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DEHYDRATION: THE STRESS FOR BACTERIOPHAGE λ

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Abstract

The effect of dehydration of different duration on air or in high vacuum was investigated. Under the tested conditions, it caused inactivation in 95%, but did not DNA damage λ1390 bacteriophage. Neither, DNA strand break nor DNA mutation were detected, but capsid proteins were destabilized.

Introduction

Viruses are the smallest, simplest form of life on earth - they are just an assembly of nucleic acids wrapped by a protein coat (or 'capsid'). Viruses are not cells, but they cannot reproduce itself outside another cell. Theri small size and simple biological construction, make them useful laboratory model systems for different kind of tests, particularly in studies of effects of extreme conditions of environment like dehydration, temperature changes, pressure, pH or different kind of chemicals. The stress during dehydration of viruses may be particularly important. The literature notes a few data of inactivation of the bacteriophage (a true parasite in bacteria) mainly as a collateral data in other studies. So, whereas T1 bacteriophage remained not more then 20% inactivated [1] or was unaffected by dehydration in vacuum, 40 to 80% of the plaque-forming units of øX174 bacteriophage [2], 25-50% of λ bacteriophage [3], and 60% of T7 [4] were inactivated. We report here the results of the preliminary experiments on the response of bacteriophage λ1390, deficient in their own recombination repair system, to dehydration on air or in high vacuum conditions. The following effects have been analyzed: surviving of bacteriophage λ and the amount of damages in bacteriophage λ DNA or capsid proteins after dehydration.

Experimental Procedure

Phage and Host Stocks. The bacteriophage λ 1390 strain and different *E. coli* bacterial strains used in this study were from Radman's laboratory (M. Radman, France). Stock of λ1390 (Red^-Gam^-) bacteriophage was prepared by confluent lysis on a plate and stored at 4 °C [5]. As host for propagation of phage strain *E. coli* P2 lysogen was used. λ1390 strain give transparent plaques on a lawn of *E. coli* plating bacteria. Counting of plaques was performed after 18 hours. Plating *E. coli* bacteria were: P2 lysogen for surviving, $recA^-$ for frequency of DNA recombination and $recA^-$ $uvrA^-$ double mutant - for bacteriophage DNA mutations.

Dehydration. For dehydration, 30µl of λ1390 stock have been applied directly at full concentration on still surfaces prepared by TritoneX-100 detergent. The samples were dried for 30 min at 37 °C, put to sterile Petri dishes and the dehydratation

was continued in room conditions or in vacuum chamber at room temperature and pressure of 10^{-5} mbar for 7 or 120 hours. After appropriate time, dehydrated phages were resuspended in 10 mM $MgSO_4$ and stored at 4°C.

Surviving and DNA test. Surviving fraction of dehydrated phages was measured by titration on layer of *E. coli* P2 lysogen strain. Possible bacteriophage DNA breaks were tested on recA$^-$ mutant of *E. coli*, and mutations in bacteriophage DNA were tested on recA$^-$uvrA$^-$ double mutant of *E. coli*.

Capside proteines test. Temperature sensitive test at 37 °C and 50 °C was performed for 10 days.

Measurement of phage survival and DNA damage rate. Survivors λ were scored by plating dehydrated bacteriophages on a lown of P2 lysogenic bacteria. The counting of plaques was performed after 18 h. The relative number of surviving bacteriophage was the ratio of both titers measured for non-dehydrated control bacteriophage and dehydrated λ. DNA damage rate was counted in the same maner but on a other bacteria lown. DNA breaks were scored by plating non-dehydrated control bacteriophage and dehydrated λ on a lown of recA$^-$ bacteria. DNA mutations were scored by plating non-dehydrated control bacteriophage and dehydrated λ on a lown of recA$^-$ uvrA$^-$ double mutant bacteria.

Results and Discussion

To provide experimental evidence that dehydration is stress, bacteriophage λ1390 strain has been exposed to dehydration on air and in high vacuum for 7h or 120h. After drying bacteriophages were rehydrated in 10mM $MgSO_4$, and the following effects have been analyzed: surviving of rehydrated bacteriophage λ and the amount of damages in rehydrated λ DNA or λ capsid proteins. Surviving test was performed by titration on *E. coli* P2 lysogen bacteria strain and surviving was similar for all tested samples: 1.3 – 4.6%. In the same time, rehydrated bacteriophage λ has been tested for possible DNA damages by titrario on recA$^-$ (for DNA breaks) and recA$^-$uvrA$^-$ double mutant of *E. coli* (for DNA mutations). Neither, DNA strand break nor DNA mutation, were detected. These data allow as to conclude: dehydration, in oxygen and oxygen-free conditions, caused inactivation of 95%, but had not DNA damaging effect on λ1390 bacteriophage.

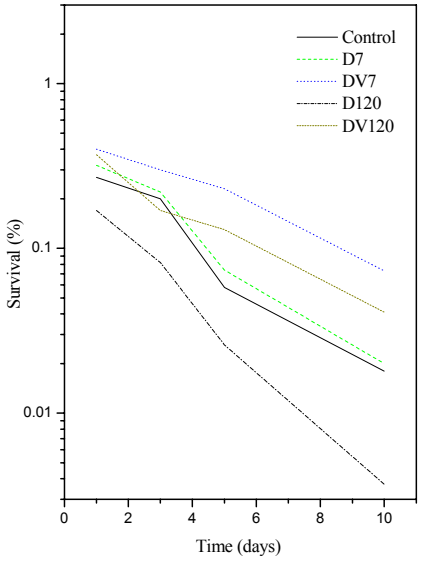

Figure1. λ capside proteine destabilization.

To test the stability of λ capsid

proteins, each sample of resuspended dry λ aliquot were transfered to 37 °C or 50 °C. Number of survivors was screened every day for 10 days. At 37°C we have not noted significant differences between samples, but at higher temperature (50 °C) differences were significant (Figure 1). The best surviving at high temperature was for samples dehydrated in vacuum and 7h on air. These three groups were more stabile than non-dehydrated control group. The worst survival was of sample dehydrated for 120h on air. Also, neither DNA strand break nor DNA mutation were caused by the high temperature in all samples.

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