A LABORATORY EXPERIMENT WITH RELEVANCE TO THE SURVIVAL OF MICRO-ORGANISMS ENTERING A PLANETARY ATMOSPHERE *

(Letter to the Editor)

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Abstract. A culture of *E. coli* was initially subjected to brief exposures to heat for durations of 30–60 s, starting with a temperature of 270 °C. A stepwise increase of this temperature from 270 °C–750 °C and a sequential culturing led to the emergence of a strain of this bacterium with a much higher resistance to flash heating than the original culture possessed. This behaviour would have an important relevance to the survival of micro-organisms upon entering a planetary atmosphere.

1. Introduction

It is well known that several classes of micro-organisms exist with capabilities of survival under conditions of extreme environmental stress. In particular, thermophilic bacteria can survive and grow in high temperature aqueous environments. Recently it has been found that some types of thermophiles can grow in water under pressure at temperatures of $\sim$ 250 °C, such as occur in vents along tectonic rifts on the ocean floor (Baross *et al.*, 1982; Baross and Denning, 1982).

In earlier papers we have reported on experiments indicating the survival of micro-organisms (*E. coli* in particular) that were subjected to high pressures in a KBr disc, and also the survival of micro-organisms that were flash-heated for a fraction of a minute in a vacuum environment (Hoyle *et al.*, 1986). The latter experiment clearly demonstrated that bacteria could survive the flash heating that they would experience on entering the Earth's atmosphere, and the former experiment showed the possibility for survival of bacteria under conditions that were totally irrelevant to the surface of the Earth. It is our point-of-view that many of these 'extreme' survival properties would have a relevance to bacteria occurring on an extraterrestrial scale, possibly on the surfaces of other planets.

Two of the present authors (FH and NCW) have argued for some years that cometary micro-organisms must arrive on planets other than the Earth within the solar system itself. Thus data from Mars, Venus, Jupiter, Saturn and most recently from Uranus can be interpreted on the basis of bacteria surviving, and even controlling the physical conditions of these planets and of their attendant satellites.

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In view of this it would be of interest to determine limits to survival imposed by the requirement that entrant bacteria must be flash-heated for brief periods (up to varying temperatures) as they become trapped in planetary atmospheres.

In the present communication we discuss new evidence for viability of cultures of *E. coli* that have been repeatedly and sequentially exposed to conditions of high temperature in a near-vacuum environment.

2. Experimental Procedure and Results

Two discs of freeze-dried *E. coli* type ATCC 25922 (DIFCO 1629-32-1) were placed inside a pair of sterilised test tubes, which were subsequently vacuumed and sealed. Next, the text tubes were suspended from a metal rack and the entire rack with the test tubes was placed inside an oven at temperature 270 °C for a duration of 60 s. At the end of this time interval the contents of the two test tubes were transferred into two 250 ml sterilised flasks each containing nutrient broth (beef extract, DIFCO Labs.). The flasks were then placed on a shaker and kept overnight at a temperature of 37 °C.

The contents of the flasks were seen to turn significantly turbid indicating the growth of cultures from the bacteria that were heated to 270 °C. The cultures were separated out by centrifuging, dried out and washed clean of nutrient. Examination using both optical and electron microscopes showed patterns of abnormal morphology very similar to that discovered earlier for micro-organisms subjected to high pressures, (Al-Mufti *et al.*, 1984). The culture from one of the two flasks was pressed into discs and set aside for later reference. The culture from the second flask was recycled through a stepwise reheating-reculturing procedure described below.

About 1 mg of the culture from this flask was put in a flask containing nutrient broth and the flask placed on a shaker at 37 °C. After 5 hr on the shaker the optical density of the flask was seen to significantly increase, indicating that extensive bacterial growth had occurred. The resulting culture was again separated out, washed and dried. Under the microscope the bacterial morphologies still seemed to be substantially deformed. Using the techniques described in our earlier paper (Al-Mufti *et al.*, 1984) this line of bacteria was sequentially cultured, harvested and regrown until normal bacterial morphologies were eventually regained. The final culture was centrifuged at 8 k rpm for 16 min and the resulting precipitate freeze-dried at –50 °C overnight.

About 2 mg of this freeze-dried sample was put in a test tube, vacuumed and sealed. The test tube was then placed in an oven at 350 °C for 30 s. The procedure described above was now repeated, yielding eventually a freeze-dried culture from bacteria that had been heated to 350 °C and had subsequently regained a normal morphology. Then 2 mg of this sample was once again heated to 400 °C for 30 s under vacuum conditions and this whole process of sequential culturing and re-

exposure to heat was repeated in step-wise fashion until an exposure temperature of 750 °C was attained for a time of 30 s. Heating to higher temperatures presented difficulties for the techniques that we had at our disposal.

The *E. coli* disc that was set aside for reference (after heating to only 270 °C) was now placed in a test tube, vacuumed, sealed and placed in an oven at temperature 750 °C for 30 s – exactly the same conditions as were applied to the final culture emerging from our step-wise heating and culturing procedure. When the contents were transferred to a new nutrient flask and a culture attempted, no viable culture could be obtained.

3. Conclusion

The inference to be drawn from our experiment is that step-wise heating and sequential re-culturing resulted in a final emergent strain of *E. coli* with a much higher resistance to flash heating than the original standard culture had possessed. Such heat resistant strains for even such well-adapted micro-organisms as *E. coli* would have a profound relevance to the survival of bacteria upon entry into the atmospheres of planets.

References

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