Deep Underground Laboratory Integrated Activity in biology (DULIA-bio)

Report of Contributions

Contribution ID: **0** Type: **not specified**

The Gollum Project: Characterising subterranean bacterial communities in depth(s)

The literature describing microorganism inhabiting the very inside of rocks –rather that pores of the surface of rocks- are scarce. The few reports analysing the microbial diversity of rock inhabitants evidence, though, a rather high diversity of microbial taxa and metabolism pathways, including bacterial groups such as green non-sulfur, sulfur or iron reducing, and also methan producers, amongst others. The Somport tunnel crosses different rocks from the late Paleozoic ages, and includes several Facies. Its length, depth and diverse ecology make it a perfect site for extremophile ecology studies. We discuss our preliminary results and our sampling strategy aiming at characterizing through high troughput sequencing (metagenomics) the resident rock-associated bacterial communities. Sampling will be carried out at different depths and rocks, which will allow an unprecedented characterization detail of the subterranean microbial communnities living in a wide range of extreme underground biocenoses.

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Contribution ID: **1** Type: **not specified**

Probing the Deep Biosphere: Mines Arte Useful But Dedicated Underground Laboratories Will Be Even Better

Several important questions regarding the deep continental biosphere remain largely unanswered, including: 1) What factors control the maximum depth limit of life? 2) What are the sources of carbon for deep life? 3) What processes regulate the energy flux for deep life? and 4) Which organisms are actively recycling C and which are inactive, e.g., spores? Opportunities to address these important questions are limited by the cost of drilling to great depth. As an alternative to drilling from the surface, we've been sampling extremely deep fracture water (1-4 km) in the Kaapvaal Craton of South Africa via existing boreholes in gold, diamond, and platinum mines. We have found that 1) deep, ancient fracture waters contain diverse, indigenous microbial communities with cell abundances of ~103-4 cells mL, 2) cell turnover times are ~1-2 years at 3 km and 55℃, 2) deep saline fracture waters, ~2.0 Ga, are rich in radiolytically generated H2 and abiogenic hydrocarbons, 3) H2 flux is accentuated by seismic activity, 4) CH4 and CO2 are the carbon sources for biomass, and 5) more complex organic C appears to be generated in situ by microbes growing on these energy sources and carbon substrates. These subsurface anaerobic ecosystems are functioning completely independently from the surface photosynthetically driven world and could even be analogs for subsurface life on other planets. Culture-independent analyses (clone libraries and pyro-tag sequencing of 16S rDNA, metagenomic sequencing, transcriptomic sequencing, and single cell genome sequencing) have revealed indigenous, novel microbial communities with active metabolic and evolutionary processes. Sulfate reduction and methanogenesis are the dominant terminal electron accepting processes. The sulfate reducer Candidatus "Desulforudis audaxviator", has been detected in other deep fracture waters, e.g., in the Great Basin Desert, Finland, and off axial marine ridge basalt, suggesting that similar H2-driven subsurface microbial ecosystems are widespread. While mines are useful for short-term studies, dedicated underground laboratories can provide platforms for long-term monitoring and experimentation. Deep laboratories that are designed for other purposes, e.g., high-energy particle physics experiments, would be ideal. Some of these may be located in geological formations with the potential for abiotic H2 formation, i.e., sites featuring serpentinization, dissolution of ultramafic rocks, radiolysis of H2O, etc. Deep drilling for geomicrobiological studies can be situated at some distance from physics experiments and thus can be entirely compatible with ongoing studies. We envision a worldwide network of underground laboratories in which deep life studies collaborate with other scientific endeavors.

