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Biological research at the deep underground low radiation background laboratory (DULB-4900) of Baksan Neutrino Observatory

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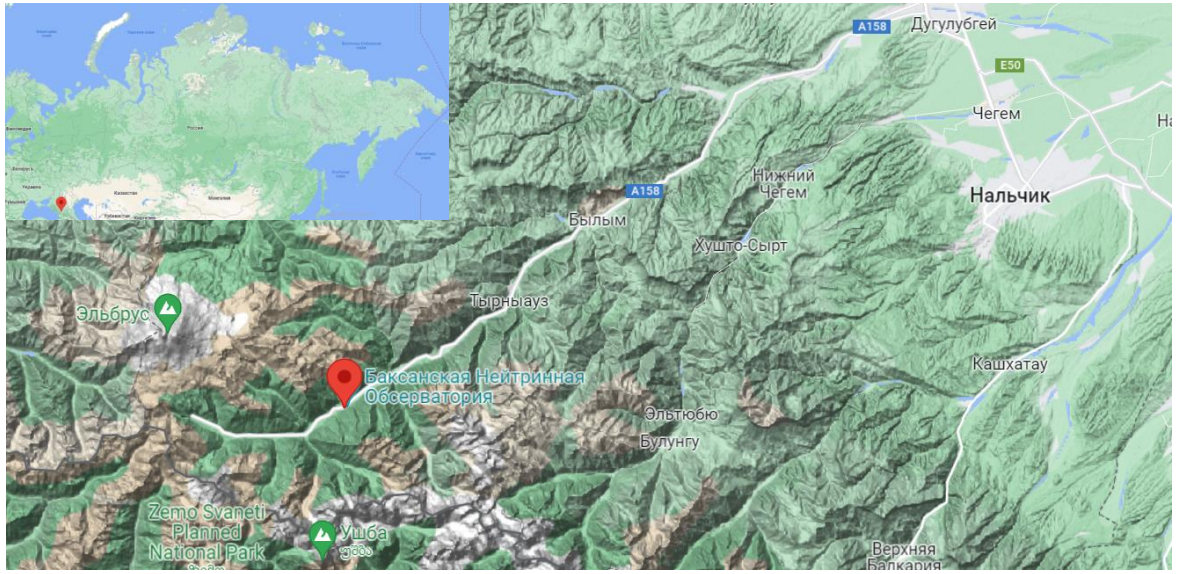
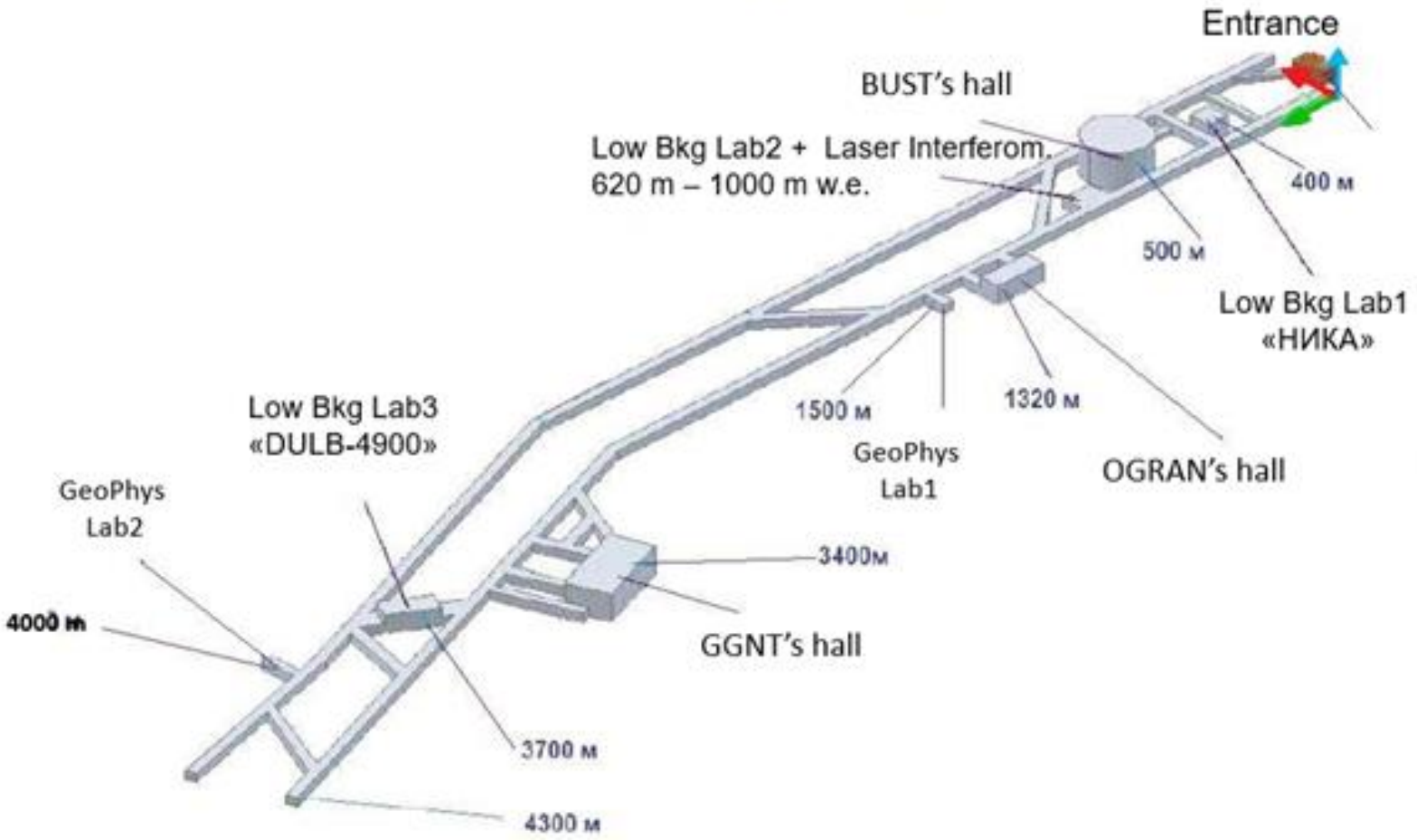
Baksan Neutrino Observatory

BNO (INR RAS) was founded in the late 60-80th in the Neutrino Village (1700 m.a.s.l.) located 25 km from the highest european mountain Elbrus (5642 m, dormant volcano) under the peak of Andyrchy mountain (3937 m). Main scientific goals of BNO are related to fields of astrophysics, particle physics and nuclear physics. Moreover newer topics of interdisciplinary research are linked to geophysics, geology and biology (since 2019)



General view of underground objects of BNO



I. Biophysical research at the deep underground low radiation background laboratory

This issue aims to register for the first time a response of complex multicellular organism (fruit fly *Drosophila melanogaster*) to the reduced environmental radiation background at a whole transcriptome level and to analyze the obtained results in terms of the impact of different types of stress.

