UNUSUAL GROWTH PROPERTIES OF A BACTERIAL STRAIN LACKING DNA POLYMERASE

Herbert S. Rosenkranz, Howard S. Carr, and Councilman Morgan

Department of Microbiology, College of Physicians & Surgeons, Columbia University, New York, N.Y. 10032

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SUMMARY

The DNA polymerase-deficient E. coli strain pol A_1^- exhibits a 5% plating efficiency when grown on synthetic liquid medium and plated on a nutritionally rich solid medium. This phenomenon is not seen when the composition of the liquid and solid media is identical. Accordingly in order to obtain meaningful results with this strain, the growth conditions must be controlled carefully.

Recently de Lucia and Cairns reported (1) the isolation of an unusual mutant of E. coli W 3110 thy^- deficient in DNA polymerase (pol A_1^-). The availability of this strain has stimulated a number of studies on the role of DNA polymerase in DNA synthesis and DNA repair (2-8). It has also led to the discovery of a number of new DNA-synthesizing enzymes (8-15) and to the development of a rapid in vivo procedure for detecting agents which react with cellular DNA (16). Many of the conclusions of these investigations are based on the enumeration of the number of pol A_1^- cells present in liquid media under varying conditions. In the present study we report that under certain experimental conditions pol A_1^- cells cannot be enumerated by spreading them onto solid agar plates.

When E. coli W 3110 thy^- pol A_1^+, the parent strain, was grown in a synthetic liquid medium (medium HA (17))supplemented with thymine (5μg/ml), the usual increase in turbidity (450 nm) was observed (Fig. 1).

When dilutions of this culture were plated on a rich nutrient agar medium (Columbia base agar (18); Baltimore Biological Labs.) and the plates incubated at 37° C for 16 hrs, the increase in the number of colonies was found to parallel the increase in turbidity (Fig. 1). When, however the same procedure was applied to the pol A_1^- strain, it was found that while the turbidity of the culture exhibited the characteristic increase, the usual increase in number of viable bacteria was not seen (Fig. 1). Actually, the number of colony-forming bacteria was only 5% that of the parent strain and it did not increase until the second hour of incubation. Soon thereafter the number of viable bacteria approached the level expected from turbidity measurements. An increase in turbidity without concurrent multiplication of viable bacteria could reflect inhibition of the final stage of binary fission such that separation is prevented and long chains (consisting of 20 bacterial units) are formed, or that the bacteria simply increase in size for several hours before dividing. Direct light microscopic examination eliminated both of these possibilities. Moreover, the ultrastructure of the pol A_1^- strain as seen in the electron microscope was similar to that of the parent strain. This suggested that *E. coli* pol A_1^- divides at the normal rate but that the daughter cells are unable to form viable progeny when plated on solid media. However, upon further incubation in liquid medium this defect is overcome.

Because the experimental procedures used involved a "shift up" from a nutritionally poor medium to a rich solid medium, the experiments were repeated growing cells in a "rich"medium and plating them on "rich" medium and, conversely, growing them on synthetic medium and plating them on the same medium supplemented with agar. It was found that when this was done that the plating efficiency of the pol A_1^- strain approached

that of the pol A^+ E. coli.

Since the only difference between E. coli pol A_1^- and its parent appears to be a deficiency in DNA polymerase, the present observation of a decreased plating efficiency during the "shift up" phase presumably reflects a function of DNA polymerase. It may be that cells deficient in this enzyme must devise a new enzymic pathway to repair spontaneously damaged DNA. This by-pass may be much slower than the reaction normally catalyzed by DNA polymerase. A shift to rich medium may induce bacterial replication before DNA repair has been completed thereby leading to cellular death. On the other hand continued incubation in the synthetic medium allows repair to "catch up" thereby increasing the efficiency of plating (Fig. 1). The exact basis of this behavior of E. coli pol A_1^- is under investigation. However in the meantime, whenever using pol A^- strains, the plating efficiency should be determined.

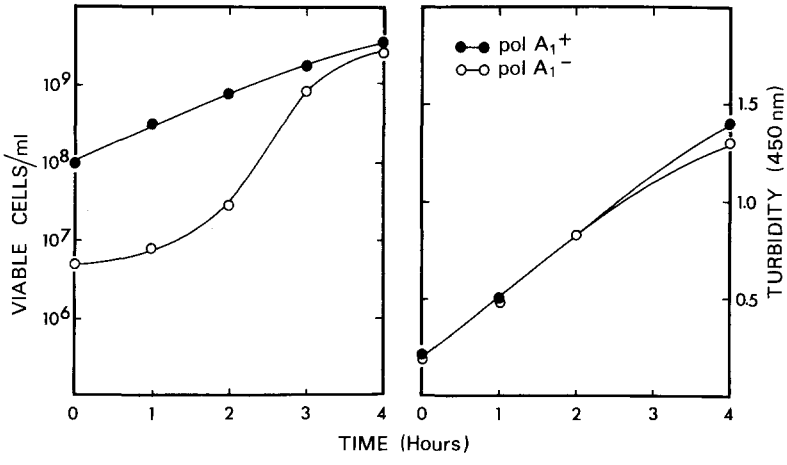

FIGURE 1: Growth properties of pol A_1^+ and pol A_1^- cells. Bacteria (E. coli W 3110 and E. coli p 3478, the pol A_1^+ and pol A_1^- strains, respectively) in the synthetic medium HA(17) supplemented with thymine (5 μg/ml) were brought to the exponential growth phase (turbidity at 450 nm ≃ 0.2), at intervals portions of the cultures were withdrawn, serially diluted in synthetic medium and 0.1 ml-samples spread on the surface of Columbia base nutrient agar plates (18). Colony-forming bacteria were enumerated after 16 hours at incubation at 37° C.

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