STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 9-10)

Influence of PDMS microtopographies on cells morphology

Simona Stroescu (Nistorescu)[1,2], Cristina Staicu[1,2], Anca Bonciu[2], Felix Sima[2], Anişoara Cîmpean[1], Valentina Dincă[2]✉

[1]University of Bucharest, Faculty of Biology, Bucharest, Romania;
[2]National Institute of Laser, Plasma and Radiation Physics, Măgurele, Ilfov, Romania;
✉***Corresponding author, E-mail: valentina.dinca@inflpr.ro.***

Abstract

The cellular adhesion and morphology influence direct cell development in the tissue engineering mechanisms (Hashemzadeh *et al*., 2020; Yeh *et al*., 2017). Our work aims at knowing the impact of substrate properties by manufacturing biocompatible microstructured in polydimethylsiloxane (PDMS). Using various roughness and topography (lines, points, or unpatterned), we systematically analyze in vitro response of the cells. Initially, we characterized the physical characteristics of the PDMS samples such as contact angle, surface free energy, roughness, and SEM images. Besides, we show how plasma can turn the PDMS surface to hydrophilic after only 5 minutes exposure. Furthermore, bioassays were performed to investigate the viability, adhesion, and morphology of the cells. In vitro biocompatibility was evaluated by visualizing the actin filaments that provide mechanical support, determine cell shape, and allow movement of the cell surface, thereby enabling cells to migrate. The wettability property has been determined to be improved for plasma-treated PDMS. Morphological observations revealed good biocompatibility with the PDMS samples, cell cytoskeleton is not affected by the substrates. Finally, we showed a correlation between roughness, surface free energy, and cell adhesion. Our results suggest that PDMS microtopographies have a very high potential for cell behavior studies and future therapies.

Keywords: cells morphology, microtopographies, PDMS, wettability.

Acknowledgements. This work was supported by PN-III-P4-ID-PCE2020-2375.

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 11-12)

Low concentration of Zearalenone affects the biochemical and immunological parameters in swine

Valeria Cristina Bulgaru[1]✉, Gina Cecilia Pistol[1], Daniela Eliza Marin[1] and Ionelia Taranu[1]

[1]*INCDBNA-IBNA, Baloteşti, Romania;*
✉***Corresponding author, E-mail:** cristina.bulgaru@ibna.ro.*

Abstract

Zearalenone (ZEA) is a mycotoxin produced by fungi of the genus *Fusarium,* that frequently contaminate wheat, barley and rye crops and affects both human and animal health. Swine is one of the most susceptible species to ZEA intoxication, this fact being due to the high intake of cereals in the diet, but also to a high sensitivity to mycotoxins. The aim of this study was to investigate the effects produced by exposure of weaned piglets to a low dose of ZEA. Some immune parameters as immunoglobulins (IgG, IgA, IgM), nitric oxyde (NO) and also biochemical parameters related to energetic (glucose, cholesterol, triglyceride), proteic (total protein, albumin, ureea, creatinine), mineral (Ca, Fe, Mg, P) profile as well as the activity of some hepatic enzymes (aspartat amino-transferase, alanil-amino-transferase, gamma-glutamil transferase and alcaline phosphatase) were assesed in piglets serum. The *in vivo* experiment was performed on 12 crossbred weaned piglets (TOPIGS-40), randomly assigned to two groups (6 animals/group): control group (C) fed uncontaminated feed and experimental group (E) fed a ZEA contaminated diet (75µg/kg feed) for 21 days. The main immunological change induced by ZEA was the decrease in IgG (P=0.0428) and NO concentration (P=0.0176). The exposure to ZEA significatly decreased the bilirubin concentration (P=0.0077) and tended to decrease the serum glycemia (P=0.07). By contrast ZEA increased the triglycerides concentration (P=0.0004) and gammaGT activity (P=0.0003). In conclusion, ZEA can affect immunological and biochemical parameters in weaned piglets, even in concentrations lower than recommended by European Comission for swine feed.

Keywords: biochemistry, immunology, swine, zearalenone.

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 13-14)

Novel combined therapy based on IL-13-PEG-LCL-SIM and PEG-EV-DOX to reduce murine melanoma aggresiveness *in vivo*

Giorgiana Negrea[1]✉, Valentin-Florian Rauca[1], Laura Pătraş[1], Lavinia Luput[1], Emilia Licărete[1], Vlad-Alexandru Toma[1,2], Bogdan Dume[1], Alina Porfire[3], Manuela Banciu[1] and Alina Sesărman[1]

[1] *Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babeş-Bolyai University, Cluj-Napoca, Romania;* [2] *Department of Experimental Biology and Biochemistry, Institute of Biological Research, branch of NIRDBS Bucharest, Cluj-Napoca, Romania;* [3]*Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania;*
✉***Corresponding author, E-mail:*** ***giorgiana.negrea@ubbcluj.ro.***

Abstract

Melanoma is one of the most aggressive type of cancer worldwide, which rapidly develops resistance to conventional treatments, resulting in metastasis and recurrence. This study aims to test the potential of IL-13-PEG-LCL-SIM and PEG-EVs-DOX to decrease the aggressiveness of B16.F10 murine melanoma by applying an innovative therapy that targets both tumor-associated-macrophages and cancer cells. Melanoma-bearing mice intravenously received the combined therapy, or the individually formulations of SIM or DOX. In order to detect the level of expression of HIF-1α, a key promoter of hypoxia and of Bcl-xL and Bax, apoptotic proteins, western blot analysis was performed. The concentration of malondialdehyde (MDA) in tumor lysates, a marker of oxidative damage, was assessed by HPLC. Our data showed a strong inhibition of tumor development for the group treated with the combined therapy. Also, there was a substantial decrease of expression of HIF-1α due to PEG-EVs-DOX therapy, which was not noticed in the case of combined therapy. However, the concentration of MDA was highly increased, indicating a disruption of intratumor ROS levels, that alters the balance needed for HIF-1α proper activity. Thus, our findings suggest that the combined active targeted therapy which was tested, strongly inhibits tumor growth. Additional studies must be performed to understand the actions of this new active therapy on other processes which maintain the tumor development, such as angiogenesis and inflammation.