The systematic studies of low background ionizing radiation effects on living organisms of different organizational level aiming to:

*** reveal fundamental principles of low background radiation effects on biological organisms**

*** distinguish regulational mechanisms of response to background doses of ionizing radiation**

*** simulate conditions of artificial and natural radiation background environments also relevant to deep space and exoplanets, that is beneficial for nuclear safety, medical and space exploration studies**

*** to improve LNT dose-risk model and etc.**

Does an absence of natural background radiation affect complex living organisms?

Nowadays, it remains unclear whether an absence or significant decrease in the level of radiation background have direct effect on living organisms, though some data obtained in low radiation background laboratories may be interpreted as predominately suppressing for biological organisms

Determination of biological effects in complex animal organisms: Experiments at LNGS

Fruit Flies Provide New Insights in Low-Radiation Background Biology at the INFN Underground Gran Sasso National Laboratory (LNGS)

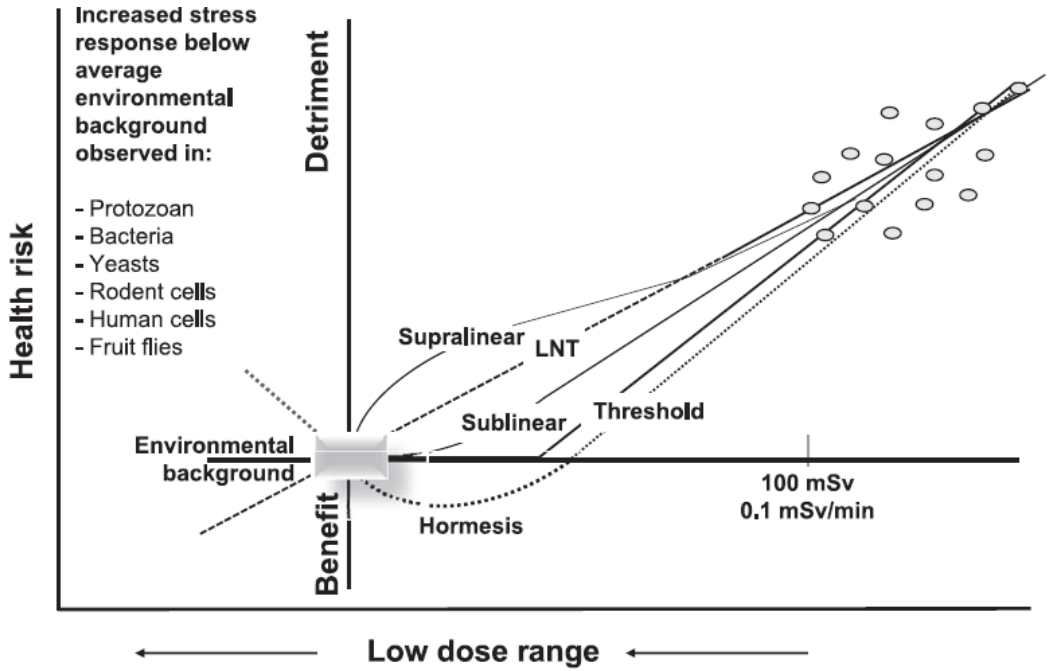
Patrizia Morciano,[a] Francesca Cipressa,[a,c] Antonella Porrazzo,[a] Giuseppe Esposito,[b] Maria Antonella Tabocchini[b,c,1] and Giovanni Cenci[a,c]

[a] *Dipartimento Biologia e Biotecnologie ''C. Darwin'', SAPIENZA Università di Roma, Rome, Italy;* [b] *Istituto Superiore di Sanita (ISS) and Istituto Nazionale di Fisica Nucleare (INFN), Sezione Roma 1, Rome, Italy; and* [c] *Museo Storico della Fisica e Centro Studi e Ricerche ''Enrico Fermi'', Rome, Italy*

…the first evidence of the influence of the radiation environment on lifespan, fertility and response to genotoxic stress at the organism level…

…changes in Drosophila growth and development are observed as soon as after 2 weeks of permanence underground, giving suggestion for possible mechanisms involved…

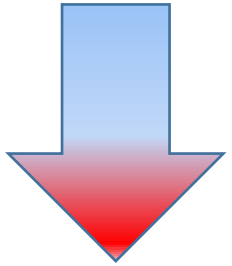
Determination of biological effects in complex animal organisms: Experiments at LNGS



..environmental radiation is necessary to trigger mechanisms that increase the ability to respond to stress...

