Content

The Research Institute of the University of Bucharest - ICUB

The Earth, Environmental and Life Sciences Division of ICUB
Biology, **I**nformatics and **S**ocial Sciences **seminars**
ICUB-BIS seminars

Friday, June 15, 2018
Venue: Faculty of Biology, The Platform of Research in Biology and Systemic Ecology,
Splaiul Independenței 91-95, Bucharest, 050095, Romania
Email: bis-seminars@icub.unibuc.ro
Web: https://lifesciencesunibuc.wordpress.com/
Theme:

From cellular to environmental sciences

11:30 - 12:00 Welcome coffee

12:00 - 12:30 Opening Ceremony
1. Laurențiu LEUȘTEAN - Opening remarks
2. Carmen CHIFIRIUC - Opportunities for research funding offered by ICUB

12:30 - 13:30 Session 1: Chair Carmen CHIFIRIUC
1. Lia-Maria DIȚU, *Faculty of Biology* - Microbial Communities Associated with Romanian Rare Plants From a Natural Habitate
2. Irina GHEORGHE, *Faculty of Biology* - Resistance, virulence markers and clonality of *Acinetobacter baumannii* community acquired and nosocomial isolates
3. Alexandru Florian DEFTU, *Faculty of Biology* - The contribution of potassium channels in microglial migration

13:30 - 13:45 Coffee break

13:45 - 14:45 Session 2: Chair Alexandru DEFTU
1. Ion-Florin TĂTUI, *Faculty of Geography* - Diagnosis of Recent Shoreface Dynamics along Delta Coast (SENBAR)
2. Maria-Laura TÎRLĂ, *Faculty of Geography* - Lithologic and structural controls on marble karst development in the central Făgăraș Mountains (South Carpathians)
3. Mihaela GRĂDINARU, *Faculty of Geology* - Microbial signatures and palaeoenvironmental conditions involved in the formation of ferruginous microbialites during the Middle-Upper Jurassic transition

14:45 - 15:00 Coffee break

15:00 - 16:00 Session 3: Chair Alexandru DEFTU
1. Aritina HALIUC, *Faculty of Geography* - Two millennia of runoff variability in Western Romania and links with large-scale atmospheric circulation
2. Mihaela LAZĂR, *Faculty of Biology* - Genetic characterization of the haemagglutinin protein of measles virus strains recently circulating in Romania
3. Sorin CONSTANTIN, *Faculty of Geography* - Exploring the remote sensing capabilities for water quality assessment in Danube Delta (DeltaRS)

16:00 - 16:15 Coffee break

16:15 - 17:00 Session 4: Chair Alexandru DEFTU
1. Melania-Maria BICA-POPI, *Faculty of Biology* - The effect of Iba-1 silencing on P2X7 receptor in BV2 microglia cells
2. Simona IGNAT, *Faculty of Biology* -
3. Mădălina Andreea BADEA, *Faculty of Biology* - Generation of 3D in vitro breast cancer models by two techniques and their biological characterization

17:00 - 17:15 Closing remarks

Microbial communities associated with Romanian rare plants from a natural habitat

Lia-Mara Ditu[1,2], Paulina Anastasiu[2], Gentiana Predan[2], Petronela Camen-Comanescu[2], Irina Fierescu[3], Anca Sarbu[2]

[1] Research Institute of the University of Bucharest, Bucharest, Romania
[2] University of Bucharest, Bucharest, Romania
[3] National Research & Development Institute for Chemistry and Petrochemistry—ICECHIM, Bucharest
E-mail: lia_mara_d@yahoo.com

Introduction: Plants do not grow axenically in nature, but host a diverse community of microorganisms, termed as plant microbiota, colonizing different niches, both inside and outside their tissues, in the endosphere and ectosphere, respectively. These microorganisms are involved in major physiological processes, such as plant nutrition and plant resistance to biotic and abiotic stress factors.

Materials and Methods: Our focus is oriented to several rare plant species from natural habitats of Romania, with localization in southern Dobrogea: *Adonis vernalis, Opopanax chironium, Asphodeline lutea. Paeonia tenuifolia* (Hagieni Natural Reserve, Constanta), *Potentilla emilii-popii* (Sipote, Constanta). In order to analyse the normal of composition associated soil microorganisms', we sampling the rhizosphere from each plant species and processed for isolation and biochemical identification of the cultivable microbial species. The physical and chemical properties of the rhizosphere samples was investigated using modern technics (Inductive coupled plasma atomic emission spectrometry (ICP-AES)), in order to determinate the availability of metals in rhizosphere and the pH values. The Plant Growth Promoting properties of some isolated bacteria strains were evaluated using sunflower germinated seeds, genotype BT017/44.