Keywords: melanoma**,** targeted therapy, tumor microenvironment

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 15)

Identifying heavy metal multi-resistant bacteria isolated from the rhizosphere of potential metallophytes

Mihai-Cristian Grimm[1,2]✉, Lucian Barbu-Tudoran[3] and Dorina Podar[1,2]

[1]Department of Molecular Biology and Biotechnology, Babeş-Bolyai University, Cluj-Napoca, Romania; [2]Centre for Systems Biology, Biodiversity and Bioresources (3B), Babeş-Bolyai University, Cluj-Napoca, Romania; [3]National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania;
✉***Corresponding author, E-mail: mihai.grimm@ubbcluj.ro.***

Abstract

Soil heavy metal pollution has attracted considerable attention due to the negative effects on human health and on ecosystems. Decontamination of heavy metal polluted soils by engineering-based remediation technologies is -expensive and invasive as they affect the topsoil and thus the micro- and macrobiota on the site. Bioremediation technologies based on using microbiota able to survive the metal toxicity has been proposed.

The aim of the study was to identify and describe the multi-resistant bacteria present in the rhizosphere of some potential metallophytes from a heavy metal polluted site in Romania. Concentration of heavy metal in soil samples was determined via Vanta pXRF analyzer - (Olympus, - USA). Culturable soil bacteria were isolated by the plate culture method using metal supplemented media. Isolates displaying metal resistance were further identified based on 16S rDNA sequence and assessed for the presence of metal resistance molecular determinants such as merA, merB, czcA, nccA, copA. Cell morphology and heavy metal accumulation was investigated by SEM-EDX.

In this study, 309 bacterial isolates were obtained based on their resistance to different metals. More than 75% of the isolates displayed multi metal resistance. The majority (93%) of the isolates were resistant to Hg^{2+} that was found in very high concentrations in soil. Most of the isolates were identified as part of the genus *Pseudomonas.* These data support the idea that the natural rhizobiota of some plants is already developed to withstand toxic concentrations of heavy metals, making them suitable candidates for new bioremediation technologies.

Keywords: Bioremediation, heavy metals, rhizosphere.

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 16-17)

Knowledge, habits and beliefs regarding usage of antibiotics - comparative study of medical and non-medical students from Nis University, Serbia

Nemanja R. Kutlesic[1]✉ and Aleksandra Jovanovic[2]

[1]University of Camerino, Department for Biosciences and Veterinary Medicine, Camerino MC, Italy; [2]University of Nis, Faculty of Medicine, Nis, Serbia;
✉***Corresponding author, E-mail: nemanja.kutlesic@studenti.unicam.it.***

Abstract

Introduction. Many studies in the developing world have reported a high level of ignorance among youth towards proper usage of antibiotics. This study wanted to examine the knowledge, beliefs and habits of Nis University students regarding this important topic and discover whether an anticipated difference in knowledge, beliefs and habits existed between medical and non-medical students.

Materials and methods. The data was acquired through an online questionnaire which addressed knowledge, beliefs and habits regarding antibiotics. Obtained data was classified into two groups and tested for statistical significance using the Chi-squared test. The study took into account the margin of error for the sample of 5%. The study adhered to principles of the Helsinki declaration.

Results. The research showed that the majority of students were able to correctly identify bacteria as the main target of antibiotics. More students from non-medical faculties thought viral infections can be treated with antibiotics (37.35% vs. 7.45% of medical, SE_D*=0.042, $p<0.05$), and identified incorrectly *Paracetamol* as an antibiotic (42.17% vs. 8.51% of medical, SE_D*=0.043, $p<0.05$).

However, a similar percentage in both groups claimed they interrupted their regimen before the prescribed time and admitted to alcohol usage.

Conclusion. While students of the medical faculty demonstrated much better knowledge and beliefs on antibiotics, their habits were not found to be significantly different. Overall, a large percentage of students from both

* SE_D = Standard error of the difference (between the two groups).

groups uses the medicines as they please. These results are similar to available studies from the developing world. Campaigns are necessary to inform students better on the subject.

Keywords: antibiotics, antibiotic resistance, appropriate use of antibiotics, public health, university students

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 18-19)

Production of polyhydroxybutyrate using renewable carbon sources by the extremely halotolerant bacterium *Halomonas elongata* DSM 2581^T

Andreea-Melisa Tripon[1], Adorján Cristea[1]✉,
Timea Kristea[1], Călin Gabriel Floare[2] and Horia Leonard Banciu[1]

[1] *Babeş-Bolyai University, Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, Cluj-Napoca, Romania;* [2] *National Institute for Research and Development of Isotopic and Molecular Technologies, Department of Biomolecular and Molecular Physics, Cluj-Napoca, Romania;* ✉***Corresponding author: E-mail:*** ***adorjan.cristea@ubbcluj.ro***.