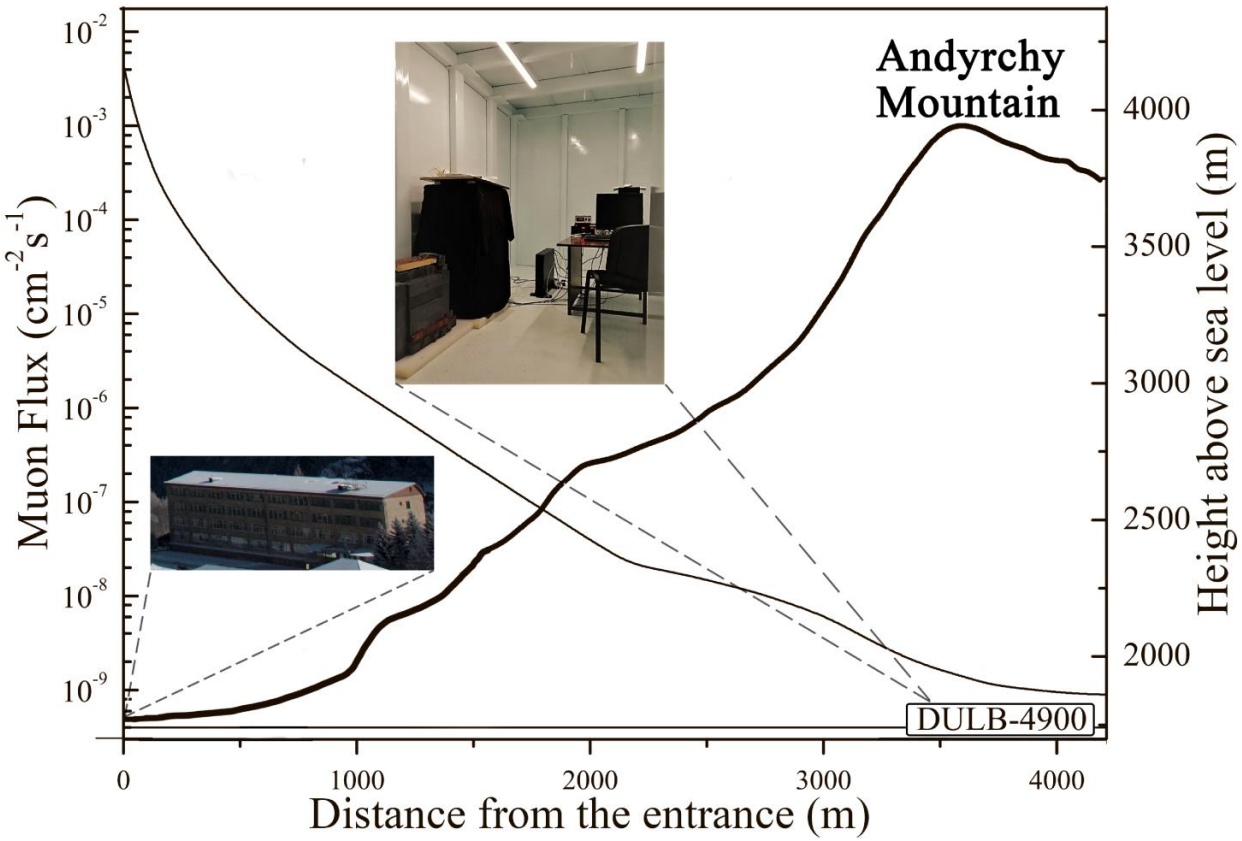
JINR

Dec. 2019-Present: **Deep underground low background laboratory DULB-4900 of Baksan Neutrino Observatory INR RAS encouraged Dzhelepov Laboratory of Nuclear Problems JINR initiative of collaboration in Life Science studies and hosted experiments concerning determination of low radiation background impact on model organisms.**



Our goal: **to determine for the first time a response of complex multicellular organism *D. melanogaster* Oregon-R line to the reduced natural background radiation at the whole transcriptome level after complete developmental cycle (14 days of exposure) in low (DULB-4900) and natural radiation background laboratories by comparison RNA-seq gene expression profiles and by comparative transcriptome analysis with data deposited at NCBI GEO and NASA GeneLab databases.**

Biological experiments in the Deep underground low background laboratory DULB-4900



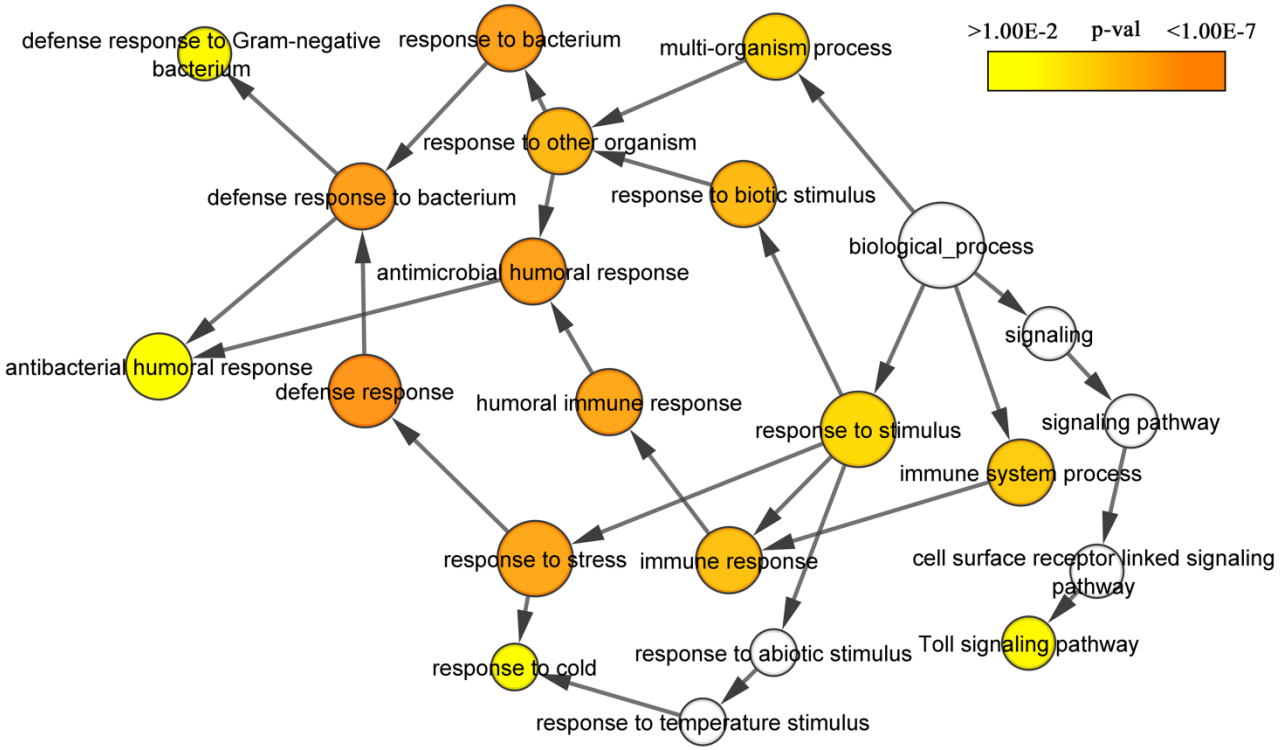
Locations and facilities at Baksan Neutrino Observatory INR RAS : institute building and the deep underground low-background laboratory DULB-4900 at the 3700 m from the entrance in horizontal tunnel under the Andyrchy mountain.