Results: The phylogenetic composition of these communities is composed by relatively few bacterial phyla, including *Actinobacteria, Bacteroidetes, Firmicutes*, and *Proteobacteria*, with variations depending on the plant species and soil characteristics. The availability of different metals (Al, Fe, Mn, Cu, Zn) in rhizospheres was influenced by the pH change and chemical compositions of root exudates. Also, the plant growth promoting testes showed that *Bacillus* strains influenced the chlorophyll pigment synthesis and plant development.

Conclusion: The major factors determining community composition of the plant microbiota were the compartment, soil type, time and environmental conditions, while the microbial community composition influences plant growth. The focus on several rare plant species from natural habitats of Romania, respectively *Adonis vernalis, Opopanax chironium, Paeonia tenuifolia, Potentilla emilii-popii, Asphodeline lutea*, will allow us to identify microbial markers of plant health and beneficial strains which can be further used in the conservation strategies, for restoring the number of individuals in their natural habitat.

Resistance, virulence markers and clonality of *Acinetobacter baumannii* community acquired and nosocomial isolates

Irina Gheorghe [1,2], Cristea Violeta Corina [*3], Luminita Marutescu [1,2], Marcela Popa [1,2], Carmen Murariu[4], Bianca Simona Trusca[5], Elvira Borcan[5], Mihaiela-Camelia Ghita[5], Veronica Lazar[1,2], Mariana Carmen Chifiriuc[1,2]

1 Department of Microbiology and Immunology, Faculty of Biology, University of Bucharest, 2 Research Institute of the University of Bucharest (ICUB), 3 Synevo Central Laboratory, Medicover, Bucharest, Romania, 4 Emergency Hospital for children "Marie S. Curie",
6 Fundeni Hospital, Bucharest, Romania.
E-mail: IRYNA_84@yahoo.com

Introduction: We aimed to identify the virulence and antimicrobial resistance features in Carbapenem Resistant *Acinetobacter baumannii* (CRAB) strains isolated from hospital settings and compare them with those isolated in the same period of time from community acquired (CA) infections in Bucharest, south of Romania.

Materials and Methods: 93 recently isolated (aug-nov 2017) *A. baumannii* strains isolated in majority from hospitalized patients (H) (n=75) and from CA infections (n=18) were identified by BD Phoenix and by mass spectrometry using MALDI Biotyper. The antibiotic susceptibility was determined by agar disk diffusion (CLSI, 2017) and automated methods (BD Phoenix and Vitek II).The virulence profiles were assessed by performing enzymatic tests for the expression of eight soluble virulence factors and PCR for biofilm encoding genes. Carbapenemases and the presence of IS*Aba1* element were searched by PCR and sequencing. Clonal relatedness was studied by PFGE (ApaI) and MLST. Plasmid analysis was performed using PCR-based on replicon typing scheme.

Results: The antibiotic resistance profiles in H and CA *A. baumannii* isolates revealed high percentages of carbapenem-resistance in both H and CA isolates. CRAB H and CA isolates revealed the intrinsec carbapenemase OXA-51 (57.33% of which 12.90% had ISAba1 immediately upstream of bla_{OXA-51}/55.55%) and the acquired carbapenemases OXA-23 (48% of which 9.67% had ISAba1 immediately upstream of bla_{OXA-23}/33.33%), OXA24 (29.33%/2.66%), IMP (26.66%/22.22%) and VIM-2 (1.33%/0%). The bla_{OXA-23} gene was identified in different plasmid types (GR2-Aci1, GR6-pACICU2), rep135040(100%) and p3S18 (100%), followed by Aci6 (67%) in H *A. baumannii* isolates. Nine major PFGE types accounted for the *A. baumannii* isolates which were associated with the worldwide spread ST2 (C, I, n=2) and the endemic ST312 (D, n=1); ST636 (H, n=1) and ST642 (E, n=1). OXA-51-like alleles corresponding to the two main sequence groups were identified as bla_{OXA66} (63.63% of the isolates) and respectively, bla_{OXA-69} (38.39%) and revealed the corresponding type of *ompA* and *csuE* sequence grouping.

Conclusion: An appropriate infection control measures are recommended in order to control the geographical spread of this very resistant and virulent clones in our country.