Abstract

Polyhydroxybutyrate (PHB) is a natural polyester which is produced under nitrogen and/or phosphorous limitation and excess of carbon (C) source. Under these conditions, the C source is converted to PHB and stored as intracellular C and energy reserves. PHB derivatives might be used as alternatives to synthetic plastics due to their biodegradability and biocompatibility. The aim of this study was to assess the ability of the extremely halotolerant bacterium *Halomonas elongata* DSM 2581^T to synthesise PHB from two renewable C sources namely industrial (IM) and commercial molasses (CM). To induce PHB production, a liquid mineral medium with high salinity (8% w/v NaCl) was used. Three C sources where used separately in the experiments (final concentration of C-source 1% w/v) (D-glucose – as control, IM and CM) and 0.1% w/v yeast extract. Batch cultures were incubated at 37^{o}C and 180 rpm. For cell count and PHB visualisation, DAPI and Nile Red staining where employed. ^{1}H-NMR spectroscopy and crotonic acid assay were used for the chemical analysis of the PHB (Cristea *et al*., 2018). The highest PHB yield was obtained after 96 h of growth on D-glucose (2.61 g/L), followed by CM (2.63 g/L after 90 h) and IM (1.07 g/L after 48 h). The weight percentage (wt%) of PHB fraction was 85% when cultivation occurred on CM, 86% on D-glucose and 35% on IM. Our preliminary results have showed that this strain could use renewable C sources (like molasses) to produce PHB that may be further applied in sustainable circular economy strategy.

Keywords: *Halomonas elongata*, high salinity, molasses, polyhydroxybutyrate, renewable source.

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Evaluation of selenium content in biofortified *Allium* species by chromatographic means

Bogdan M. Boşca[1]✉ and Augustin C. Moţ[1]

[1]*Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University, Cluj-Napoca, Romania;*
✉***Corresponding author, E-mail:*** ***bogdan.bosca@stud.ubbcluj.ro.***

Abstract

Selenium is an essential trace element in animals and humans. It has chemical properties similar to sulfur. In plants, selenium is involved in several important biochemical routes, including antioxidative processes (Germ *et al*., 2007). In animals and humans, compared to other micronutrients, there is a much thinner line between the normal concentration and the toxic amount of selenium. The inorganic forms of selenium (selenate, selenite) present a much higher toxicity than its organic combinations (e. g. selenocysteine, selenomethionine) (Herrero Latorre *et al*., 2013). Thus, a sensitive method is required for detection of selenium species in biological samples. A distinct method is represented by the formation of piazselenol. This kind of complexes represent the product of reaction between an aromatic *o*-diamine and Se(IV) species, in acidic conditions. This study describes an HPLC-based optimized, sensitive and selective method for determination of selenium in biological samples. The analyzed *Allium* species were grown in our laboratory in a phytochamber. Before the analysis procedures, the biofortified *Allium* samples have been mineralized and pre-concentrated using solid-phase microextraction technique. This analytical step affords an excellent precision and accuracy as it removes the interferences form a typical biological sample, thus allowing a limit of detection as low as 0.5 ng/mL Se.

Keywords: *Allium,* biofortification, piazselenol, selenium determination

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 21-22)

The influence of abiotic factors on phosphate solubilizing bacteria

Loredana-Elena Mantea[1]✉ and Marius Ştefan[1]

[1]*Alexandru Ioan Cuza University, Faculty of Biology, Biology Department, Iaşi, Romania;*
✉***Corresponding author, E-mail:*** ***mantealoredana9@gmail.com.***

Abstract

Phosphorus is one of the most important macronutrients needed for optimal plant growth and development. Although P compounds are relatively abundant in agricultural soils, the concentration of soluble phosphorus accessible to plants is very low. The use of phosphate solubilizing bacteria as biofertilizers is a sustainable alternative for improving agricultural productivity globally, but the efficiency of these bacteria can be affected by some abiotic factors, such as: pH, temperature and salinity. In this context, the purpose of this study was to identify bacterial strains that have the ability to solubilize tricalcium phosphate ($Ca_3(PO_4)_2$) under abiotic stress. Bacterial strains were isolated from the rhizosphere of maize using the serial dilution method. The bacterial isolates were further analyzed for their ability to solubilize tricalcium phosphate in Pikovskaya liquid medium. Ten out of fifteen isolated bacterial strains solubilize $Ca_3(PO_4)_2$ in quantitative assay and the amount of phosphorus varied between 9.82 and 17.06 µg P/ml. The strain that solubilized the highest amount of $Ca_3(PO_4)_2$ was further subjected to abiotic stress (pH and temperature). When the bacterial strain P2.1S grew in medium with an acidic pH (4.9) it solubilized a lower amount of $Ca_3(PO_4)_2$ compared to that solubilized at pH 7.2, respectively pH 9. The highest amount of phosphorus solubilized by the P2.1S strain was recorded at 28°C. In conclusion, the bacterial strain P2.1S solubilizes $Ca_3(PO_4)_2$ regardless of pH and temperature values tested, but more studies are needed before this bacterial strain can be used in agriculture.

Keywords: bacteria, pH, temperature, tricalcium phosphate.

Acknowledgements. This work was supported by Microbiology Laboratory, Faculty of Biology, Alexandru Ioan Cuza University of Iaşi.