Biological experiments in the Deep underground low background laboratory DULB-4900

Background component	Data source	Ground laboratory in the institute building, BNO (INR, RAS)	Chamber of DULB-4900, BNO (INR, RAS)
Gamma, nGy h^{-1}	NaI(Tl) crystal scintillation detector [32]	120	0.02
Neutrons, nGy h^{-1} ($cm^{-2}s^{-1}$)	Helium proportional counter [32, 33]	3.45 (4.67×10^{-3})	~0 (3.8×10^{-7})
Muons and cosmic rays, nGy h^{-1} ($cm^{-2}s^{-1}$)	Determined by the altitude (m.a.s.l.) and covering rock massive (m.w.e) [32]	24.4 (2.0×10^{-2})	~0 (3.0×10^{-9})
Radon, nGy h^{-1} (Bq m^{-3})	Experimental set-up to continuously measuring the radon activity [33, 34]	1.19 (35)	0.85 (25)
Nutrition medium ^{40}K, nGy h^{-1} (Bq kg^{-1})	Spectrometer SNEG	15.5 (6.7)	15.5 (6.7)
Total dose rate, nGy h^{-1}	Estimation	164.5 (190.7—based on UNSCEAR data)	16.4

Experiment was performed simultaneously in both laboratories at equalized environmental conditions except radiation background: ~190 nGy/h in surface laboratory and ~ 16.4 nGy/h in DULB-4900.

Transcriptome analysis (RNA-seq): Gene ontology biological process term enrichment analysis



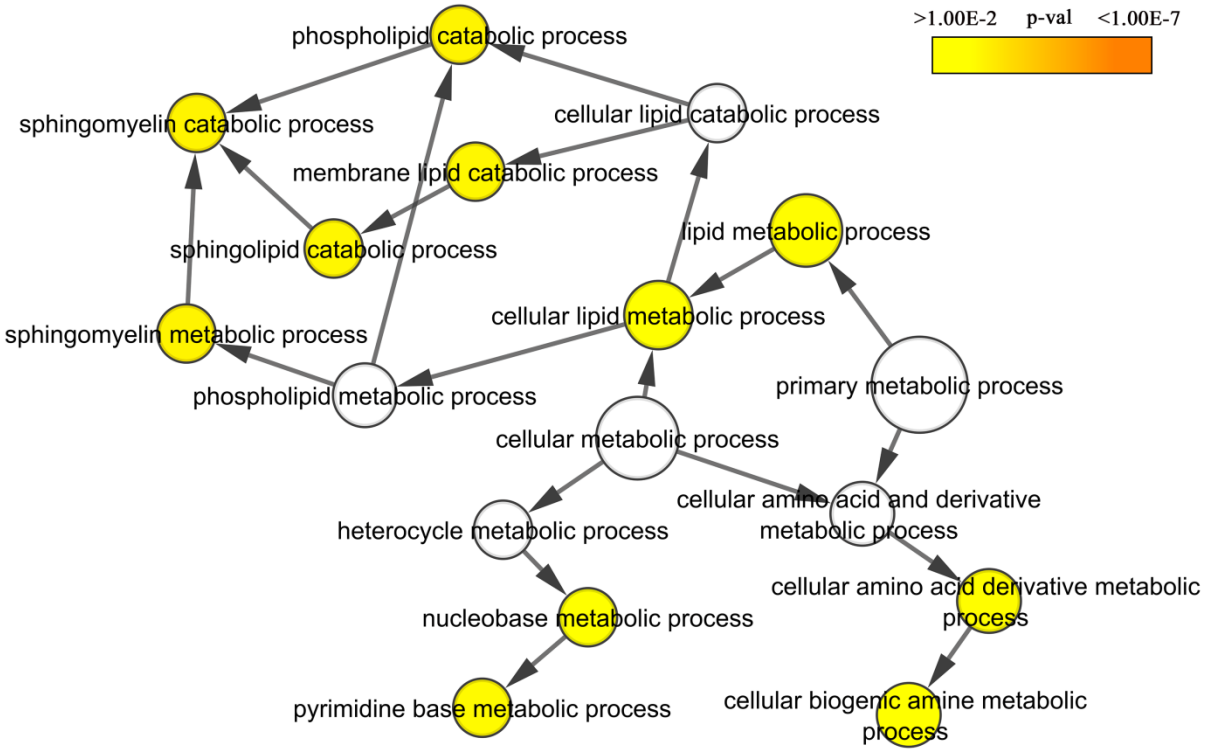
Enriched biological processes for up-regulated genes (activated expression)

In this work, the input of low background radiation was estimated at the level of multicellular complex organism by comparing RNA-seq gene expression data for LB-flies (low-background) and NB-flies (natural-background).

The relatively small list of 76 significantly altered genes (FDR<0.05 FC>1.5) for response of organisms to conditions of DULB-4900 was obtained.

