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Aug 19 – 22, 2024 Guildhall, York

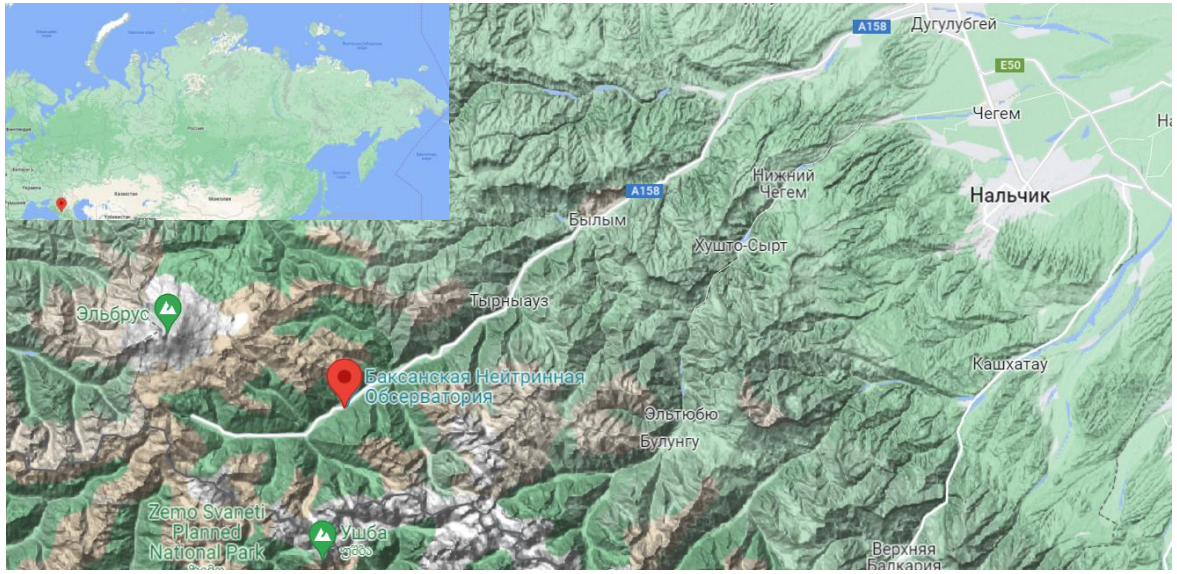
Biological researches at the deep underground low radiation background laboratory (DULB-4900) and tunnel of Baksan Neutrino Observatory: biophysics and microbiology

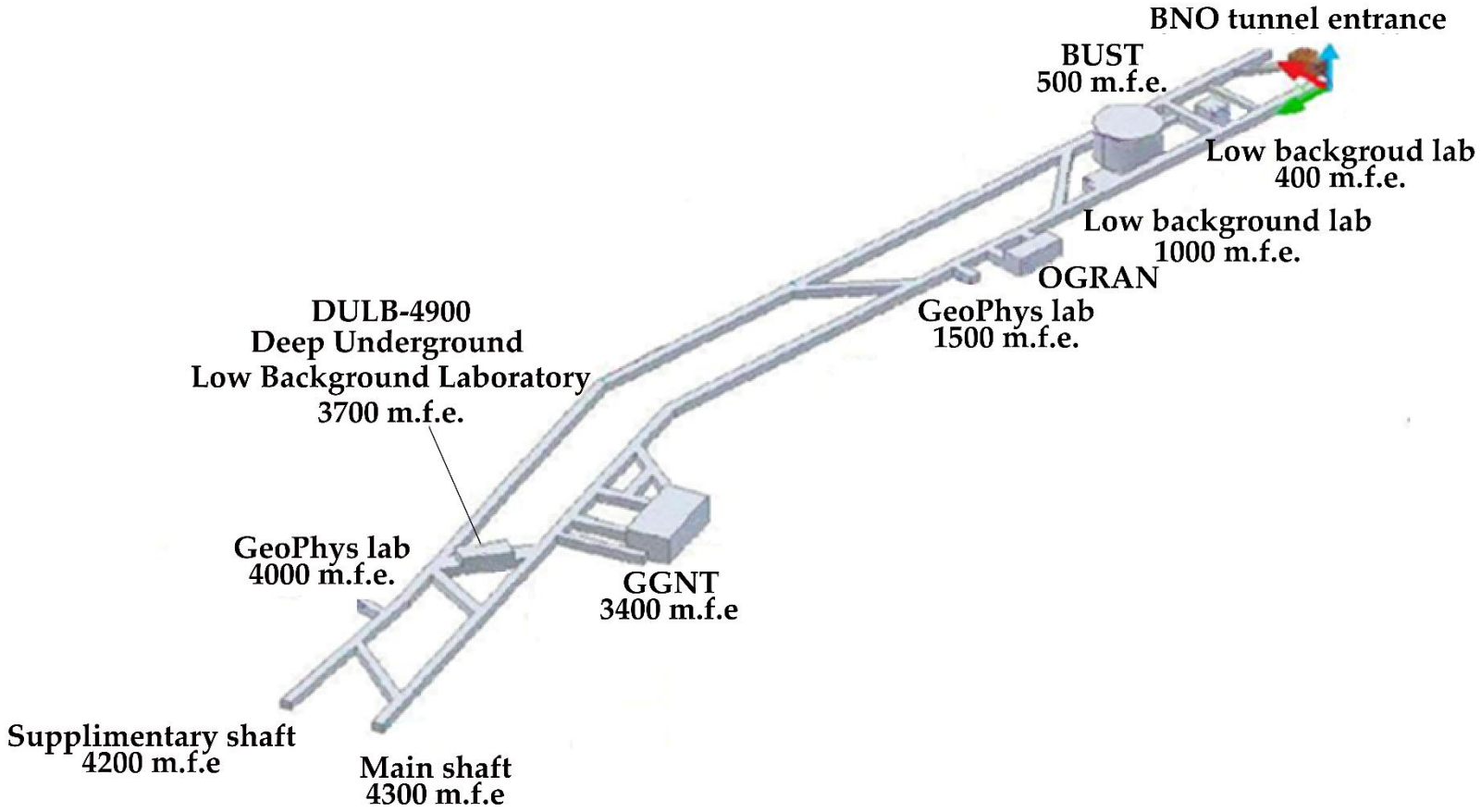
Zarubin Mikhail[1], Kravchenko Elena[1], Gangapshev Albert[2]