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 23-24)

Simultaneous determination of torulene, torularhodin and β-carotene in *Rhodotorula mucilaginosa* using UV-Vis spectroscopy and chemometric approaches

Anca-Elena Anghel[1]✉, Ioana Grosu[2] and Augustin Cătălin Moţ[3]

[1]Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, Department of Chemistry, Cluj-Napoca, Romania; [2] National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania; [3] Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, Chemometrics and Bioanalytical Chemistry Laboratory, Analytica Research Center, Cluj-Napoca, Romania; ✉***Corresponding author**, **E-mail:** **ancu.anghel@gmail.com**.*

Abstract

Carotenoids are pigments of biotechnological importance, with antioxidant and radical-scavenging properties, confered by their extensive system of conjugated double bonds. They are synthesized via the mevalonate pathway in red yeasts, such as those belonging to the genus *Rhodotorula,* which have been explored as natural pigment-producing living factories, a safer and more consumer-friendly alternative than chemically synthesized carotenoids.

The aim of this work was to develop a method for the simultaneous, non-destructive determination of the main carotenoids produced by *Rhodotorula mucilaginosa* (β-carotene, torulene and torularhodin), using Principal Component Regression (PCR), a multivariate chemometric technique.

The fungal pigments were purified after chemical cell wall disruption via normal-phase column chromatography. PCR was used to obtain multiple regression equations for the determination of each carotenoid, using the UV-Vis spectra of carotenoid mixtures as input data. The method was validated on samples of known pigment concentration and tested on real samples, prepared by subjecting yeast cells to sodium selenate stress, to investigate the antifungal activity of selenocompounds.

An inverse correlation between total carotenoid content and seleno-compound concentration was observed. Intermediary selenate concentrations appear to shift the carbon flux in the biosynthetic pathway of carotenoids, favoring carotene synthesis in the detriment of xanthophylls.

To our knowledge, no previous method for the simultaneous determination of *Rhodotorula mucilaginosa* carotenoids using PCR has been developed.

The method could be used in industrial settings, when optimization of the culture media for the overproduction of a specific carotenoid is desired. It could also facilitate future studies regarding selenium-mediated fungal toxicity.

Keywords: carotenoids*, R. mucilaginosa*, simultaneous determination, selenocompounds.

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 25)

Danube water physico–chemical parameters in relation with ichthyofauna diversity

Cristian-Emilian Pop[1]✉, Abdulhusein Jawdhari[2] and Nicolae Crăciun[2]

[1]University of Bucharest, Faculty of Chemistry, Department of Organic Chemistry, Biochemistry and Catalysis, Bucharest, Romania; [2]University of Bucharest, Faculty of Biology, Bucharest, Romania; ✉***Corresponding author, E-mail:*** ***cristian.pop@s.unibuc.ro.***

Abstract

Aquatic habitats are sharply deteriorating all over the world due to increasing human impact. The Danube River not an exception especially due to its high economically importance and therefore intense exploatation. Biodiversity inventories are needed to understand the impact of human activities on the Danube ecosystem and to detect potential early warning signals for catastrophic ecological changes in the species communities. In this work we used known physico–chemical indicators (Ilie *et al*., 2017) and fish diversity to assess the ecotoxicological status of the Danube River from eleven locations. Electrofishing was performed in linear 500 m transects, upstream and downstream over shore, as well as over the main water body. Most of the high levels of inorganic pollutants found could be explained by point and diffuse sources scattered along the sector such as discharged household and farm wastewater, as well as nearby agricultural areas where fertilizers have been used and then leaked in the water stream during rainfalls (Ivan *et al*., 2021). Although the Danube water analysis was found to be in what are considered normal ranges, heavy metals concentrations shown a slight correlation with the current status of fish communities which we surveyed, therefore requiring a more detailed investigation for future prospects.

Keywords: ecotoxicology; ichthyofauna; pollution.

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 26-27)

Preliminary results of a new approach for *in-vitro* culture conservation of the sand bindweed (*Convolvulus persicus*): seeds

Valentin Adrian Kiss[1]✉ and Liliana Jarda[2]

[1]*Romanian Institute of Science and Technology, Cluj-Napoca, Romania;*
[2]*Alexandru Borza Botanical Garden, Babeş-Bolyai University, Cluj-Napoca, Romania;*
✉***Corresponding author, E-mail: kissvalentinadrian@gmail.com.***

Abstract

Convolvulus persicus is a critically endangered species, endemic to the embryonic shifting dunes of the Caspian Sea and the Black Sea. The risk factors for the populations of *C. persicus* in Romania have an anthropic origin (Kiss and Szatmari, 2020). At the same time, natural risks are also present, such as the solidification of the sands. The efficient approach of plant conservation involves the combination of *ex-situ* and *in-situ* strategies, having as the main objective the maintenance of genetic diversity. In our case, *ex-situ* conservation presents a viable and even indicated alternative. *In-vitro* conservation has been conducted before by Holobiuc on this species using fragments of the stem or root (Holobiuc *et al*., 2015) but not seeds. The study aims to obtain an optimal protocol for *ex-situ* conservation using seeds, as well as the multiplication of plant *in-vitro* culture. Seeds from 20 individuals were used and kept over the winter at 18-20 °C, as well as a month at 4 °C. To initiate the *in-vitro* culture, the seeds were disinfected and inoculated into culture vessels containing 50 ml of MS solid medium supplemented with 20% sucrose. The infection rate after 30 days from inoculation is 8%. The germination rate is 1%. Only one of the seeds germinated, and after 20 days from germination, it was passed on a medium supplemented with phytohormones. In conclusion, we can say that germination of *C. persicus* is quite difficult and it is easier to induce *in-vitro* cultures from fragments of the stem or root as was done in Holobiuc's study.

Keywords: critically endangered, *in-vitro*, seeds, endemic, *ex-situ* conservation.

Acknowledgements. This work was supported by Alexandru Borza Botanical Garden.

