadenosine, ▲; or deoxyguanosine, ○. Every point represents the average of two different experiments. The stippled curve is the reference curve from figure 1.

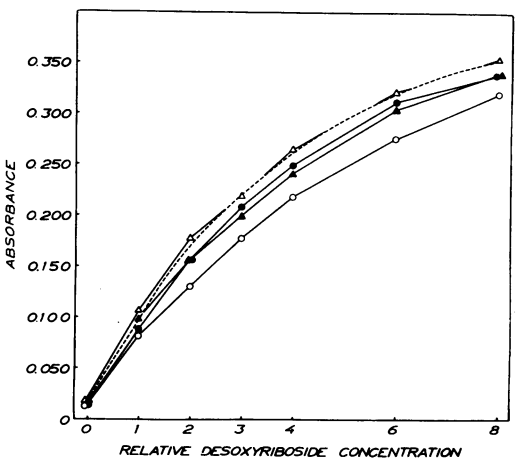


*Figure 4.* Standard curves obtained when both inoculum and assay medium contain thymidine, △; deoxycytidine, ●; deoxyadenosine, ▲; or deoxyguanosine, ○. Every point represents the average of two different experiments. The stippled curve is the reference curve from figure 1.

## DISCUSSION

The present work is part of an investigation aimed at establishing the optimal conditions for the DNA determination assay utilizing the

deoxynucleoside-requiring *Thermobacterium acidophilum*. From our results it appears that the composition of the growth medium with respect to deoxynucleosides influences the growth of this microorganism, and thus the standard curves. For discussion of the possible errors arising from the composition of the assay medium, we simply assume that our medium containing all four nucleosides is very similar in composition to the digests of DNA samples offered to the bacteria. In the usual procedure the bacteria are grown in an inoculum containing thymidine, and are transferred partly to a standard curve medium containing thymidine (figure 4), and to tubes with DNA samples containing all four nucleosides (figure 2). Comparing these curves it is seen that they coincide. This seems to be fortuitous since among the four deoxyribosides, thymidine is the only one for which this holds. No methodological error is thus involved in using thymidine in the standard curve and inoculum media.

From a more general point of view our results confirm the finding of Hoff-Jørgensen (1951) that all four nucleosides may be utilized by the bacteria. However, we have not been able to confirm that they can sustain growth to the same extent. On the contrary, irrespective of the composition of the inoculum medium, we found the deoxyguanosine curve to lie below the thymidine curve. If a thymidine standard were used for microbiological assay of deoxyguanosine, the values obtained would be about 30 per cent too low (figures 3 and 4).

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SUMMARY

The role of deoxyribosides in the microbiological deoxyribonucleic acid determination using *Thermobacterium acidophilum* (*Lactobacillus acidophilus*) has been studied. The composition of the inoculum medium with respect to deoxyribosides has little influence. The deoxyriboside content of the standard curve medium has a conspicuous effect. The thymidine curve is above and the deoxyguanosine curve below the others. All four deoxynucleosides, thymidine, deoxyadenosine, deoxycytidine, and deoxyguanosine, may be utilized by the bacteria, but they do not support growth to the same extent.

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