[1]Joint Institute for Nuclear Research, Dubna, Russia
[2]Institute for Nuclear Research, Moscow, Russia
mzarubin@jinr.ru

Baksan Neutrino Observatory

BNO (INR RAS) was founded in the late 60-80th in the Neutrino Village (1700 m.a.s.l.) located 22 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)




BNO tunnel entrance
BUST
500 m.f.e.
Low backgroud lab
400 m.f.e.
Low background lab
1000 m.f.e.
OGRAN
GeoPhys lab
1500 m.f.e.
DULB-4900
Deep Underground
Low Background Laboratory
3700 m.f.e.
GeoPhys lab
4000 m.f.e.
GGNT
3400 m.f.e
Supplimentary shaft
4200 m.f.e
Main shaft
4300 m.f.e

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

At the first stage of biological researches, the aim was to register the response of complex multicellular organism (fruit fly *Drosophila melanogaster*) to the low radiation background for the first time at the 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 complexity aim 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)

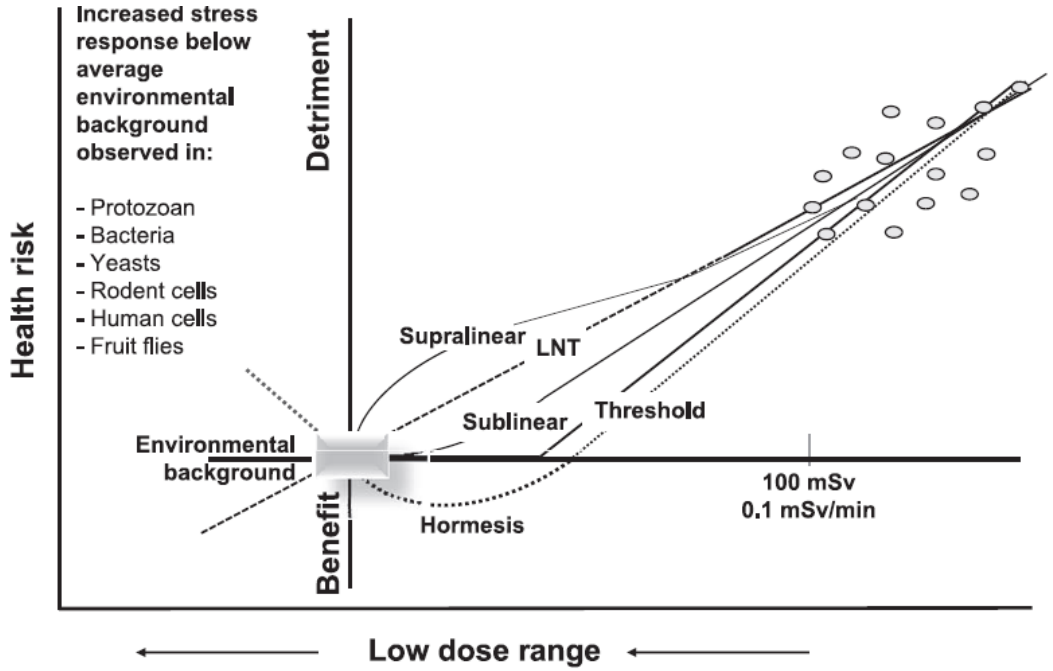
Patrizia Morciano,[a] Francesca Cipressa,[a,c] Antonella Porrazzo,[a] Giuseppe Esposito,[b] Maria Antonella Tabocchini[b,c,1] and Giovanni Cenci[a,c]

