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## Abstract EANA2021-60

### *Ignicoccus hospitalis* – understanding its extraordinary radiation tolerance and an unsolved archaeal repair system

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*Ignicoccus hospitalis* is an obligate anaerobic, hyperthermophilic and chemolithoautotrophic archaeal microorganism that has exhibited an extraordinarily high tolerance against ionizing radiation (1). It was demonstrated by Koschnitzki, 2016 that *I. hospitalis* cells can remain viable after exposure to X-ray doses up to 12 kGy and it can completely repair DNA damages within one hour (2). *I. hospitalis* has a D10-value of ~5 kGy but it can remain metabolically active after being exposed up to 118 kGy (3). This exceptional radiotolerance is unexpected since ionizing radiation is not present in its natural environment - a submarine system of hydrothermal vents (4).

Given that DNA damages induced by high temperature are similar to those induced by ionizing radiation (5), we hypothesize that the radiation tolerance of *I. hospitalis* is a consequence of the intrinsic biological properties it uses to cope the extreme conditions of its habitat.

To unravel the mechanisms involved in the radiation tolerance of *I. hospitalis*, two approaches are currently being followed: exploring the intracellular-specific protection and monitoring the gene regulation of the DNA repair process.

Having multiple genome copies (polyploidy) might allow microbes for genomic DNA protection, maintenance, and repair at extreme conditions (6). The possibility of polyploidy in *I. hospitalis* was addressed. The number of genome copies per cell under different growth stages was calculated based on the quantitation of the total DNA content and the cell density from a series of culture aliquots. It was found that during the beginning of Log phase, *I. hospitalis* cells have  $0.85 \pm 0.35$  genomes/cell, in the middle of Log phase this value doubles to  $1.78 \pm 0.27$  genomes/cell, and at the stationary phase it drops again to  $0.59 \pm 0.37$  genomes/cell.

Compatible solutes have been extensively studied for their role in cellular protection against severe injuring influences like osmotic stress or heat shock, and for their function as radical scavenging molecules (7). A combination of different cultivation setups, like supra-optimal growth temperatures ( $92.5 \pm 95$  °C) and high salinity ( $3 \pm 5$  %w/v) were tested to influence the accumulation of compatible solutes. Then, desiccation survival was used as an indication of their presence within the cells. No cell survival after desiccation was detected, meaning there isn't significant compatible solutes accumulation.

An alternative intracellular protection mechanism in some microorganisms is based on the intracellular manganese/iron ratio (Mn/Fe). For instance, it has been reported that *Deinococcus radiodurans* accumulates high amounts of intracellular Mn and low levels of Fe (8). The determination of intracellular content of these two transition metals is currently ongoing, and it will be measured by ICP-MS.



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A set of transcriptomics experiments are currently in progress in order to investigate the up-or-down-regulation of genes related with DNA repair mechanisms. We will use dRNA-seq analysis to contrast different irradiation conditions with pre-selected time points during the DNA repair process and optimal conditions.

This project will help to gain knowledge on the DNA repair mechanisms in Archaea, and to better understand the limits of life.

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