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Contribution ID: **2** Type: **not specified**

The underground biology at the Gran Sasso National Laboratory: from Pulex to Cosmic Silence

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on behalf of the Cosmic Silence collaboration

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In 1995, Satta and co-workers, taking advantage of the opportunity given by the presence of the underground Gran Sasso National Laboratory (LNGS) of the Italian National Institute of Nuclear Physics (INFN), carried out experiments aimed at investigating the possible modulation of the mutagenic potential of chemical agents in yeasts kept for 120 generations in extremely low radiation environment (LRE). This work showed that low radiation background impairs biological defence of the yeast Saccharomyces cerevisiae to chemical radiomimetic agents. Since then, in the framework of a wider collaboration, the biological underground research activity at LNGS continued. The so called Pulex experiments (with reference to the MACRO experiment, running in those years underground LNGS) further investigated the influence of the radiation environment on the metabolism and the stress response mechanisms of cultured rodent and human cells maintained in parallel, for an equal number of generations as yeasts, in conditions of different environmental radiation. The overall data have shown that, similarly to yeasts, mammalian cells maintained underground LNGS develop a different biochemical behaviour, compared to those maintained in reference radiation environment (RRE). In particular, cells cultured in the LNGS underground laboratory were less preserved from DNA damage induced by chemical and physical agents, and showed a reduced Reactive Oxygen Species (ROS) scavenging power than cells cultured in the external laboratory, e.g. set up at the Istituto Superiore di Sanità, Rome.
More recently, the Cosmic Silence Project started with the aim to deepen the investigation of the molecular mechanisms involved in the environmental radiation response of biological systems by using sensitive in vitro (A11 hybridoma cells derived from transgenic pKZ1 mouse model) and in vivo models (Drosophila melanogaster and pKZ1 transgenic mice), showing different levels of complexity in the phylogenetic tree.
Data on A11 cells, kindly donated by Prof. Pamela Sykes, Flinders University, Adelaide, Australia, obtained after 1 months of culture in LRE and RRE corroborate the hypothesis that environmental radiation contributes to the development and maintenance of defence mechanisms. Furthermore, the results obtained also show that modulation of the gamma component intensity of the underground environmental radiation does not significantly influence the biological response. Characterization of the radiation field by measurements and simulations, with special emphasis to the neutron component, is in progress in the LRE and RRE laboratories.
Presently, besides the cell culture laboratory, a new facility for housing living organisms is under construction underground at LNGS. Once it will be ready, the first step will be to start experiments using Drosophila melanogaster. Drosophila melanogaster is indeed a well-established model for genetic analyses on development, aging as well as DNA damage response. This organism shows several advantages in laboratory practice, due to its relatively short reproduction time (ten-days generation time), high fertility with many progeny. Finally, a large number of developmental processes are conserved between fruit fly and vertebrates, thus making Drosophila a great candidate as complementary organism for the Cosmic Silence experiment. Eventually, once the license from

the competent Authorities will be obtained, experiment will be carried out using the pKZ1 mouse model that represents the gold standard in this field.

Contribution ID: **3** Type: **not specified**

Metabolic effects of working deep underground

My research group uses the fruit fly, Drosophila melanogaster, to study genetics and metabolism and with a particular focus on stress responses and genetic variability. We have recently become interested in the metabolic effects of working deep underground.

The fruit fly, one of the best model species for over a century, is also a very good model for other aspects of biology, including physiology and metabolism. Small with a short lifespan, the species shares most of its genes, biochemistry, and metabolism, with humans. For the last 3 years, my research has been developing the fruit fly as a system for metabolomic, the combination and interactions of the 1000s of small metabolites (biomolecules such as sugars, lipids, and amino acids), studies. Recently, we have begun a project quantifying the metabolomic effects of deep mining, using SNOLAB as a test facility, to understand how humans respond to working deep underground. The project is ongoing, but initial results are exciting with approximately 10% of metabolites changing from a single trip underground. Ultimately, this research will help us better understand how our bodies respond to changes in pressure and point the way to simple methods, such as changes in diet, that could help limit the harmful long-term consequences of working deep underground.