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 28-29)

Spread status of *Corythucha arcuata* (Heteroptera, Tingidae) in Romania

Virgil Ionel Oltean[1]✉, Alexandru Sabin Bădărău[1] and Olivia Elena Pistrui[1]

[1]*Babeş-Bolyai University, Faculty of Environmental Science and Engineering, Cluj-Napoca, România;* ✉***Corresponding author, E-mail: olteanvirgil@gmail.com.***

Abstract

Corythucha arcuata (Say, 1832), commonly named the Oak lace bug (OLB), is an insect that belongs to the order Heteroptera, family Tingidae, native to North America. The allogeneic species is known to be invasive and its presence was first reported on the European continent in May 2000, in the region of Lombardy, Italy (Bernardinelli and Zandigiacomo, 2000), then reached Turkey (Mutun, 2003) and spread further in most southern European countries: Bulgaria (Dobreva et al., 2013), Croatia (Hrašovec et al., 2013), Hungary (Csóka *et al.*, 2013), Slovenia (Jurc and Jurc, 2017), Slovakia (Zubrik *et al.,* 2019), progressing very rapidly and affecting very large areas of *Quercus* forests. Also, the species was detected for the first time in the southern Romania in 2016 (Chireceanu *et al.,* 2017), and after a relatively short period, the presence of the insect was reported in several areas of Romania.

The aim of the research was to evaluate the current spread status and level of infestation of the species. We used direct observation method and evaluation of leaves in situ. We also used drone photography as a technique for large areas evaluation The field work was carried out in the vegetative periods of 2020 and 2021. We made over 50 observations in *Querqus* forests and our results shows that the species was detected in over 50% of the survey points, some of the areas being highly infested.

Keywords: biodiversity, conservation, invasive species

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 30-31)

Global warming promotes adaptive changes in the freshwater cyanobacterium *Microcystis aeruginosa*

Maria Nicoară[1]✉ and Bogdan Drugă[1]

[1]National Institute of Research and Development for Biological Sciences (NIRDBS), Institute of Biological Research, Cluj-Napoca, Romania; ✉***Corresponding author,***
E-mail: maria.nicoara@icbcluj.ro.

Abstract

Global warming has a substantial impact on aquatic ecosystems, especially on microalgae, influencing their growth and physiology (Padfield *et al.,* 2015; Sandrini *et al.,* 2015; Schaum and Collins, 2014). In this study, three strains of freshwater cyanobacterium Microcystis aeruginosa have been grown in two different conditions (22°C: A - ambient temperature and 26°C: H - the estimated temperature for the end of the century) for a period of 12 months. In order to observe their response to global warming, thermal reaction norms were calculated in a temperature range of 20-40°C. The results showed that after 100 generations, the H lineage gained remarkable competitive skills, being able to grow even at 38°C, whereas the A lineage did not survive. Moreover, after being re-incubated in the ambient temperature, the development of H strains was reduced, representing an irreversible change suggesting adaptive evolution. This study highlights the necessity of adaptive evolution experiments during a long period of time and with multiple strains, in order to understand the effects of climate change upon aquatic ecosystems.

Keywords: adaptive evolution, cyanobacteria, freshwater, global warming, *Microcystis*.

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 32-33)

Diversity of cultivable heterotrophic bacteria from three Romanian karst caves

Nicoleta-Patricia Vaniga[1]✉, Diana Felicia Bogdan[1,]✉, Oana Teodora Moldovan[2] and Horia-Leonard Banciu[1]

[1]Babeş-Bolyai University, Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, Cluj-Napoca, Romania; [2]Emil Racoviță Institute of Speleology, Cluj-Napoca Department, Cluj-Napoca, Romania; ✉***Corresponding authors, E-mails:*** ***nicoleta.vaniga@stud.ubbcluj.ro; diana.bogdan@ubbcluj.ro.***

Abstract

Karst caves are peculiar ecosystems accommodating specific biota that readily include microbial communities with key roles in the biogeochemical cycling of main elements and thus, in the functioning of trophic webs (Barton and Northup, 2007). In caves, macroscopic colonies of microorganisms are frequently encountered on the walls, ceilings, in aquatic sediments and on the surface of speleothems. To assess the cultivable microbial diversity in timely, accurately and cost-effective manner, the molecular (marker gene-based) identification of isolates is presently preferred as preceding detailed genotypic and phenotypic characterization. The revealing of microbial diversity in the cave ecosystem is crucial to the understanding of their ecological roles and, in addition, to the discovery of novel taxa and metabolic capabilities. The aim of this study was to isolate and evaluate the taxonomic diversity of bacterial strains from three Romanian karst caves, namely Cloşani, Ferice and Muierilor, located in different geographic areas. To achieve this goal, sample plating and isolation on non-selective, oligotrophic solid culture media and subsequent molecular identification of bacterial isolates have been employed. In total, 41 strains were isolated from soil sediments of the above-mentioned caves. These underwent genomic DNA extraction, 16S rRNA gene amplification, and Sanger sequencing. The retrieved 16S rRNA gene sequences were analyzed and a phylogenetic tree was constructed by bioinformatic tools. The isolates were assigned to *Proteobacteria* (37% of total number of isolates), followed by *Firmicutes* (36%), *Actinobacteria* (26%), and *Bacteroidetes* (2%). *Pseudomonas glareae* (Cloşani), *Aeromonas* sp. and *Polaromonas jejuensis* (Ferice) and *Streptomyces sp.* (Muierilor) were the most frequently recovered isolates. *Bacillus sp.* and *Paenibacillus sp* were retrieved in all caves, while *Flavobacterium sp*. was found only in Cloşani samples. Well-known as bioactive compound-producers, *Actinobacteria* members isolated from the floor sediments of the

explored cave soils were assigned to *Arthrobacter* sp. (Cloșani and Ferice), *Paeniglutamicibacter kerguelensis* (Ferice), and *Streptomyces* sp. (Muierilor). Our findings indicated that the diversity of cultivable bacteria varied among the studied caves, probably due to the geographic distances and slightly different physicochemical setting of each particular cave. However, further detailed investigations are needed to reveal the full picture of bacterial diversity and its roles in the tested cave ecosystems.