Potassium ionic channels in microglial cells: the role of Kv1.3, Kv1.5 and Kir2.1 in migration

Alexandru-Florian Deftu[1,2], Ruxandra-Elena Anton[1], Mihai Ghenghea[1], Violeta Ristoiu[1]

[1]Department of Anatomy, Animal Physiology and Biophysics, University of Bucharest
[2]The Research Institute of the University of Bucharest (ICUB)

E-mail: alex.deftu@bio.unibuc.ro

Introduction: Microglia are the resident immune cells in the central nervous system, they have many functions with implication in the physiology and pathology of this system. Microglia have several types of ion channels that adjusts cellular activity. The Kv1.3 potassium channel is considered a marker of inflammation and can be a target to modulate microglial activity. UK-78282 is a piperidine compound that specifically blocks Kv1.3 channels. Through this study, we have seen the effect of blocking the Kv1.3 channels with different concentrations of UK-78282 and how microglia migration is influenced.

Materials and methods: BV2 cells were seeded at 2×10^4 in DMEM supplemented with 10% FBS and 1% P/S in the presence of 10 µM UK-78282, and were incubated for 3 h or 6h at 37ºC and 5 % CO_2. To test the blocking effect on microglia migration, we used inserts with 8 µm pores. The cells were cultured in a volume of 200 µl and 600 µl of medium was pipetted into the well with or without the Kv1.3 blocker, following the incubation time set. Subsequently, the cells were fixed with 4% PFA, the nuclei were dyed with Hoechst (# 33342) and the images on the diagonal of the insert were captured with a 10x objective using an Olympus fluorescence microscope.

Results: Following the incubation of BV2 cells for 3 h with 10 µM UK-78282, a decrease in the migration rate (273.9 ± 39.36, N = 22) compared to the control condition was observed (403.8 ± 44.96, N = 22, P <0.05). On the other hand, incubation of BV2 cells for 6 h with 10 µM UK-78282 did not result in a reduction of the migration rate (251.1 ± 57.56, N = 15) compared to the control condition (302.9 ± 50.58, N =5, P > 0.05).

Conclusion: Specific Kv1.3 potassium blockade with 10 µM UK-78282 inhibits the migration of BV2 cells only after short intervals, suggesting a transient activation of these channels during microglia migration.

Microbial signatures and palaeoenvironmental conditions involved in the formation of ferruginous microbialites during the Middle-Upper Jurassic transition

Grădinaru Mihaela, Lazăr Iuliana, Petrescu Lucian, Ducea Mihai

University of Bucharest, Faculty of Geology and Geophysics, 1 N. Bălcescu Bd, 010041 Bucharest, Romania.
E-mail: mihaela.gradinaru@unibuc.ro

Microbial activity plays an important role in precipitation of iron compounds and in formation of the ferruginous microbialites that are interesting do to their relative stratigraphic scarcity, and especially for their disputed genesis. Iron mineralization related to the activity of microbial mats had long been a subject of many controversial discussions and continues to be a topic of high research interest. Iron stable isotopes and rare earth elements provide powerful indicators of biologic processes, being extensively studied to trace the history of microbial metabolisms and the redox evolution of the oceans.

The aim of the present paper is to reports the first data concerning the detailed geochemical and mineralogical analyses, correlated with petrographic and scanning electron microscope (SEM) examination of the Middle Jurassic condensed sections, containing ferruginous microbialites, located in the Southern Carpathians.

The X-ray diffraction data of the ferruginous microbialites showed that goethite and hematite are the most ferriferous mineral phases. Iron isotopes data of the studied samples, reflect that δ^{56}Fe with predominant positive, near zero, or even low negative values, could provide evidence for the presence of microaerophylic iron-oxidizing bacteria. SEM studies reveal that different types of filamentous bacteria and fungi were the significant framework builders of the ferruginous laminae, contributing to the formation of the ferruginous microbialites.

Multidisciplinary analyses present valuable results about a possible microbial involvement in the genesis of the studied ferruginous microbialites preservation from the Southern Carpathians that can provide important information on the chemistry of ancient marine sedimentary environments. Such studies are of importance as they could reflect the effects of the regional and global changes documented for the Middle-Upper Jurassic time interval in different settings of the peri-Tethyan areas.

Two millennia of runoff variability in Western Romania and links with large-scale atmospheric circulation

Aritina Haliuc[1]

[1] The Research Institute of the University of Bucharest (ICUB)
E-mail: aritinahaliuc@gmail.com

In the context of future climate projections to global warming and their effects on bio-hydro-atmosphere and human society, a better understanding of past and present climate conditions is crucial. Taking into consideration that instrumental data for key climatic and environmental variables are limited to the last 150 years, longer, indirect data about extreme climatic changes are extracted from natural archives.

Lake sediments are continuous natural archives which store information about past climatic changes of local-to-regional origin and thus, provide a powerful tool for high-resolution investigations at different time-scales. Geochemical (major and minor elements) and other characteristics (magnetic, grain-size, loss-on-ignition) of lacustrine sediments can be used as proxies for total detrital material and can provide insights on runoff activity which can be further related with rainfall variability.