Contribution ID: **4** Type: **not specified**

Deep science at the UK's Boulby Underground Laboratory

For more than a decade UK astrophysicists have been operating experiments to search for Dark Matter (the 'missing mass in the Universe') 1100m below ground in a purpose-built 'low-background' facility at Boulby potash mine in the North East of England. This facility - the Boulby Underground Laboratory - is one of just a few places in the world suited to hosting these and other science projects requiring a 'quiet environment', free of interference from natural background radiation. The race to find Dark Matter continues and Boulby currently hosts a number of internationally important projects dedicated to this cause. In the meantime the range of science projects looking for the special properties of deep underground facilities is growing and projects currently operating at Boulby range from astro & particle physics to studies of geology and geophysics, environmental radioactivity & radio-dating, Carbon Capture and Storage (CCS) and studies of life on Earth (and beyond!). This talk will give an overview of the Boulby Underground Laboratory, the science currently supported and plans for science at Boulby in the future.

Contribution ID: **7** Type: **not specified**

New underground laboratory in Pyhäsalmi Mine – CallioLab

Opportunities and possibilities on scientific experiments in the Pyhäsalmi Mine, Finland and the new underground laboratory (CallioLab) are presented. CallioLab is located in the Pyhäsalmi Mine, in central Finland. The Pyhäsalmi Mine is a copper and zinc mine being the oldest operating mine in Finland. The infrastructure of the site is excellent offering unique facilities and wealth of opportunities for a variety of scientific and commercial purposes. The deepest operational level is currently at the depth of 1430 meters (4000 m.w.e.). If the deepest site is not needed, there are several suitable locations also at higher levels up to shallow sites in the mine with already large caverns available. There are currently two experiments running in the lab in the field of astroparticle physics.

The current situation in the CallioLab is briefly explained as well as the instructions for the open call process. We are looking for collaborations from different scientific fields showing interest in the potential of the CallioLab and which could possibly use the existing or new halls in the CallioLab for their experiments.

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Contribution ID: **8** Type: **not specified**

Petri-dishing the Dirt: An Overview of Astrobiology Projects at Boulby

Astrobiology seeks to understand the origin, evolution and distribution of life in the Universe. The Boulby Mine in North East England offers a rare environment for advance in these areas, without exposure to the hazards of space.

The UK Centre for Astrobiology at the University of Edinburgh runs the BISAL facility (Boulby International Subsurface Astrobiology Laboratory) with the Boulby Underground Science Facility, taking advantage of the extreme environments in the mine to learn more about life in the deep subsurface and the instrumentation needed to probe such habitats remotely.

Some of the work in Boulby is focussed on the identification and analysis of the geobiology, chemistry and physics of these deep subsurface environments, such as brine geochemistry, characterising the microbial communities by culture and culture-independent techniques, and examining how they contribute to deep subsurface biogeochemistry. This can not only tell us about how Earth's microorganisms can adapt to life these in extreme environments, but allows us to assess the habitability of extraterrestrial environments. Another upcoming project entails looking at how radiation, and the lack thereof, can affect microbe viability, using the shielding from cosmic and geological sources of radiation that Boulby provides.

Additionally, utilising Boulby as an analogue site for testing techniques and instruments being developed for the exploration of other planets is realised in the MINAR project (Mining and Analogue Research). Various analytical instruments being built for planetary exploration have been tested in this logistically difficult and isolated environment providing vital information and insight into the challenges of robotic space exploration and sampling. As MINAR occurs in an active mine, technological spinoffs from MINAR are shared with the mining community, thus aiding mine environmental assessment such as ore quality and mine structural geology.