Keywords: karst cave; microbiota; phylogeny; 16S rRNA gene; cultivable diversity

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 34-35)

Downregulated microRNAs as mediators of retinoic acid-dependent transcriptome homeostatic mechanisms in mouse embryos

Silvia Alda[1], Andreea-Daniela Andreescu[1] and
Ioan Ovidiu Sîrbu[1]✉

[1]Victor Babeş University of Medicine and Pharmacy from Timisoara, Biochemistry Department, Timişoara, Romania; ✉***Corresponding author, E-mail: ovidiu.sirbu@umft.ro.***

Abstract

Retinoic acid (RA) is an important transcriptional gene expression regulator which functions as a ligand for retinoic acid receptors (RAR), which bind retinoic acid response elements (RARE) within the regulatory regions of target genes. During antenatal development, RA signaling starts at late gastrulation stages and is required for cell differentiation, cell migration, axial elongation and organogenesis.

In mouse embryos, the most important source of RA is RALDH2, expressed in the paraxial mesoderm starting with E7.5. RA has been shown to travel over long distances and to activate gene expression and orchestrate morphogenetic events in axial and paraxial tissues of neuroectodermal and mesodermal origin. Several *ex vivo* and *in vivo* studies have described the impact of RA on transcriptome homeostasis; however, little is known about the post-transcriptional mechanisms altered by RA in the context of vertebrate embryo development.

The aim of this study was to provide a better understanding of these mechanisms. Here we used Exiqon qRT-PCR arrays to analyze the small RNA profile in E8.5 *raldh2-/-* embryos and identified a set of 26 downregulated microRNAs. MicroRNAs are small non-coding RNAs that regulate gene expression at post-transcriptional level. In order to identify the signaling pathways putatively modulated by RA-microRNAs interactions during early organogenesis stages, we combined mirWalk3.0 target prediction algorithms (for 3'UTR, 5'UTR, and CDS regions), complex network analysis, and DAVID gene ontology analysis.

Further, more complex investigations are needed in order to understand the transcriptomic impact certain microRNAs have on RA signaling during mouse embryogenesis.

Keywords: mouse embryo, gene regulation, miRNA, retinoic acid

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 36-37)

Upregulated microRNAs as mediators of retinoic acid-dependent transcriptome homeostatic mechanisms in mouse embryos

Alexandru Alexandru[1], Cristiana-Smaranda Ivan[1] and Ovidiu Sîrbu[1]✉

[1]*Victor Babeş University of Medicine and Pharmacy from Timisoara, Biochemistry Department, Timişoara, Romania;* ✉***Corresponding author**, **E-mail:** ovidiu.sirbu@umft.ro.*

Abstract

A major transcriptional gene activator, retinoic acid (RA), exerts its function by binding to retinoic acid receptors (RAR), which recognize retinoic acid response elements (RARE) within the regulatory regions of target genes. During embryo-fetal development, RA signaling orchestrates axial elongation, organogenesis, cell differentiation, and cell migration, starting with late gastrulation stages.

In mouse embryos, RA is synthesized starting with E7.5 by RALDH2 expressed in the paraxial mesoderm and diffuses over long distances to activate gene expression and initiate morphogenetic events in the adjacent tissues. The impact of RA on mouse embryo transcriptome homeostasis has already been described; however, little is known about the RA-dependent post-transcriptional mechanisms of gene expression regulation in the context of vertebrate embryo development.

MicroRNAs are small non-coding RNAs that operate as endogenous post-transcriptional gene expression regulators. Here, we used Exiqon qRT-PCR arrays to identify the set of 11 upregulated microRNAs in *raldh2-/-* mouse embryos at E8.5. By combining mirWalk3.0 target prediction algorithms (for 3'UTR, 5'UTR, and CDS regions), complex network analysis, and DAVID gene ontology analysis, we identified and described the signaling pathways putatively modulated by RA-microRNAs interactions during early organogenesis stages of mouse embryo development.

Our work provides a conceptual framework for future, more complex investigations of microRNAs' role as mediators of RA signaling during mouse embryogenesis.

Keywords: mouse embryo, gene regulation, miRNA, retinoic acid

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 38-39)

Photooxidation of skin cells with titanium oxide systems excited with visible light

Iulia Moigrădan[1]✉, Ştefania M. Milin[1] and Maria Suciu[1,2]

[1]Babeş-Bolyai University, Faculty of Biology and Geology, Cluj-Napoca, Romania; [2]National Research and Development Institute for Isotopic and Molecular Technologies, Cluj-Napoca, Romania; ✉***Corresponding author**; **E-mail:** iuliamoigradan@gmail.com.*