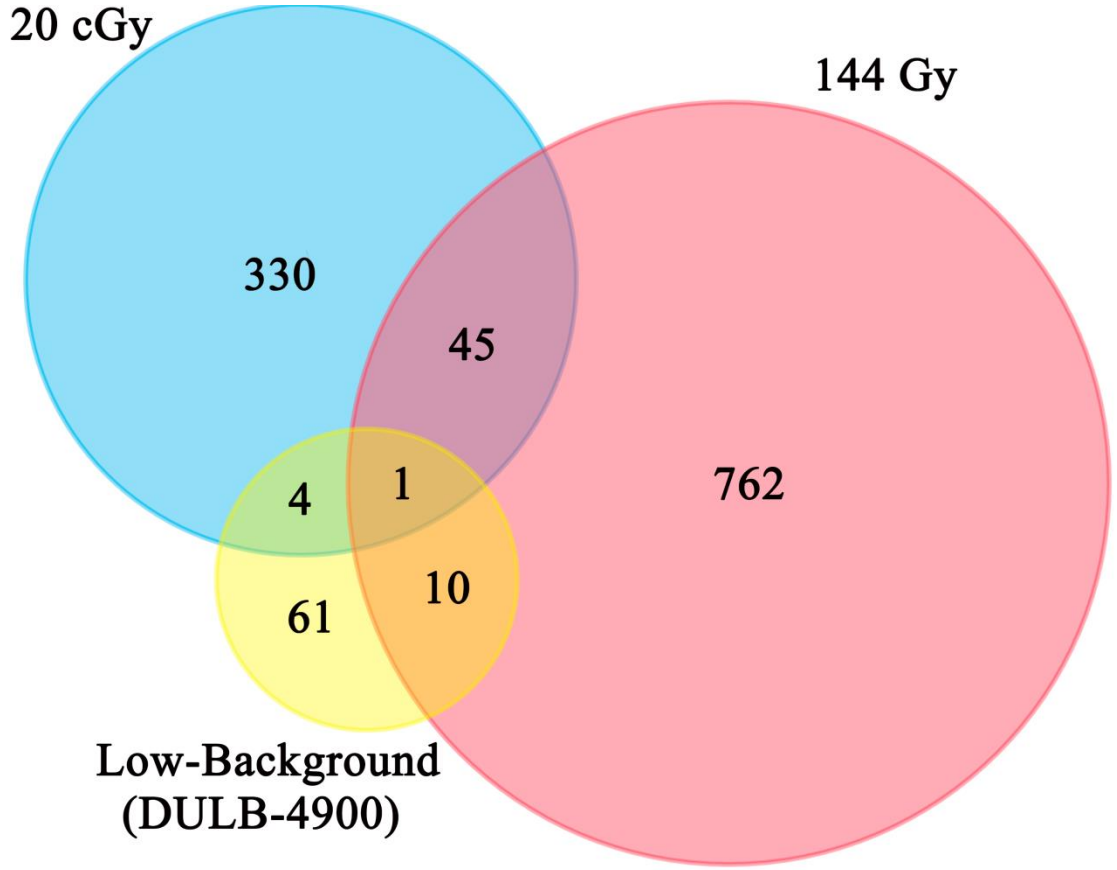
For up-regulated genes overrepresented biological process GO terms relate to activation of the immune system process (19,4%, p-value < 0.01) and response to stimulus (45,2%, p-value < 0.01), that is a consequence of a violation of cellular homeostasis.

For down-regulated genes biological process GO can be roughly combined into a group of cellular metabolism (56,8% of down-regulated in LB-flies genes, p-value < 0.05). Also several genes as *Vmat*, *nAChRbeta1*, *tutl*, *hll*, *Shmt* taking part in neural signal transmission were down-regulated in LB-flies.



Enriched biological processes for down-regulated, genes (suppressed expression)

Comparative transcriptome analysis: effect of DULB-4900 vs responses to low and high doses of ionizing radiation

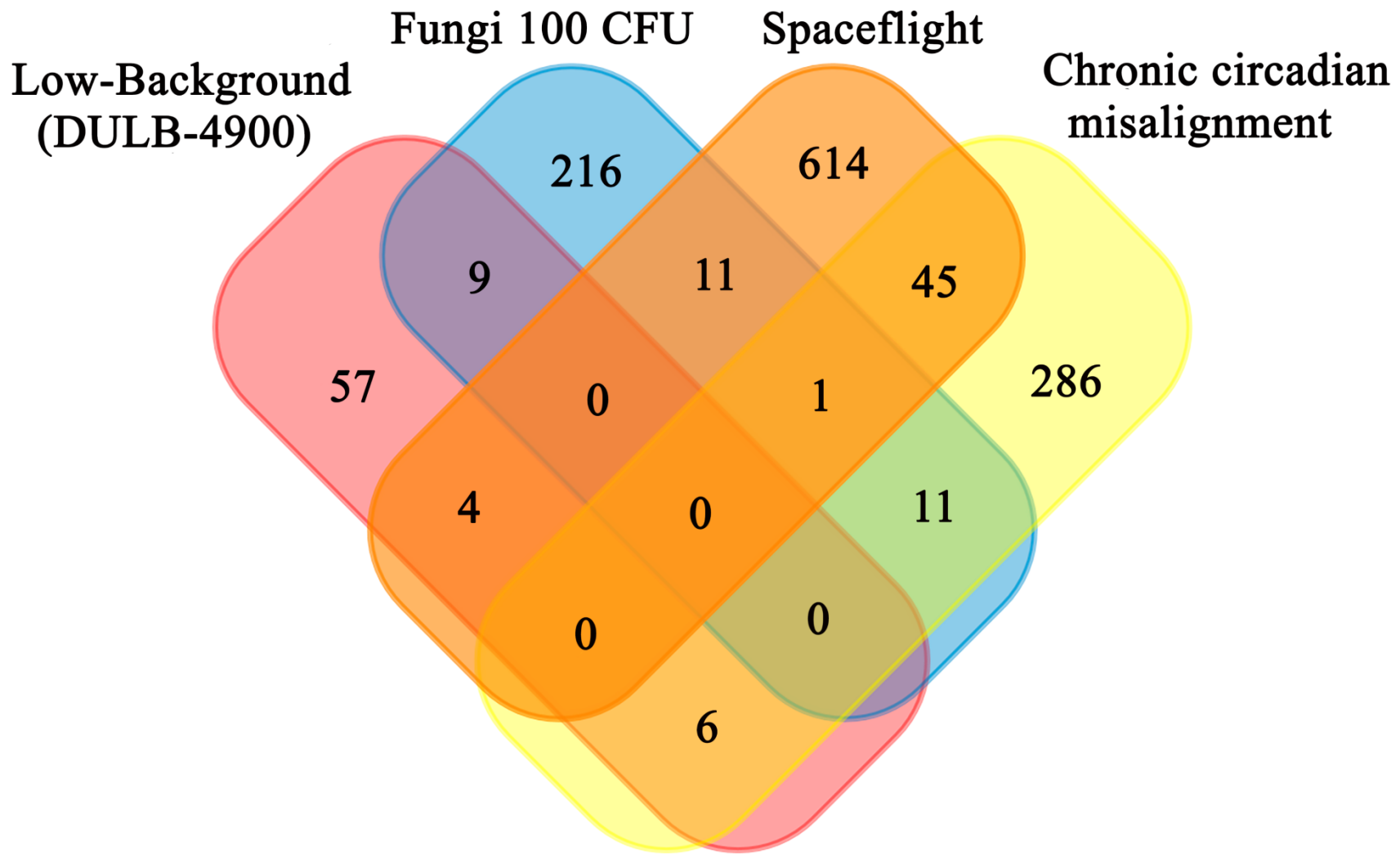


Venn diagram representing the quantity of shared genes for *D.melanogaster* developed in DULB-4900 and after gamma irradiation (0.2 and 144 Gy)

Common biological processes for flies exposed to DULB-4900 and after irradiation are related to immune response, defense response, transmembrane transport and cellular metabolic process, that is often an indicator of *D. melanogaster* general stress response.