Contribution ID: **9** Type: **not specified**

Is the Deprivation of Natural Radiation a Biological Stress? Low Background Radiation Experiments (LBRE) at the U.S. Waste Isolation Pilot Plant (WIPP)

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The LBRE project was initiated in 2009 at the U.S. Waste Isolation Pilot Plant (WIPP) taking advantage of the shielding provided by the 650 m deep repository, with the objective to study the response of cells to radiation doses from the "other side of background." Added to the natural low-rad nature due to the salt composition of the Salado formation, is the use of a pre-World War II steel vault which allows us to reduce background radiation by an approximate factor of 400 (down to 0.2 nGy/hr), as modeled by Monte Carlo N-Particle (MCNP) analyses. We have employed two types of controls in which cells are exposed to normal, background levels of radiation: they are grown aboveground (Smith et al. 2011) or they are grown underground with a natural source (KCl) of radiation added back to approximate background levels. For comparative purposes we have developed a system to test low levels of above background radiation by deploying an array of 60Co coupons to monitor low level radiation above background. Initial results suggest that below background levels are more stressful than above background levels. Results also indicate that when deprived of natural levels of radiation, the radiation sensitive bacterium, Shewanella oneidensis, up-regulates the expression of genes belonging to three different families of stress response genes for DNA repair, reactive oxygen stress (ROS) scavenging and metal efflux pumps. After documenting the stress response, when we return the cells to radiation-sufficient conditions, the stress is allayed and the cells return to normal, control levels of gene expression and growth (Castillo et al. 2015). We have recently expanded our screening capabilities with next-generation sequencing to study the genome-wide response (transcriptome analysis) with an RNA-Seq approach. Preliminary data shows the differential regulation of 272 and 172 known-function genes in both S. oneidensis and D. radiodurans, respectively, in response to radiation deprivation. As an example, S. oneidensis patterns of gene expression suggest that radiation-shielding at WIPP increases the transcription of genes related to protein synthesis, signal transduction and transport processes while decreasing the transcription of genes involved in protein assembly.

Contribution ID: **10** Type: **not specified**

Low Background Radiation Intensity Appears to be Harmful to Life

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Summary

Very little is known about the influence of environmental radiation on living matter. In principle, relevant information can be acquired by analyzing possible differences between parallel biological systems, one in a reference radiation environment (RRE) and the other in a low-radiation environment (LRE). We took advantage of the unique opportunity represented by the cell culture facilities at the underground Gran Sasso National Laboratories (LNGS) of the Istituto Nazionale di Fisica Nucleare. We tested immortalized Human lymphoblast (TK6) cells and Chinese hamster lung (V79) cells against cell doubling time, mutation frequency, spontaneous and induced by X-rays micronuclei production, enzymatic activity against ROS. Our results corroborate the hypothesis that environmental radiation contributes to the development and maintenance of defence mechanisms in organisms living today

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Contribution ID: **11** Type: **not specified**

Microbes at extreme environments and the Spanish Network on Extremophiles

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Microorganisms are the creatures living under the most extreme conditions on Earth and the main candidates to inhabit other planets. Our research team studies the microorganisms on a variety of environments with a focus on extreme environments. We attempt to understand the functioning and role of microorganisms and to apply this knowledge into biotechnology. Currently, we are working on different topics.
For instance, we are analyzing the microbial diversity of a hot spring environment showing a temperature gradient of 50ºC. One isolate from this environment belonging to the Phylum Thermotogae, which is able to grow optimally at 80ºC, is being further analyzed through its genome project.
Other aspect being studied is the effect of periodic high temperature events on the microbes from temperate soils. The influence of temperature, pH and water content on thermophilic microbial communities and their enzymatic activity is under study and it is providing with interesting consequences on the actual role of some thermophiles in soils.
Microorganisms in nature could behave differently than in laboratory cultures. The growth and survival of extremophilic microorganisms is being analyzed under a variety of conditions to understand how microbial life is preserved in variable environments. Other investigations involve acidophilic communities, hyperthermophiles, anaerobic reactors, comparative studies of protein structure from thermophilic and mesophilic enzymes and analyses of various microorganisms growing under unique conditions.

The Spanish Network on Extremophiles involves most of Spanish researchers working on the study of extremophiles. Most aspects of research on extremophiles are covered within this network. These topics include halophiles, acidophiles, thermophiles, xerophiles, psychrophiles, and these researcher teams offer broad expertise on different techniques and disciplines such as microbiology, physiology, biochemistry, genomics, taxonomy, molecular biology, biotechnology, among others. Meetings are organized periodically (generally on an annual basis) to share our last findings and to propose new research collaborations.