[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



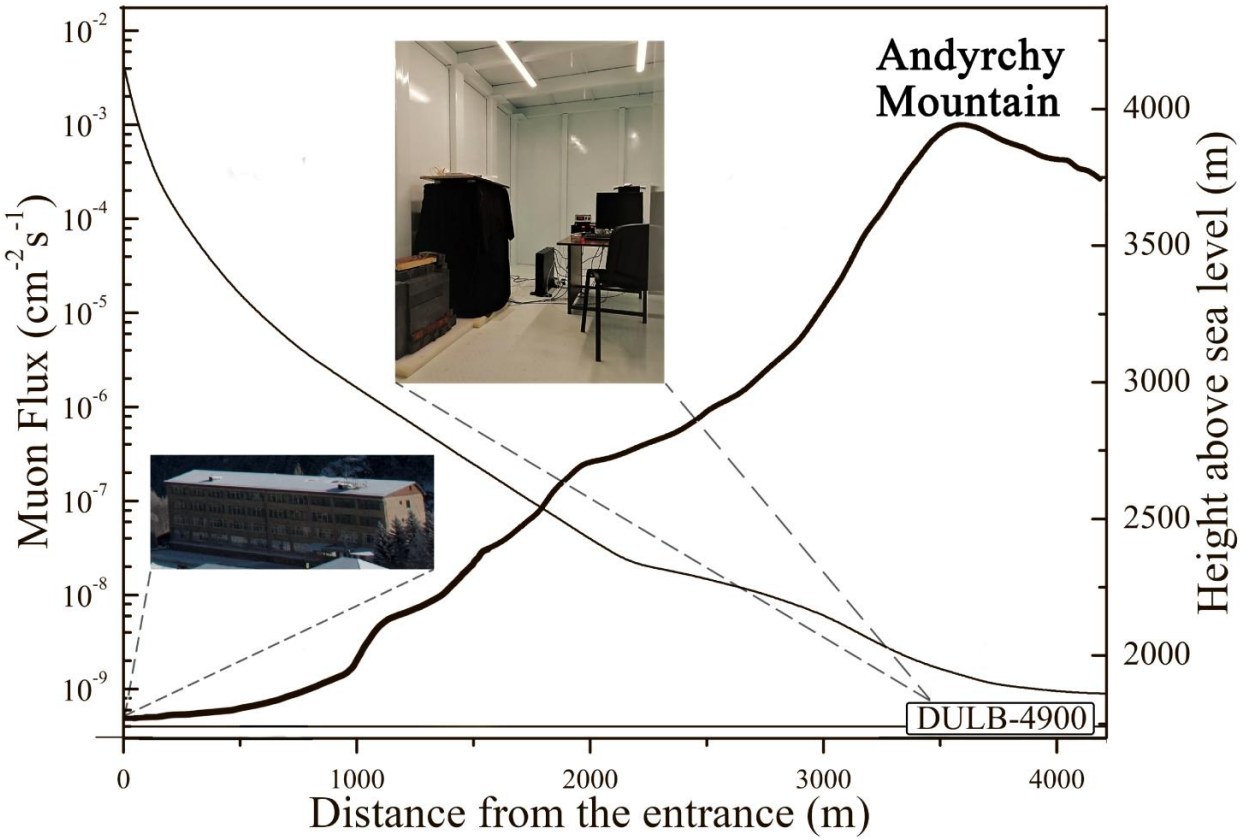
P. Morciano et al. 2018

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

Nov. 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 background radiation impact on model organisms**

First experiment: **to determine the response of complex multicellular organism *D. melanogaster* Oregon-R line to the reduced natural background radiation for the first time 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



Involved locations and facilities of Baksan Neutrino Observatory:

(i) surface institute building

(ii) deep underground low-background laboratory DULB-4900 at 3700 m from the entrance of horizontal tunnel under the Andyrchy mountain

(iii) 3 low background laboratories in total

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

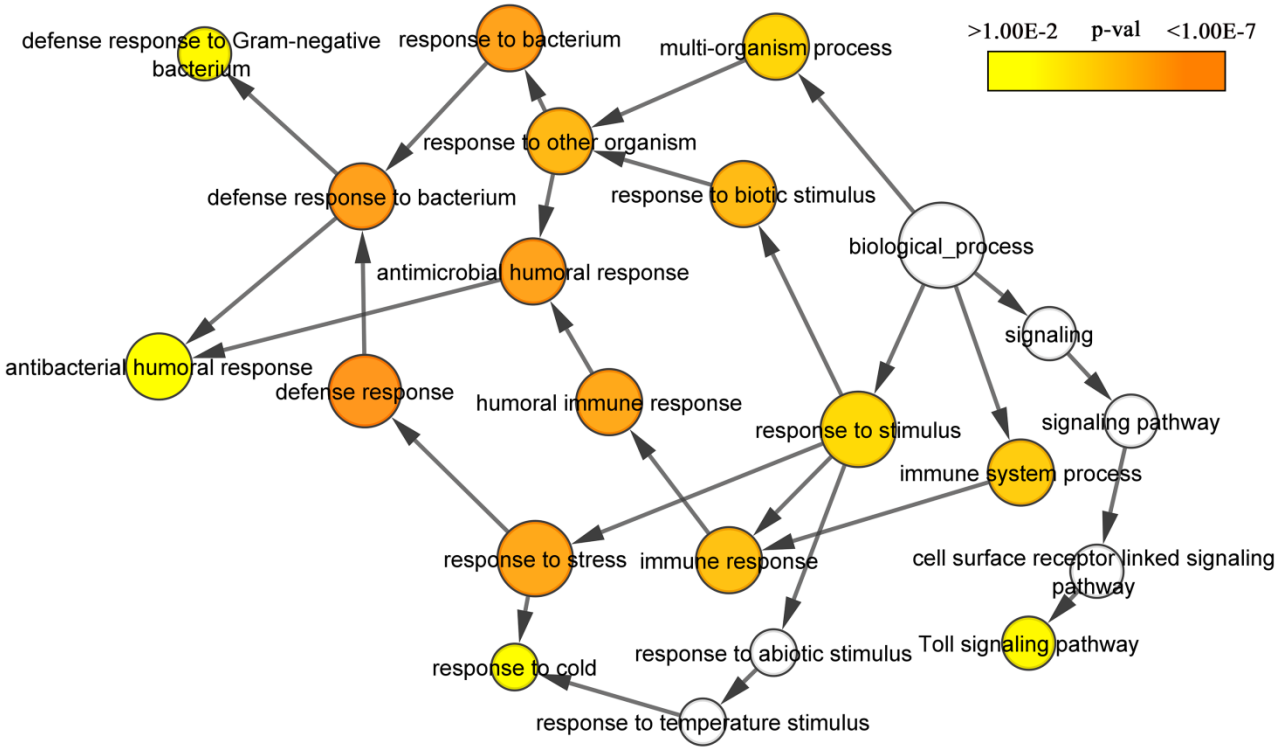
Background component	Data source	Ground laboratory in the institute building, BNO (INR, RAS)	Chamber of DULB-4900, BNO (INR, RAS)
Gamma, nGy h^{-1}	NaI(Tl) crystal scintillation detector [32]	120	0.02
Neutrons, nGy h^{-1} ($cm^{-2}s^{-1}$)	Helium proportional counter [32, 33]	3.45 (4.67×10^{-3})	~0 (3.8×10^{-7})
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 two laboratories at equalized environmental conditions except radiation background:

(i) ~190 nGy/h in surface laboratory

(ii) ~ 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)