Abstract

The interest for nanoparticles increased in the last few years thanks to their unique proprieties and wide use. Although, there are some concerns about the exposure to these nanoparticles because, in certain quantities, can be toxic. In the current study, the effects of graphene nanoparticles doped with titanium dioxide (TiO_2) and silver (Ag) or TiO_2 and copper (Cu) were identified on two human cell lines, A375 and HaCaT, in the absence and presence of visible light. Cytotoxicity and oxidative stress were investigated in the two cell lines with the help of three assays: testing cell viability, measuring the quantity of lactate dehydrogenase (LDH) released and the level of nitric oxide (NO). More precise, the two cell lines were treated with TiO_2/Ag/TRGO and TiO_2/ Cu/TRGO at five different concentration (0.01-1 mg/mL) and incubated for 24 hours in the absence or presence of visible light; after the treatment, three specific assays were made. The findings of this research proved that the toxicity induced by the nanoparticles, in the absence or presence of visible light, damaged the A375 cell line much more compared to the HaCaT cell line. In addition, the results also showed that the treatment TiO_2/Ag/TRGO at high concentrations had the most aggressive effect. In conclusion, this study proved the antitumoral effect of nanoparticles doped with TiO_2/Ag or TiO_2/Cu and opened an opportunity for potential treatments of various diseases using photooxidation. However, due to the fact that the nanoparticles used in this study are known to be present in many everyday products that could come in contact with the human skin, further studies are recommended to be made regarding the toxicity of these nanoparticles on the HaCaT cell line.

Keywords: A375; cytotoxicity; graphene; HaCaT; nanoparticles.

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 40-41)

CRISPR-Cpf1 system and its utility in editing the *Paenarthrobacter nicotinovorans* genome

Iustin-Tiberius Munteanu[1], Fakhri Kallabi[1] and Marius Mihăşan[1]✉

[1] *Alexandru Ioan Cuza University of Iaşi, Faculty of Biology, BioActive Research Group, Iaşi, Romania;* ✉***Corresponding author, E-mail: marius.mihasan@uaic.ro.***

Abstract

Paenarthrobacter nicotinovorans is a Gram-positive bacterium that is best known for its ability to metabolize nicotine. The stain has proven its potential for converting nicotine containing waste into useful chemicals (Hritcu and Mihasan, 2019; Yu *et al.,* 2017). Its applications in biotechnology are hampered by the lack of reliable gene editing systems that would permit rational engineering of the nicotine degradation pathway.

CRISPR systems have been extensively used for genomic editing of eukaryotic cells and proved to be reliable and accurate. The applicability of CRISPR system for genomic editing of *Paenarthrobacter* strains remains elusive. The main goal of this work is to evaluate the applicability and functionality of the CRISPR-Cpf1 system in *P. nicotinovorans.* CRISPR-Cpf1 is a class 2 type V CRISPR system known to work in the closely related *Corynebacterium glutamicum* strains (Jiang *et al.,* 2017). The draft genome of *P. nicotinovorans* was used to screen for the presence of incompatible CRISPR systems using CRISPRs web server (https://crispr.i2bc.paris-saclay.fr/). A number of 4 CRISPRs candidates were found on different contigs, but none were related to CRISPR-Cpf1. Hence, we concluded that the system might work in this strain and the pJYS3_ΔcrtYf plasmid containing a functional a CRISPR-Cpf1 system was electroporated into *P. nicotinovorans*. No transformants were obtained upon selection with kanamycin, indicating that the pJYS3 replicating origin might not be functional in *P. nicotinovorans*. Next, the CRISPR-Cpf1 genes from pJYS3_ΔcrtYf were amplified by PCR and ongoing work aims to clone these genes into a plasmid known to work in *P. nicotinovorans* – pART2 (Sandu *et al.,* 2005).

Keywords: CRISPR, genetic engineering, *P. nicotinovorans*

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 42-43)

Effects of graphene materials on A375 and HaCaT cell lines due to exposure to visible light

Ştefania Milin[1]✉, Iulia Moigrădan[1], Crina Socaci[2], Sergiu Gabriel Macavei[2] and Maria Suciu[1,2]

[1]Babeş-Bolyai University, Faculty of Biology and Geology, Departament of Molecular Biology and Biotehnology, Cluj-Napoca, Romania; [2] National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania;
✉ ***Corresponding author, E-mail: stefania.milin@stud.ubbcluj.ro.***

Abstract

The wide scale use of nanoparticles (NPs) due to their unique properties and important applications in sensor devices, clothing, alimentation and cosmetics makes human being more prone to the exposure of NPs and its potential to adverse effects. Exposure is mainly through skin. Therefore, the aim of the present study was to investigate the effects of graphene oxide nanoparticles complexed with titanium dioxide and copper or copper oxide (TiO_2/CuO/GO and TiO_2/Cu/TRGO) on A375 and HaCaT cell lines exposed to visible light. We explored the cytotoxicity and oxidative stress induced by nanoparticles. Cell viability, nitric oxide (NO) levels and extracellular release of lactate dehydrogenase (LDH) were assayed in A375 and HaCaT cells after 24 hours incubation with 0.01-1 mg/ml TiO_2/CuO/GO and TiO_2/Cu/TRGO nanoparticles. The results showed that nanoparticles under light iradiation reduced cell viability, induced nitric oxide generation and impaired cell membrane integrity of A375 and HaCaT in a dose dependent manner. It is valuable to inform that HaCaT cells appeared to be slighty more susceptible to TiO_2/CuO/GO treatment than A375 cells, TiO_2/Cu/TRGO nanocomposite has the potential for antitumor treatment by photooxidation, as green and blue lights intensify the toxicity. These results provide a basic comparative toxic effect of TiO_2/CuO/GO and TiO_2/Cu/TRGO nanoparticles on normal keratinocytes and cancerous epitelial cells. Considering the diverse results, further studies using different conditions are recommended.

