THE ACTIVATION OF PROPHAGE IN E.COLI B BY HIGH PRESSURE

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The present study was undertaken as part of an investigation of the effects of high pressure on intracellular phage development. E.coli B was grown at 37° C under heavy aeration in Difco nutrient broth with 5 g. Difco casamino acids added per litre. When the cells were in the logarithmic phase of growth the culture was divided in two parts, one of which was transformed into spheroplasts with the aid of lysozyme - versene (Repaske, 1958). About half of the spheroplast suspension was then osmotically shocked by being suspended in physiological saline. The two samples and also the part of the material which consisted of untreated cells were then exposed, at a density of 2-5 x 10^7, for five minutes to 2000 atm. at 37° C. Non-pressure treated portions of the material served as controls. Without diluting them, all the preparations were then immediately (within five minutes of the exposure to pressure) plated against E.coli B on tryptose-agar plates with the top-layer agar method. The Petri dishes were incubated at 37° C for 16-18 hr. and the results noted. It was found that the viable cell count dropped about 100 times as a consequence of the pressure treatment and that the spheroplasts yielded plaques (47, 5, 32 in different experiments). Pressure treatment of whole cells and of shocked spheroplasts yielded no plaques and the same was true for the controls. In one experiment it was possible to obtain five plaques from whole cells exposed to 2000 atm. at 37° C for five minutes but only by treating them with lysozyme - versene after the pressure treatment.

When ten single colonies of the E.coli B culture were isolated, pressure-treated, and plated individually against the homologous clone of cells it was found that a single experiment might

fail to yield plaques but upon repeated trials most of the clones were found to liberate phages. One clone was selected for a more thorough study. In this case bacteria, obtained from the periphery of a plaque, were shown to be immune to the phages obtained by the pressure-treatment of the spheroplasts. It was further shown that those bacteria when plated together with the original E.coli B culture lysogenized these. When exposed to pressure, with or without lysozyme-versene treatment, the lysogenic bacteria were found to release phages that were virulent to the original E.coli B culture. To our mind this constitutes a strong indication that E.coli B carries at least one prophage which in fact has also been suspected by Cohen (Cohen, 1959). The plaques, which vary somewhat in size, have an average diameter of 5 mm. and do not resemble the "T-phage-types". It was possible to transfer the released phages on E.coli B and to observe them in the electron microscope. In one experiment not only the rubber-enclosed glass vial used for holding the material but also the apparatus for high pressure was sterilized. Under these conditions the risks of contamination are very small and should of course be detected in the controls anyhow. The experiment yielded the typical plaques as expected.

The nature of the phenomenon is obscure. The cytoplasm of the cells exposed to these high pressures might well be subject to a gel-sol transformation (Marsland,1958) which can increase the solubility of different cell constituents. Prophages might be liberated from their chromosomal sites and activated in this freed state as a consequence of the pressure strain on the replicating genome. It might also be that an important phage constituent is of cytoplasmatic origin and is activated by association with a specific chromosomal site rendered accessible by the pressure treatment. The results might also be explained by the fact that many of the spheroplasts when exposed to 2000 atm. disintegrate and may thus liberate particles able of self-reproduction. This explanation, however, presupposes that the pure disintegration effect of pressure is different in some way from osmotic disruption. In order to mature, the particles, at least for some time, seem to require an intact inner cell structure. The prophages must, however, have been changed - in one way or the other - presumably genetically, because it has been shown that as a rule they can infect the carrier clone of cells. If

the mother bacteria yield just one or a large number of phages is not known at the present time.

Experiments are now being performed on different lysogenic bacteria to see if it is a general phenomenon or restricted to *E. coli* B.

References:

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