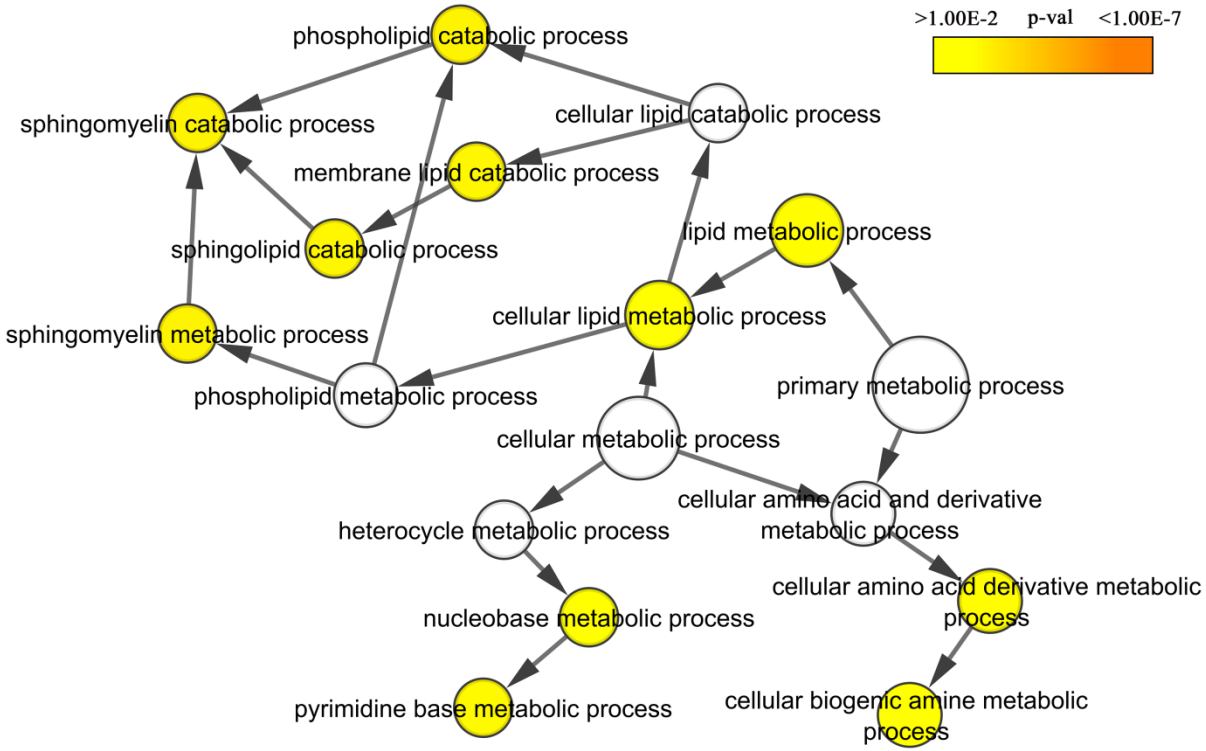
Effects of low background radiation in multicellular complex organisms was estimated by comparing RNA-seq gene expression data for LB-flies (low-background) and NB-flies (natural-background)

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

For up-regulated genes overrepresented biological process GO terms were related to the activation of the immune system process (19,4%, p-value < 0.01) and response to stimulus (45,2%, p-value < 0.01)

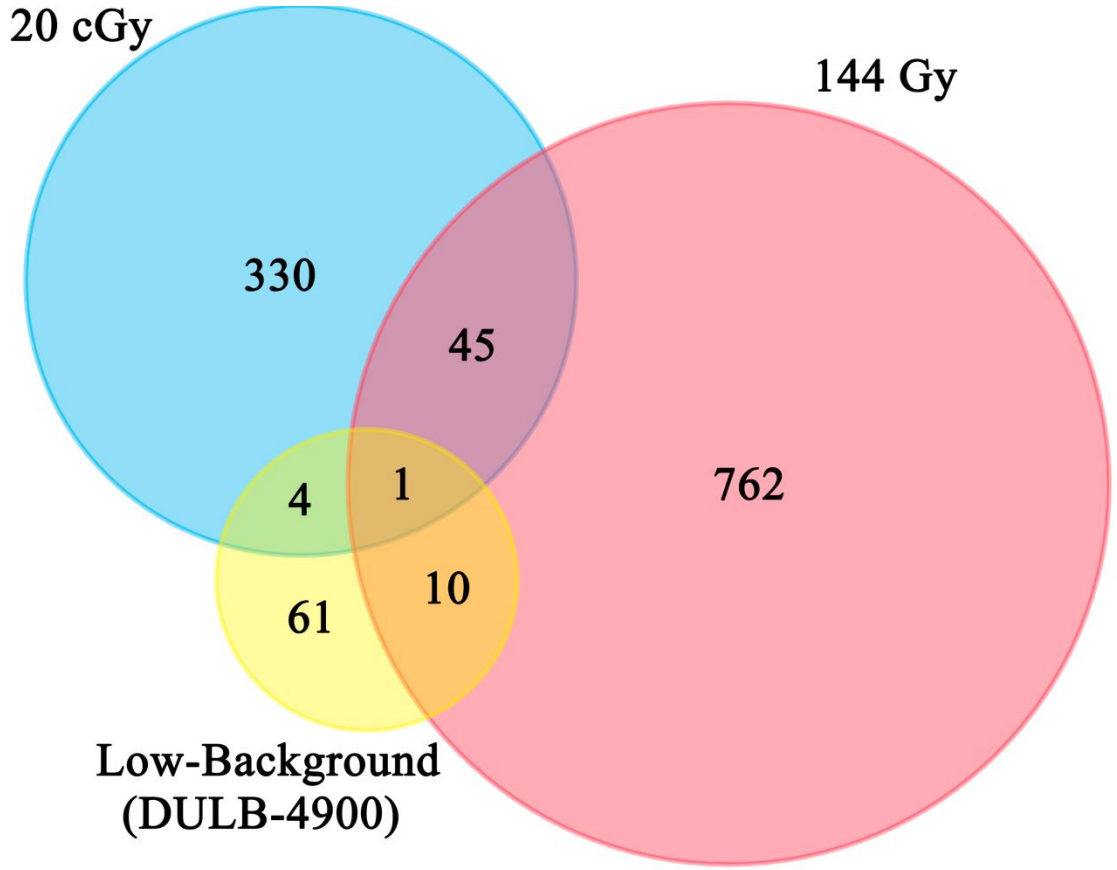
For down-regulated genes biological process GO can be roughly combined into a group of cellular metabolism (56,8% of down-regulated in LB-flies genes, p-value < 0.05)

