Differential tolerance of biofilms and planktonic cells of *Deinococcus geothermalis* to desiccation and to simulated space and Mars conditions

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The formation of biofilms is one of the most successful survival strategies of bacteria on Earth. Aggregated and embedded in a matrix of extracellular polymeric substances (EPS), biofilm cells are often more tolerant to environmental stress than single, planktonic cells of the same species. If microorganisms were to travel through space or to reside on a Mars-like planet, their survival in these hostile environments might be enhanced if they are organised in biofilms. The aim of this study was to test this hypothesis.

Deinococcus geothermalis DSM 11300 was chosen as a model organism due to its high intrinsic tolerance to both desiccation and radiation. Biofilms generated at the solid-air interface of membranes placed on R2A agar medium and, for comparison, membrane-deposited planktonic cells grown in R2A broth were air-dried overnight and exposed to various stressors relevant for space and Mars environments, including prolonged desiccation, vacuum, simulated Martian atmosphere, low and high temperatures, and UV radiation.

The EPS of *D. geothermalis* were isolated using a cation-exchange resin extraction method. They contained significant amounts of proteins and polysaccharides, both of which might promote the retention of water under dehydrating conditions, as well as relatively low amounts of extracellular DNA (eDNA), which could have a structural role within the EPS matrix. In biofilms, the EPS were of distinct spatial arrangement. Environmental scanning electron microscopy provided evidence for an EPS layer covering the uppermost layer of cells. Three different polysaccharide fractions – presumably galactosides – of different spatial distribution and arrangement were identified within the matrix using fluorescently-labelled lectins. When tryptic soy agar (TSA) was used as a nutrient source instead of R2A, biofilms of different morphology and EPS composition were formed. High peptone concentrations in the TSA medium (20 g l^{-1}) caused the cells to form highly cohesive aggregates. Dispersal of these cell aggregates could be achieved by treatment with proteinase K, suggesting the involvement of proteins, possibly in the form of adhesins or type IV pili, in the cell-to-cell attachment of the organism.

Following exposure to stress conditions, the viability of the organisms was assessed and compared to non-exposed cells. Since many bacteria are able to enter a viable but non-culturable (VBNC) state as a response to stress, cultivation-independent viability markers

(membrane integrity, ATP levels, presence of 16S rRNA) were analysed in addition to the determination of colony counts. During prolonged desiccation, biofilms sustained viability significantly longer than planktonic cells: Compared to non-desiccated samples, a desiccation period of 56-61 days reduced the culturability of biofilms and planktonic cells to 5.6% and 0.8%, respectively. Membrane integrity was maintained to a high degree in biofilms, whereas more than 60% of planktonic cells showed signs of membrane damage following desiccation. Whilst biofilm cells sustained their initial ATP levels, planktonic cells experienced a 1-log reduction in ATP upon dehydration.

When desiccated biofilms and planktonic cells of *D. geothermalis* were exposed to vacuum, artificial Martian atmosphere, repeated thaw-freeze cycles, or extreme temperatures (-25 °C; +60 °C), their viability in terms of culturability and membrane integrity remained unchanged compared to dry but non-exposed controls. UV irradiation – either monochromatic (254 nm; ≥ 1 kJ m^{-2}) or polychromatic (200-400 nm; > 5.5 MJ m^{-2} for planktonic cells and > 270 MJ m^{-2} for biofilms) – significantly reduced the culturability of *D. geothermalis* in both its biofilm and planktonic form. Survival seemed to be insignificantly enhanced when the cells were irradiated in artificial Martian atmosphere instead of space-like vacuum.

Under both desiccation and UV irradiation, biofilms exhibited a decline in culturable cells whilst total cell counts and cultivation-independent viability parameters remained relatively stable. This suggests that a part of the population became VBNC as a response to these stressors. Induction of the VBNC state might confer an increased tolerance towards stress to *D. geothermalis*.

In conclusion, a significant fraction of the population of *D. geothermalis* sustained viability under all stress conditions tested, with biofilm cells often being more stress-tolerant than planktonic cells. It seems that the increased stress tolerance of biofilms is a result of the induction of a VBNC state and the protective effect of the EPS matrix. Judging from the results obtained in this study, *D. geothermalis* might survive in space or on Mars for a limited period of time, especially if shielded against the harmful extraterrestrial UV radiation.