No observed changes in expression of genes that may be specifically involved in response to radiation

Comparative transcriptome analysis: effect of DULB-4900 vs responses to various environmental factors



Venn diagram representing the quantity of shared genes for *D.melanogaster* developed in DULB-4900 and exposed to several environmental factors

Conclusion on biophysical and radiobiological studies at DULB-4900


PLOS ONE

First transcriptome profiling of *D. melanogaster* after development in a deep underground low radiation background laboratory

Mikhail Zarubin, Albert Gangapshev, Yuri Gavriljuk, Vladimir Kazalov, Elena Kravchenko

Published: August 5, 2021 • https://doi.org/10.1371/journal.pone.0255066

For fruit flies exposed to DULB-4900, not so many differentially expressed genes (78) with 1.6-32 times altered expression were identified

In DULB-4900, up-regulation of genes related to immune response, response to stimuli and down-regulation of genes involved in primary metabolic processes were observed. That approves activation of immune response and response to stimuli


Biological Effects of Low Background Radiation: Prospects for Future Research in the Low-Background Laboratory DULB-4900 of Baksan Neutrino Observatory INR RAS

M. P. Zarubin, O. A. Kuldoshina & E. V. Kravchenko

Physics of Particles and Nuclei **52**, 19–30 (2021)

Changes in gene expression reflect an adaptive response to DULB-4900 conditions that not typical and stressful for terrestrial organisms, possibly due to the chronic lack of external natural stimuli

Comparative transcriptome analysis of obtained data and transcriptome profiles from NCBI GEO and NASA GeneLab databases reveals low similarity of responses to irradiation or several deep underground environmental stresses

Perspectives of biophysical and radiobiological studies at Baksan Neutrino Observatory

Our work is the first initiative of biological studies at Baksan Neutrino Observatory INR RAS. We expect, Baksan Neutrino Observatory possess outstanding potential for interdisciplinary studies on tasks of biophysics, radiobiology, astrobiology and medicine

Further genetic experiments with *D. melanogaster* devoted to long-term effects of low-background conditions and chronic low dose irradiations are highly demanded. And RENOIR project at LNGS might be the next step in this field

For further step we will focus on biological impact of cosmic component of natural background radiation and it's components (especially muons): experiments at DULB-4900 accomplished by chronic low dose irradiation experiment at muon torch of high energy accelerator facility U-70 (IHEP)

II. Investigation of extremophile organisms inhabiting deep underground spring in the far unused parts of BNO tunnel (~4.5 km)

Unique properties of far parts of Baksan Neutrino Observatory makes highly perspective the search of extremophile organisms

* Deep underground location (~4.5 km) beneath Andurchy mountain (~2.3 km)

*One of the deepest microbiomes in Caucasus region and Russia

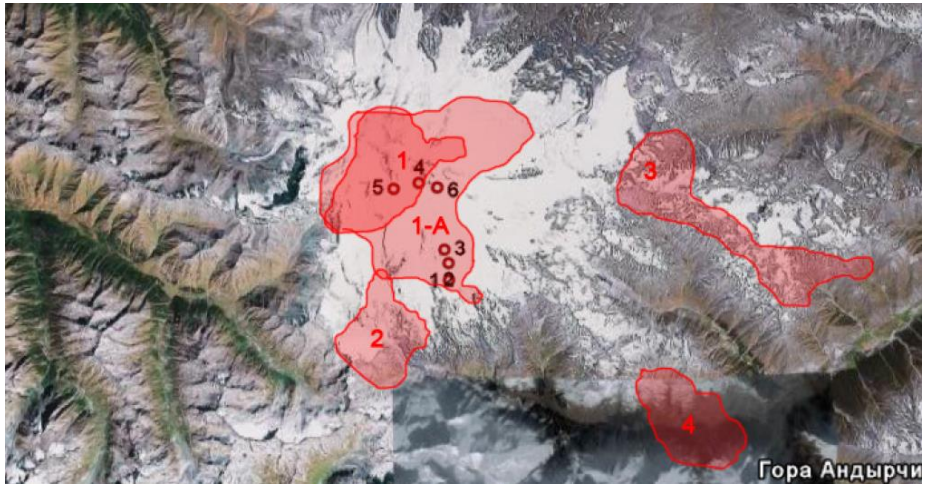
* Isolated ecosystem

* Nearby Elbrus volcanic center

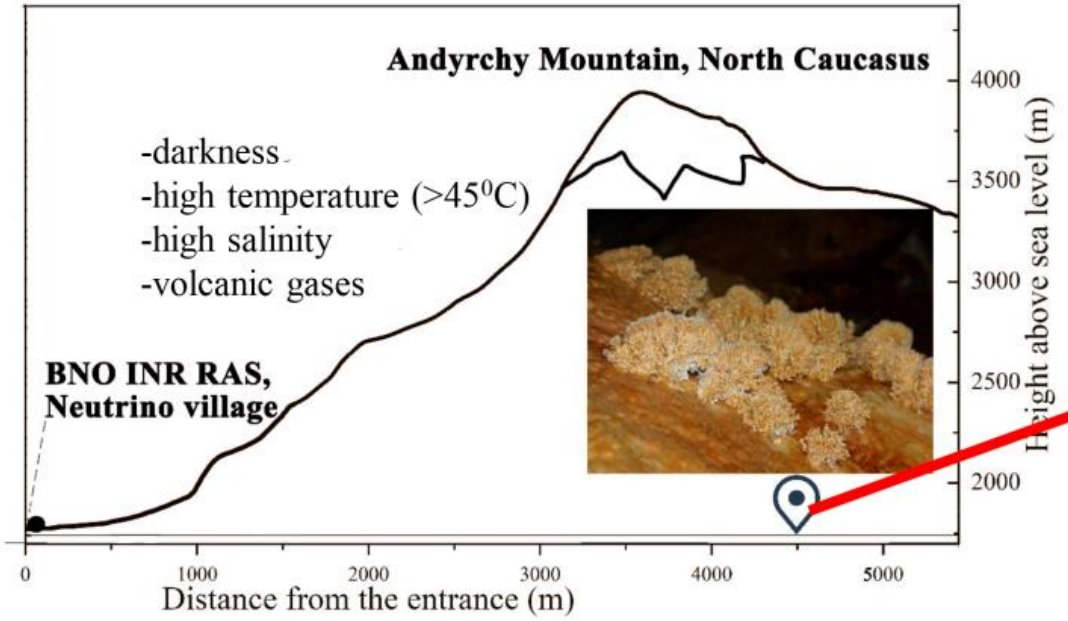
* Complex geothermal system in the region