Keywords: A375, cytotoxicity, graphene, HaCaT, nanoparticles;

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 44-45)

Epigenetic changes induced by commonly used metal and metal oxide nanoparticles

Bogdan Andrei Miu[1]✉ and Anca Dinischiotu[1]

[1]*University of Bucharest, Faculty of Biology, Department of Biochemistry and Molecular Biology, Bucharest, Romania;* ✉***Corresponding author, E-mail: miu.bogdan-andrei@s.bio.unibuc.ro.***

Abstract

Nanotechnology is one of the fastest developing fields in science and engineering. Based on market-estimated size and online repositories listing nanoproducts (e.g., Nanodatabase), it is obvious that silver, titanium dioxide and silicon oxide nanoparticles (NPs) are widely used in day-to-day items such as cosmetic and skincare products or clothing materials. However, consumer products containing NPs may become a source of air pollution, raising concerns regarding human health. Toxic effects of NPs are relatively well documented, although experimental data regarding epigenetic alterations is limited. Our review study aims to provide a general description of the epigenetic changes induced by these three commonly used NP types. We considered recent *in vivo* and *in vitro* studies and discussed which molecular pathways associated with DNA methylation and histone post-translational modifications were impaired by NPs exposure. Knowledge gaps related to the subject were also highlighted. Our work could contribute to the improvement of knowledge about NPs toxicity by compiling the related data available so far and clearly illustrating general NPs effects observed on key molecules from epigenetic signalling pathways. Epigenetic changes play a crucial role in triggering different human disorders. Considering the widespread of NPs, all their toxic effects, including epigenetic impairment, need to be documented to completely assess their safety. We concluded exposure to NPs affects genes involved in establishing and maintaining the normal epigenetic pattern. It remains unknown whether epigenetic changes occur as an indirect consequence of other NPs toxic effects such as oxidative stress and inflammation. These data may be considered in developing appropriate public policies for nanomaterials market.

Keywords: DNA methylation, histone modification, epigenetic, nanoparticle.

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STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 66, 1, 2021 (p. 46-47)

Subcloning and expression of recombinant glucose-dehydrogenase from *Bacillus subtilis*

Andrea Popa[1]✉, Sebastian Emanuel Gherman[1] and Iulia Lupan[2]

[1]Babeş-Bolyai University, Cluj-Napoca, Romania; [2]Interdisciplinary Research Institute on Bio-Nano-Sciences, Cluj-Napoca, Romania; ✉***Corresponding author, E-mail:*** ***andreapopa3101@gmail.com.***

Abstract

Glucose-dehydrogenase (GDH) is an enzyme that catalyzes the oxidation of glucose to gluconate, reducing NAD(P)+ to NAD(P)H + H+. This enzyme is of particular interest due to the fact that it can be used to regenerate the NAD cofactor using a cheap substrate such as glucose. The aim of this study was the fusion of recombinant GDH gene with the His-Tag at the N-terminus. For this purpose, the recombinant GDH gene was subcloned into the pET28a expression vector and expressed in *E. coli* BL21(DE3) cells. The recombinant protein was expressed both in soluble form (10% of total proteins) and in inclusion bodies. The recombinant GDH was purified by Ni-agarose affinity chromatography and tested for enzymatic activity (glucose and NAD). In conclusion, after subcloning the ORF did not change, the protein was fused with His-tag, and this fusion did not affect the activity or solubility of the enzyme. The results obtained in this study may be used to optimize glucose-dehydrogenase production so that it would increase its biotechnological importance.

Keywords: cofactor regeneration; glucose-dehydrogenase; recombination; subcloning; vector

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CRISPR/Cas9 – mediated gene silencing of OXCT1 in HeLa cells favors cell proliferation

Marta Biesiekierska[1]✉, Maciej Studzian[1], Paweł Hikisz[1], Souad Nasser[2], Luciano Pirola[2] and Aneta Balcerczyk[1]

[1]University of Lodz, Faculty of Biology and Environmental Protection, Department of Molecular Biophysics, Lodz, Poland; [2]INSERM Unit 1060, CarMeN Laboratory, Lyon 1 University, France;
✉***Corresponding author, E-mail:*** ***marta.biesiekierska@edu.uni.lodz.pl.***

Abstract

Extensive evidence suggests that the utilization of ketone bodies as an alternative source of energy to carbohydrates may have a beneficial effect on cancer treatment due to the fact that the major ketone body – β-hydroxybutyrate – directly affects inflammatory processes. The rate-limiting enzyme in ketone bodies catabolism is succinyl-CoA:3-ketoacid-coenzyme A transferase 1 (SCOT1), encoded by the OXCT1 gene. Our previous studies showed an elevated OXCT1 expression in various cancer cell lines, including cervical cancer cell line HeLa. The main aim of the research was to knockout the OXCT1 gene from HeLa cells using the CRISPR/Cas9 technique in order to analyze the proliferation rate and possible new functional characteristics of this model.

The knocking-out procedure of OXCT1 was performed by CRISPR/Cas9 and gDNA transient transfection, followed by a Surveyor test to scan for the occurrence of the DNA mutation. The SCOT1 protein ablation in multiple clones was determined by Western blotting and immunofluorescent microscopy. Moreover, we performed a cytometric analysis of the cell cycle phase distribution and analyzed the gene expression of selected cell cycle determinants, including p21 and cyclins.

We observed that OXCT1 knockout increases the proliferation rate of HeLa cells, suggesting that OXCT1 gene may be non-essential for cell proliferation or even involved in attenuation of cell proliferation, suggesting that a switch to an alternative energy source to glucose/carbohydrates in cancer cells may render them less susceptible to proliferation.

Keywords: cancer cells, ketone bodies, metabolism

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