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 acute doses of ionizing radiation

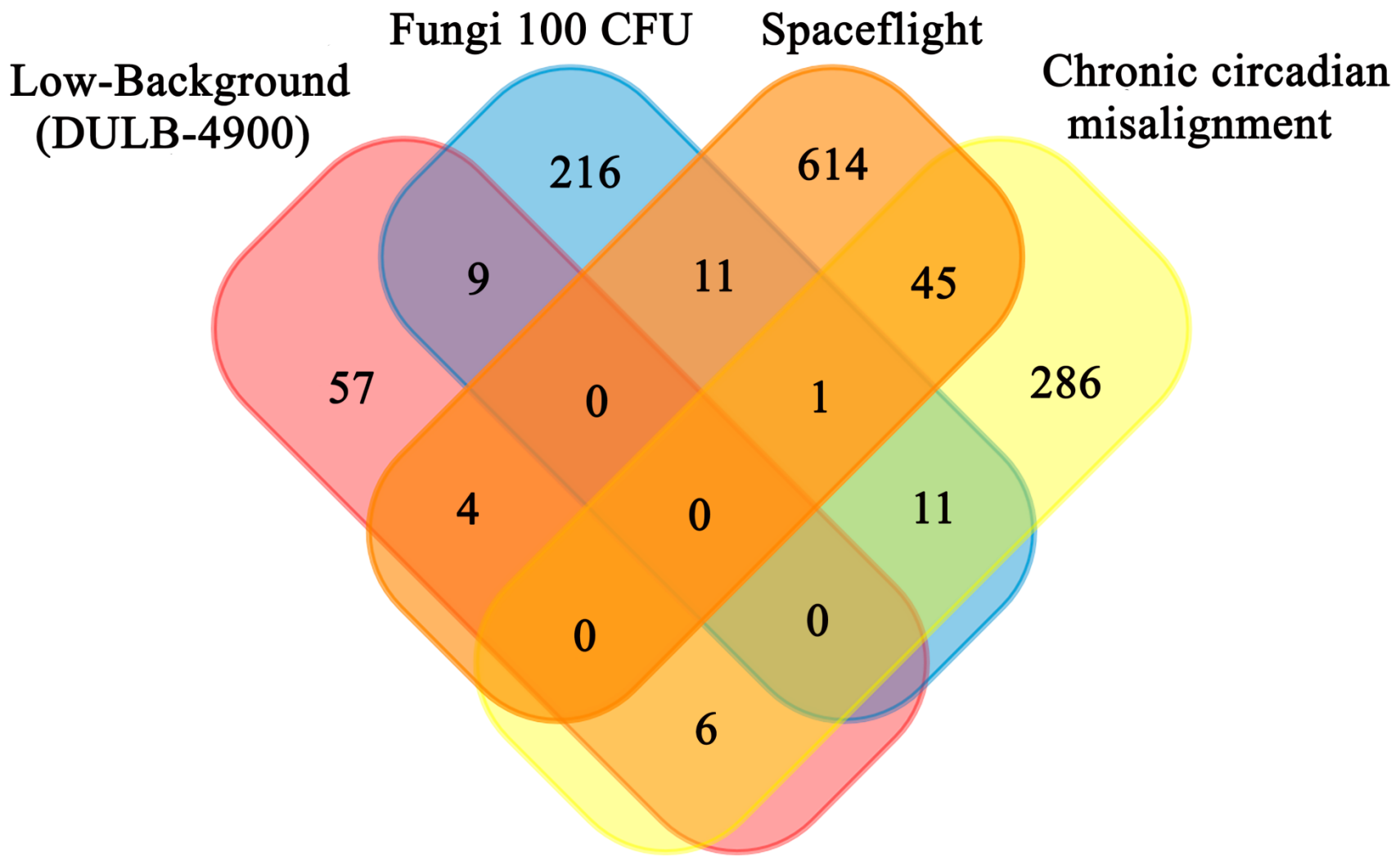


Venn diagram representing the quantity of shared genes for *D.melanogaster* developed in DULB-4900 and after gamma irradiation (0.2 and 144 Gy)

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

https://doi.org/10.1371/journal.pone.0255066

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)

https://doi.org/10.1134/S1063779621010056

For fruit flies exposed to DULB-4900 conditions, not so many differentially expressed genes (76) 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