Contribution ID: **12** Type: **not specified**

Geomicrobiology of the Iberian Pyrite Belt Subsurface

R. Amils and the IPBSL team Centro de Astrobiología(CSIC-INTA) and Centro de Biología Molecular Severo Ochoa (UAM-CSIC)

Although Darwin predicted life in the subsurface, the first paper describing life associted to deep basalt aquifers has les than 20 years. Terrestrial subsurface geomicrobiology is a matter of growing interest. On a fundamental level it seeks to determine whether life can be sustained in the absence of radiation, whereas it also aims to develop practical applications in environmental biotechnology. Subsurface ecosystems are also of interest in astrobiology. In spite of its interest, information concerning microbial abundance, diversity and sustainability in the subsurface is still scarce, mainly due to methodological limitations. We will report our experience on two drilling projects, MARTE and IPBSL devoted to understand the origin of Río Tinto, an extreme acidic river of 92 km in the western part of Spain.

Contribution ID: **13** Type: **not specified**

Some Lessons Tought by Searching for Life in the Universe

Juan Perez Mercader (Harvard U.)

Actively searching for Life in the Universe requires that we understand "what" we are looking for and "how" to search for it. This is a transdisciplinary pursuit that involves observational, experimental and phenomenological work in areas such as Interstellar Chemistry, Planetary Exploration, Origin of Life, Evolution of Life and the frontier between Physics, Chemistry, Biology and Engineering. In the last decades we have put together a picture of the Evolution of the Universe where on the basis of a few basic principles we understand many features of how it has changed through its history and evolved into the morphologies we observe today: we even have sets of equations that encapsulate this. We have also learned that chemical evolution takes place in the Interstellar Medium, and are beginning to understand many details of the formation of planetary systems and their planets from chemically and gravitationally evolved building blocks. In parallel we have developed a deep understanding of the Co-Evolution of Life in our planet, and discovered an extraordinary number of features and properties of extant and extinct living systems so that the accidental from the regular can begin to be disentangled. Planetary discovered made in the last few years imply that the limits of habitability are wider than suspected just a decade or so ago. This is an important part of the theme for planetary exploration where technologies, strategies and instruments are opening new vistas. Even though our understanding of Life and Living Systems is still far from the more simplicity-based understanding that we have for the Universe at large scales, the above is helping to make make progress in pinning down the accidental from the regular in Biology and, eventually, can hope to be able engineer living systems from the "Top-down". In this talk we will give an overall review of this exciting area.

Contribution ID: **14** Type: **not specified**

Examining the biological effects of ultra-low background radiation exposure within SNOLAB

The aim of our research is to examine the effects of prolonged exposure to ionizing radiation levels well below natural background. Our research group, along with others, have demonstrated that exposure to low-dose radiation slightly above background levels can have beneficial effects on biological systems. We hypothesize that natural background radiation is essential for life and maintains genomic stability in living organisms, and that removal of background radiation could have detrimental effects. This research will be conducted within SNOLAB in Sudbury, Ontario, Canada. SNOLAB is located within an active Nickel mine 6,800 feet (approximately 2 km) below the Earth's surface. The laboratory is a class 2000 clean room and has approximately 50 million times less cosmic radiation and 100 million times less radioactivity than would be found in the aboveground environment. Initial experiments will utilize both a cell culture and a whole organism (lake whitefish) model. Cell cultures will be grown for many generations underground and the rate of spontaneous DNA damage and mutations will be compared to surface controls. Low background adapted cells will also be tested for their response to an induced stress. We have previously shown that exposure to low-dose radiation can stimulate growth in developing lake whitefish embryos. Embryos will be raised within SNOLAB and in our surface control laboratory to examine how an ultra-low background radiation environment impacts survival, development rate and growth.