* Low content of organic materials and lack of energy sources for heterotrophic organisms

* High temperatures and salinity, presence of volcanic fluids and gases



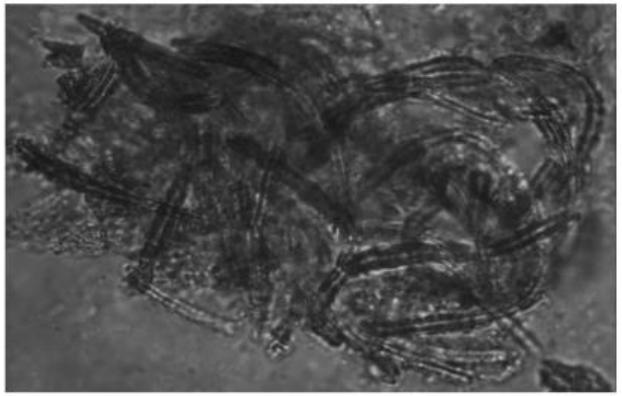
Study of one of the deepest microbiomes obtained in Caucasus region and Russia

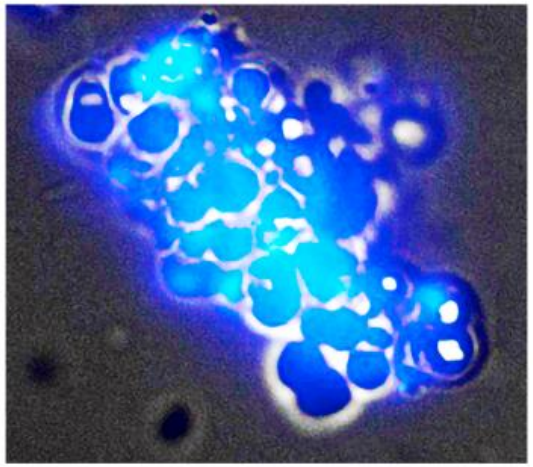


Total DNA extraction

New generation sequencing (whole genomes)

31 million paired reads of DNA fragments





5 NEW genus of bacteria

6 NEW species of bacteria

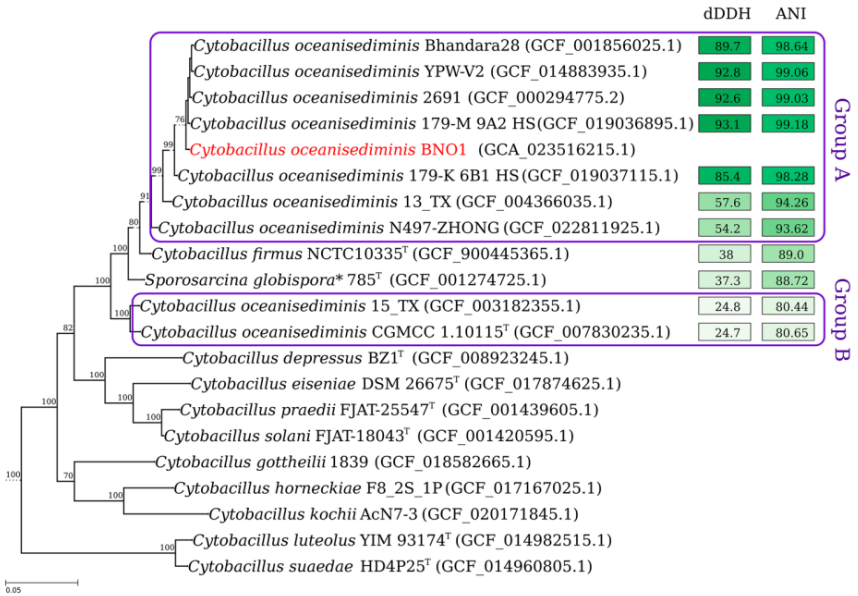
New spices and genus have been named in honour of JINR and INR RAS

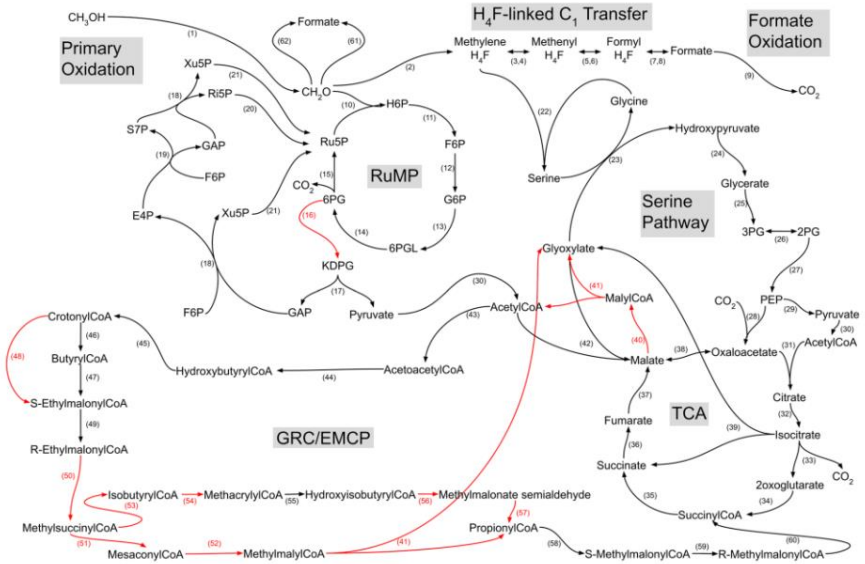
First results: Cultivated novel extremophile specie *Cytobacillus pseudoceanisediminis* and it's complete bacterial genome