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

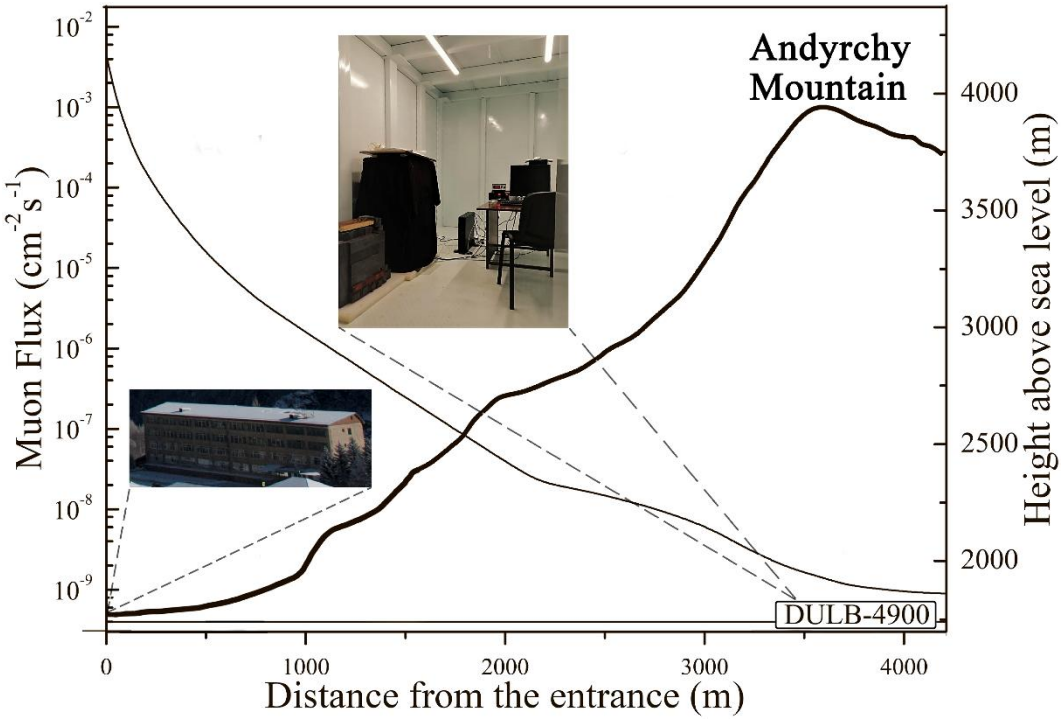
Our work is the first initiative of biological studies at Baksan Neutrino Observatory INR RAS. We expect, Baksan Neutrino Observatory possess outstanding potential for interdisciplinary studies on tasks of biophysics, radiobiology, astrobiology and medicine

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

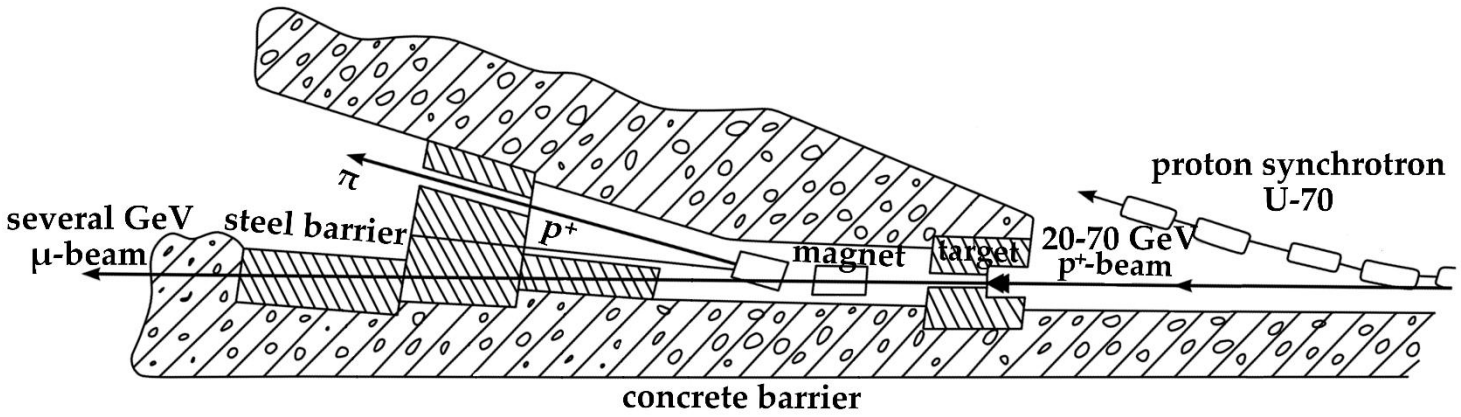
BIOMUON experiment (Bioμ): biological impact of high energy muons being also the component (cosmic) of natural background radiation. Experiments at DULB-4900 are accomplished by chronic low dose irradiation experiment at muon beam of proton synchrotron U-70 (NRC KI IHEP)

BIOMUON experiment (Bioμ): biological impact of high energy muons (>1 GeV) - since Dec. 2021

Chronic exposure to nearly completely reduced natural background (or residual muons) at low background laboratories of Baksan Neutrino Observatory



Chronic low-dose irradiation (high background) experiment at muon beam of proton synchrotron U-70 (NRC KI IHEP)



Preparing the manuscript for the submission: Review of BIOMUON experiment

II. Microbiological studies at unique ecosystems of Baksan Neutrino Observatory (>2 km underground)

Tunnels of Baksan Neutrino Observatory are highly perspective for studies of Deep Life and extremophile organisms in granite rock environment

*One of the deepest (2.1-2.3 km) easy-accessible locations for biology in Caucasus region and Russia

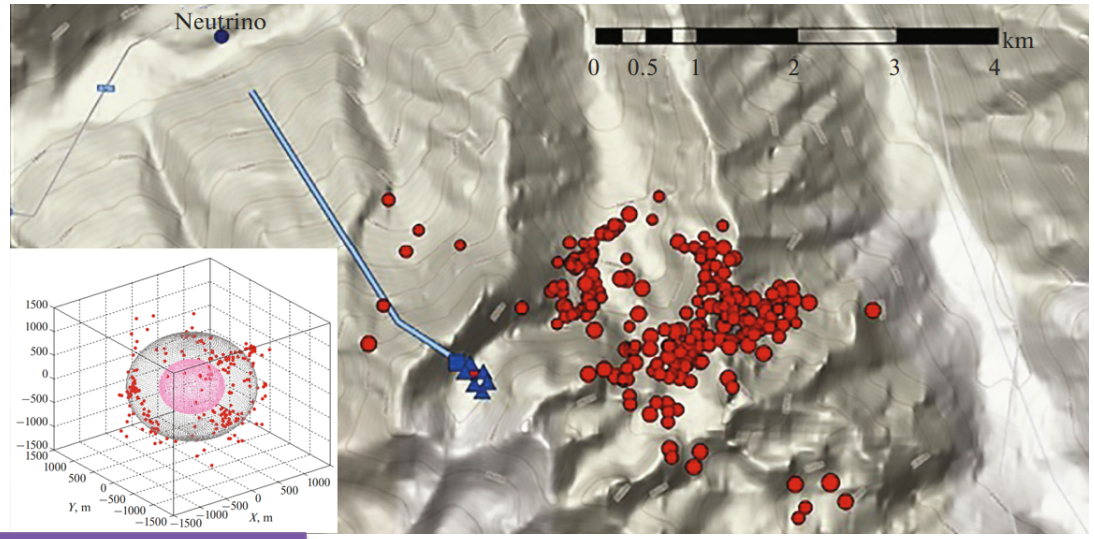
* Nearby Elbrus volcanic center and it's small chamber