Here we discuss a new, high-resolution sedimentary record from Lake Ighiel (924 m a.s.l.), located in Apuseni Mountains, in an attempt to reconstruct runoff variability over the last two millennia and test possible link with large-scale atmospheric circulation. Based on ^{14}C dating, X-ray fluorescence scanning (µ-XRF) measurements, long-core sedimentary logging, environmental magnetic proxies (susceptibility, natural and induced remanences), loss-on-ignition and grain-size we document centennial-to-decadal runoff phases, proxy for rainfall activity. The resolution of our investigation proves that the commonly known wet/dry and/or warm/cold periods (e.g., Roman Warm Period, Little Ice Age) are interrupted by decadal-to-centennial shifts.

Our record shows increased rainfall during the first half of Roman Warm Period whilst important runoff episodes characterize the Dark Ages Cold Period and Little Ice Age. The first part of MCA appears wet followed by an apparent drought period; this two phases during MCA are also evident in other record in NE and SW Romania. It appears that North Atlantic Oscillation plays a significant role in the rainfall variability of the region but there are also other controls that must be considered.

Genetic characterization of the MF-NCR region of measles virus strains recently circulating in Romania

Lazar Mihaela[1,2]
[1]National Institute for Research and Development Medico-Military "Cantacuzino"
[2]ICUB – The Research Institute of the University of Bucharest
E-mail: mlazar@cantacuzino.ro

Recently, there has been a resurgence of measles in Romania. The ongoing outbreak began in January 2016, with more than 13,000 cases. Fifty-three deaths have been confirmed with measles, most of them in infants. This is the highest number of measles-related deaths since the introduction of the measles vaccine in 1979.

Measles virus genotyping is important for surveillance to document chains of measles virus transmission, discriminate between imported or indigenous viruses and monitor elimination programs. Current WHO guidelines on genotyping of measles virus recommend sequencing a 450-nucleotide (nt) region of the nucleoprotein gene (N-450) and the hemagglutinin gene (H). The standard phylogenetic analysis does not always provide sufficient specificity to differentiate between measles viral strains within one genotype. The aim of this project is to determine the utility of sequencing the MF-non coding region (MF-NCR) for analyses of measles transmissions and sources during the most recent Romanian measles outbreaks in order to distinguish between endemic transmission and multiple importations of measles viruses that belong to the same genotype. Specimen types used for laboratory testing are nasopharyngeal swabs collected from the both continuous and discontinuous outbreaks as per standard public health surveillance practice. Analysis of the preliminary results shows **that importation of measles resulted in subsequent transmission within Romania to non-vaccinated individuals.**

This project will enable to measure how much the MF changes over the course of an outbreak, to determine whether extended sequencing can distinguish between separate outbreaks of the same genotype (based on the same N-450 lineage) and to determine the level of MF variability that would allow for differentiation between and within outbreaks.

Exploring the remote sensing capabilities for water quality assessment in the Danube Delta (DeltaRS)

Constantin Sorin

Research Institute of the University of Bucharest
E-mail: sorin.constantin@icub.unibuc.ro

The Danube Delta, the largest wetland in the European Union, became subject of anthropic pressure more than a century and a half ago. Danube Delta is a well preserved wetland that can continue to maintain its current status only if the suspended sediments input remains at a level that can contribute to overcome the sand and mud washing by waves and currents. A proper distribution of the mineral loads within the delta is mandatory for its survival, in the context of sediment shortage and constant sea level rise. Water quality monitoring in this area is not only necessary for a proper management of the protected area, but also a fundamental requirement of the Water Framework Directive of the European Union (2000), that aims to achieve good qualitative and quantitative status of all water bodies in the European Union.

The very large area of the Danube Delta (more than 5000 km2) makes in-situ sampling of water quality parameters a very challenging task. In order to provide a synoptic overview of the region, with complete spatial coverage over the entire water surface (even in the most remote areas), satellite data can be successfully used. The study addresses the capabilities offered by two of the main high and medium resolution satellite sensors available today: Sentinel-2 and Landsat 8. The analyzed period is from 2013 up to the most current available remote sensing data. The two parameters of interest that were taken into consideration are turbidity and suspended particulate matter (SPM). Turbidity represents an optical property of the aquatic environment that causes the light rays to be scattered and absorbed rather than transmitted in a straight line through the water column. SPM, on the other hand, is the exact quantity of solid material per volume of water (in g/m3). It can be used to compute sedimentation rates and sediment dynamics.