Cytobacillus pseudoceanisediminis sp. nov., A Novel Facultative Methylotrophic Bacterium with High Heavy Metal Resistance Isolated from the Deep Underground Saline Spring

Kirill Tarasov, Alena Yakhnenko, Mikhail Zarubin, Albert Gangapshev, Natalia V. Potekhina, Alexander N. Avtukh & Elena Kravchenko

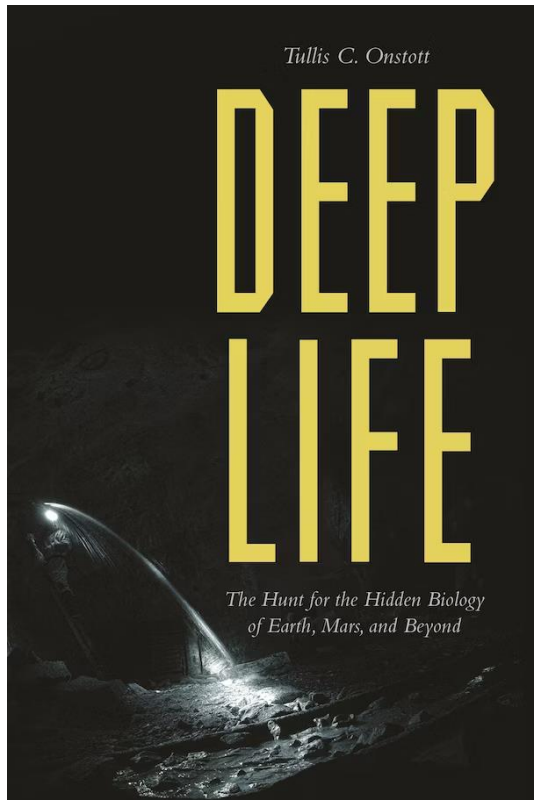
Current Microbiology **80**, Article number: 31 (2023)

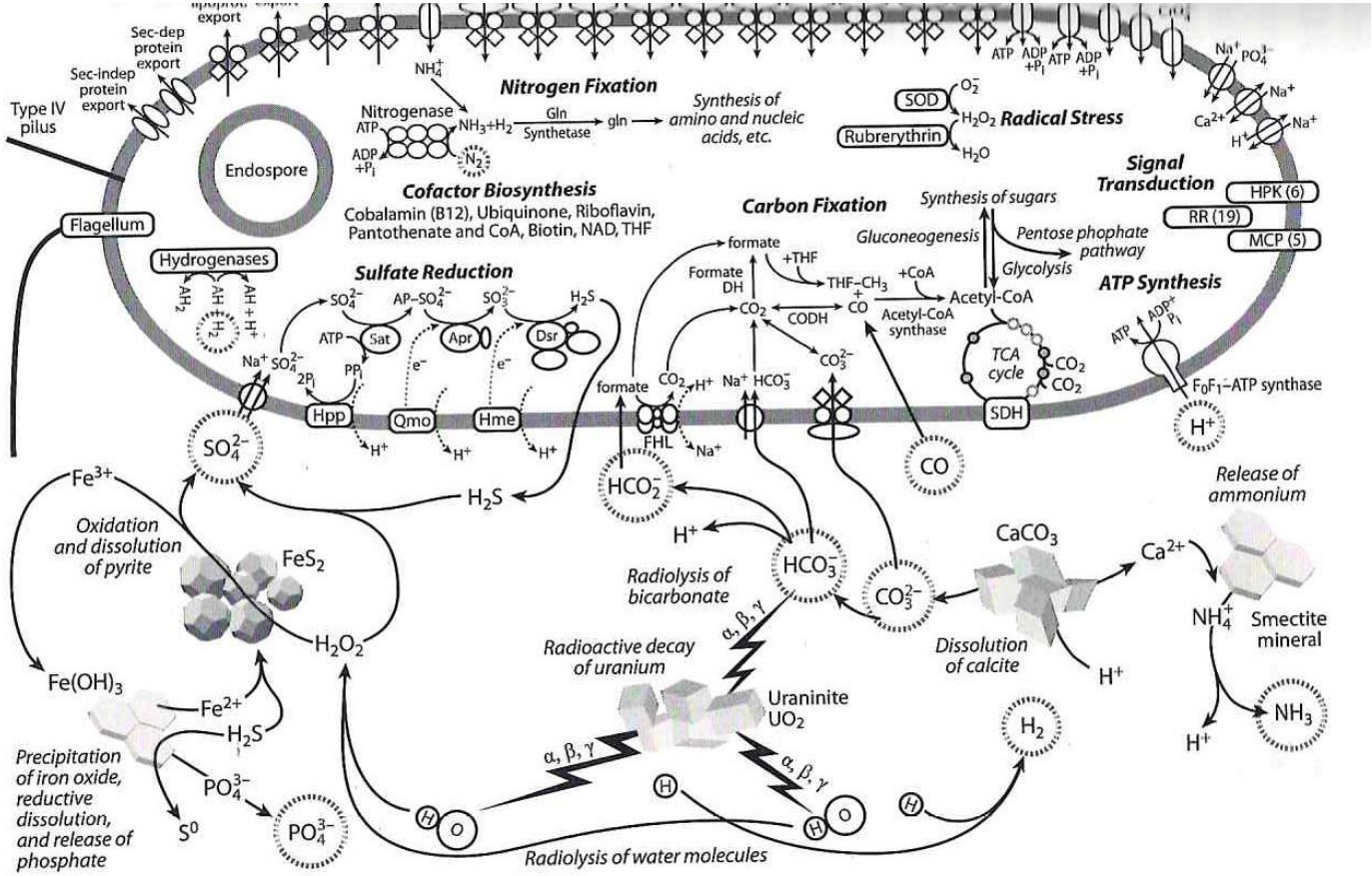




Soon to published: metagenome of deep underground spring (~4.5 km)

Perspective of deep underground biological studies at PAUL and South Africa





Example: works by Onstott Tullis (1955 – 2021) and colleagues at deep underground mines of RSA

… unraveled a complex high-energy pathway that coupled abiotic radiolytic splitting of water molecules and subsequent biotic electron transfer from H_2 to oxidants by a new bacterial species given the name Desulforudis audaxviator, which lives kilometers below the land surface in the gold-bearing Witwatersrand formation in South Africa… — Gerhard Kminek

Thank you for your attention!