* Complex geothermal system in the region and multiple springs of differing nature are in the tunnels

* In springs: high temperatures, salinity, presence of volcanic fluids and gases, CO_2-/^{222}Rn/H_2S-rich waters

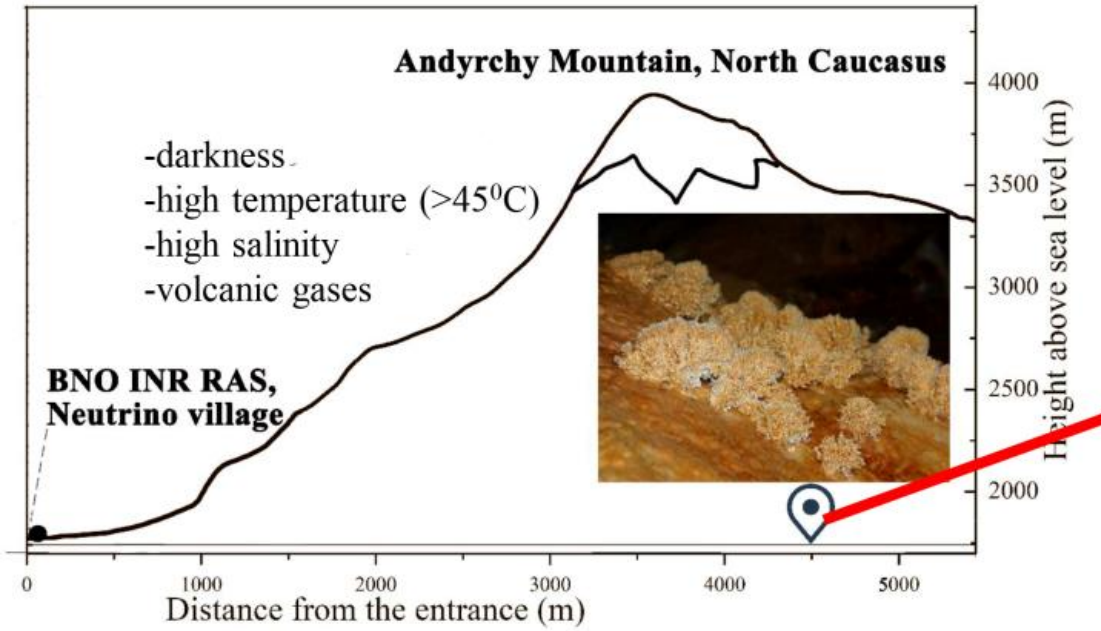
* Spring ecosystems are relatively isolated from the surface (fluids and gases are mainly of deep underground origin!)

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



DOI: 10.1134/S0742046323700173

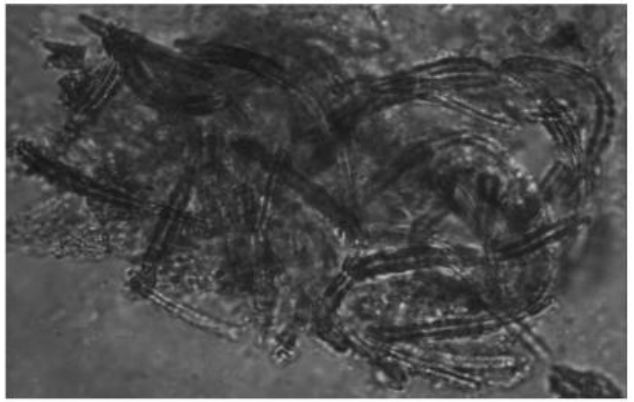
Ecosystem and metagenomic studies of deep underground springs

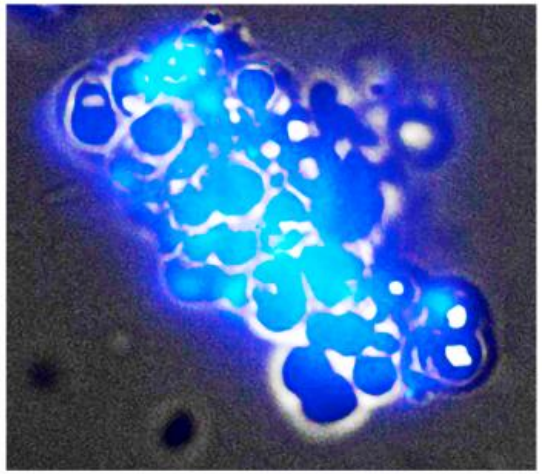


Total DNA extraction

New generation sequencing (whole genomes)

31 million paired reads of DNA fragments





5 NEW genus of bacteria

6 NEW species of bacteria

One of the deepest metagenomic studies in Caucasus region and Russia

Preparing the manuscript for submission: metagenome of biofilm community from deep underground spring