The results revealed that satellite information can be successfully used to derive these two basic water quality parameters, with good accuracy. In order to achieve such performance, several critical issues have to be properly accounted for, such as i) contamination by targets with higher reflectance (straylight effects); ii) shallow waters - bottom contribution to the overall reflectance values; iii) floating vegetation - can be avoided using indices such as the Floating Algal Index; iv) sunglint, which appears at specific sensor view angle and can contaminate large areas of the satellite image.

Iba-1 silencing in BV2 microglia cells and the effect on P2X7 receptors

Melania-Maria Bica-Popi[1], Alexandru-Florian Deftu[1,2], Violeta Ristoiu[1]

[1]Department of Anatomy, Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Romania
[2]The research Institute of the University of Bucharest *(ICUB)*
E-mail: melaniabicapopi@yahoo.com

Introduction: Iba-1 is a cytoskeleton protein specifically found in macrophages and microglia cells. Iba-1 is involved in many cellular functions. Transmembrane ionotropic receptor P2X7 is a non-selective cation channel with high Ca^{2+} and Na^+ permeability. Its activation by Benzyl-ATP (BzATP) involves quick alteration of cytoskeleton. Our goal was to see if the Iba-1 silencing could interfere with P2X7 function. This alteration was measured by calcium microfluorimetry on BV2 cell line.

Materials and Methods: BV2 cell line was maintained in DMEM + 10% FBS + 1%P/S. BV2 microglia cell line was transfected with scramble siARN (#01955) or with silencing siRNA (#18491). The P2X7 response at 300 μM BzATP for 20 seconds was measured by calcium microfluorimetry with a ratiometric fluorescent marker Fura-2 and quantified in Imaging Workbench 4.0.

Results: The amplitude response analysis when 300 μM BzATP was applied for 20 seconds show an increase of calcium influx after Iba-1 silencing (1.041 ± 0.053, N=194) in comparison with the control condition (0.8545 ± 0.031, N=270, $P < 0.05$). Area under the curve analysis also confirm that the calcium influx increases after Iba-1 silencing (6.102 ± 0.25, N=194) in comparison with the control condition (4.813 ± 0.23, N=270, $P < 0.05$).

Conclusion: Our main conclusion was that P2X7 response is enhanced after Iba-1 silencing. In further experiments we want to show the signaling pathway between P2X7 and Iba-1.

Generation of 3D *in vitro* breast cancer models by two techniques and their biological characterization

Madalina Andreea Badea, Mihaela Balas, Anca Dinischiotu

Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Bucharest, 91-95 Splaiul Independentei, Bucharest, R-050095, Romania
E-mail: badea_andreea08@yahoo.com

Introduction: Spheroids are cellular aggregates characterized by many cell-cell and cell-extracellular matrix interactions, with a specific structure: proliferative cells at the spheroids' periphery followed by quiescent cells and the center defined by hypoxic cells organized in a necrotic core. Spheroids are generated through various techniques: hanging drop, microfluidic methods or methods based on non-adherent surfaces (liquid-overlay). The aim of this study was to generate breast cancer spheroids using two culture techniques and to characterize their biological properties.

Materials and Methods: Triple negative breast cancer cell line (MDA-MB-231) was used to generate spheroids by hanging drop and liquid-overlay (single and multiple spheroids) techniques. Morphology, diameter and spheroids' viability were evaluated for 6 days. Nitric oxide (NO) production, intracellular reduced glutathione (GSH) content and lactate dehydrogenase (LDH) level were analyzed by colorimetric methods. Hypoxia (Nrf2 and Hsp70) and proliferation capacity (PCNA) were assessed by Western blot analysis.

Results: Within 6 days, spherical or ellipsoidal morphology of spheroids was characterized by a high compaction of cells. The diameter of spheroids generated by hanging drop and liquid-overlay-single spheroid techniques was dependent by cell density and decreased in time, while multiple spheroids had various diameters. The biological evaluation showed that spheroids generated through both techniques are characterized by a decrease of PCNA expression and a necrotic center developed over time. A low cellular viability and a high release of LDH in a time-dependent manner were correlated with high level of NO production in culture medium. The presence of necrotic center was confirmed also by a significant increase of Hsp70 and Nrf2 proteins expressions. Instead, GSH content decreased in spheroids generated through hanging drop technique while the spheroids generated through liquid-overlay technique were characterized by an increase of GSH level.

Conclusion: These results show that through both techniques similar breast cancer 3D models were generated, expressing the characteristics of multicellular spheroids – spherical and ellipsoidal morphology with a necrotic center induced by hypoxia. However, through liquid-overlay technique were generated spheroids with a high viability and proliferation capacity.