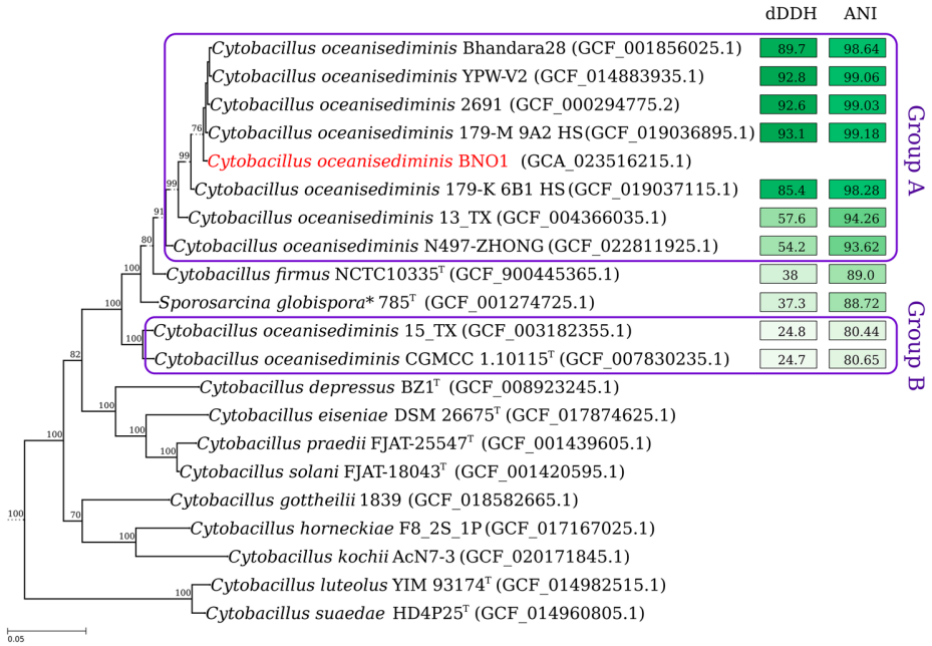
First results: cultivation and complete bacterial genome of novel extremophilic specie *Cytobacillus pseudoceanisediminis*

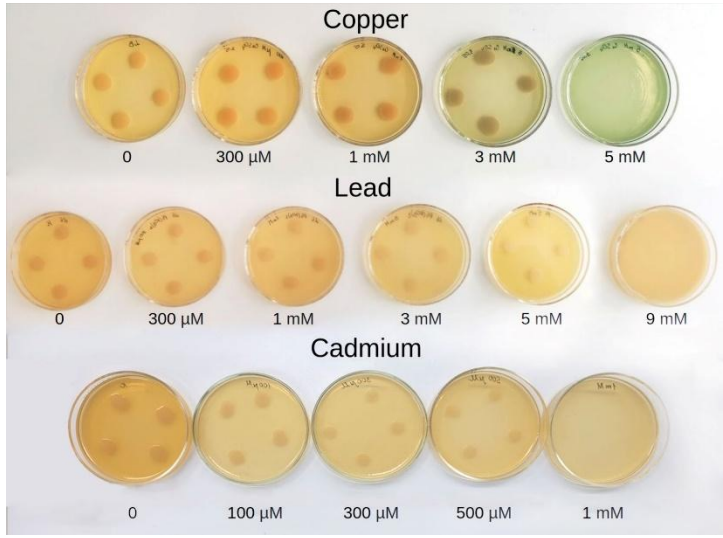
Cytobacillus pseudoceanisediminis sp. nov., A Novel Facultative Methylotrophic Bacterium with High Heavy Metal Resistance Isolated from the Deep Underground Saline Spring

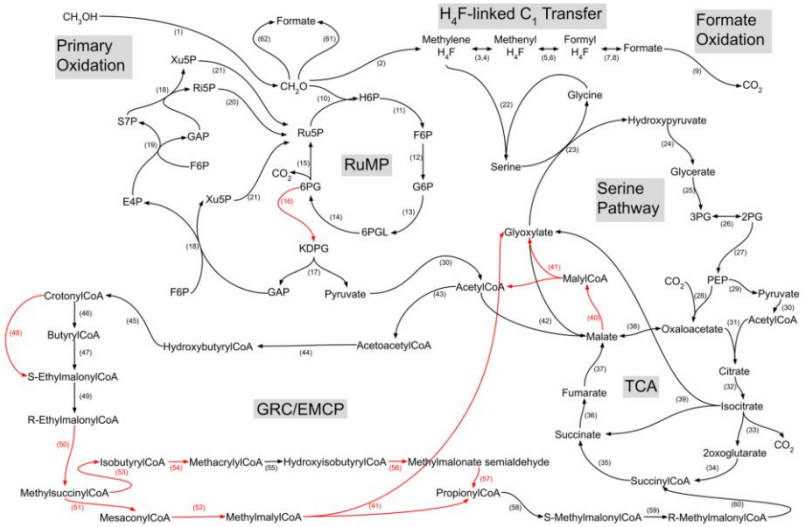
Kirill Tarasov, Alena Yakhnenko, Mikhail Zarubin, Albert Gangapshev, Natalia V. Potekhina, Alexander N. Avtukh & Elena Kravchenko

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

https://doi.org/10.1007/s00284-022-03141-8







Rock biology profiling in Andyrchy mountain: results of July-August 2024 expedition

DLNP JINR Sector of Molecular Genetics of the Cell

Rock biology profiling in Andyrchy mountain: results of July-August 2024 expedition

Perspectives of biological researches in Baksan Neutrino Observatory

Biophysics

BIOMUON experiment (Bioμ): experimental studies of biological impact of high energy muons (> 1 GeV) and applied researches with these particles. Combined researches in DUL (DULB-4900) and at the accelerator facility (high energy muon beam). Biological impact of secondary cosmic radiation

Microbiology, biology of extremophiles, astrobiology

Rock biology profiling in Andyrchy mountain: the group of experiments dedicated to the exploration of unique microbial communities in Baksan Neutrino Observatory, analysis of depth-distributions of microorganisms, comparison with global deep rock biosphere, etc.

Hosting of astrobiology analogue studies in Baksan Neutrino Observatory (?)

Cooperative studies with other DULs are important to obtain more solid knowledge

Participants:

JINR
Elena Kravchenko
Mikhail Zarubin
Kirill Tarasov
Alena Yakhnenko

BNO INR RAS
Albert Gangapshev
Yuri Gavriljuk
Vladimir Kazalov

NRC KI IHEP
Vladimir Pikalov

Thank you for your attention!