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The Annual International Conference of the
Romanian Society for Biochemistry & Molecular Biology
8 – 9 June 2017, Timişoara

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NEW FRONTIERS IN CHEMISTRY: METADATA

New Frontiers in Chemistry (New Front. Chem.) is an open-access fee-free peer-review international academic journal addressing the modern trends and frontiers in fundamental, applicative and multidisciplinary chemistry, paralleling the ever-expanding chemical data, methods, and compounds available and continuously produced, towards cross fertilizing the multidisciplinary ideas as coming from Chemistry combined with Mathematics, the Natural and Applied sciences (Physics, Biology, Medicine, Agriculture, Environmental, Engineering), and beyond.

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NEW FRONTIERS IN CHEMISTRY:

SPECIAL ISSUE EDITION

The Annual International Conference of the
Romanian Society for Biochemistry & Molecular Biology
8 – 9 June 2017, Timișoara

Special Editors:

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NEW FRONTIERS IN CHEMISTRY:

SPECIAL ISSUE EDITION

The Annual International Conference of the
Romanian Society for Biochemistry & Molecular Biology
8 – 9 June 2017, Timişoara

Conference Sections:
• Correlation Structure–Properties in Biological Models - Drug Design
• Biochemistry and Molecular Biology in Clinical Medicine and Pharmacy
• Biochemistry and Molecular Biology in Plants and Animals Models
• Biotechnologies and Biochemical Interactions in Environmental Sciences

Satellite Training Events, 7th June, 2017:
• Intensive course in clinical practice: „Real Time Polymerase Chain Reaction" (for details please follow this link)
• Use of UPLC MS in Biochemistry peptide separation (Prepared by Chromaktiv, partner of Waters) (for details please follow this link)

Thursday, 8th June, 2017:
8:00-10:00 Participants registration
10:00-10:15 Opening Conference 2017
10:15-10:50 Keynote Plenary Lecture:
Angel RAYA-CHAMORRO – Biomedical applications of induced reprogramming
10:50-11:25 Keynote Plenary Lecture:
Rainer FISCHER – Latest Developments in Molecular Farming – Production of Biopharmaceuticals in Plants
11:25-11:45 Short Talk:
Katja SCHREITER, Market Development Manager Applied Genomics – miRNA solutions @QIAGEN
11:45-12:00 Coffee Break
12:00-12:20 Short Talk:
Jasmin MOSS – IMAG(in)E SIMPLICITY: Cellular imaging and High Content Analysis tools by ThermoFisher Scientific
12:20-12:40 Short Talk:
Ayca ULGEN – Single-Cell Analysis Made Simple with Bio-Rad workflow
12:40-13:00 Short Talk:
Lutz BÜCHNER – CESI-MS method in proteomics, metabolomics and biopharmaceutical biosimilarity assessment. SCIEX Separations
13:00-14:30 Lunch
14:30-17:00 Sections Presentations (different Conference Rooms)

17:00-18:00 Poster Session & Sponsors Stands
18:00-19:00 General Meeting
19:30-22:00 Festive dinner

Friday, 9th June, 2017:
9:00-9:35 Keynote Plenary Lecture
Adrian ANGHEL – The Drug Discovery Program at Translational Oncology Research Laboratories. An integrated approach
9:35-10:10 Keynote Plenary Lecture
Andrei-Jose PETRESCU – Computational Assisted Research in Molecular Life Sciences
10:10 -10:25 Coffee break
10:25-11:00 Keynote Plenary Lecture
Costel C. DARIE, – Applications of mass spectrometry in biomedical research
11:00-11:20 Short Talk
Rainer ROZENICH – Current worldwide trend in regulation for the biosimilar industry (Waters: Separations – Business)
11:20-11:40 Short Talk
Matthias SZESNY – Increasing arginine production in C. glutamicum by rational strain design using a combination of metabolomics and proteomics (Bruker)
11:40-12:00 Short Talk
Name Speaker – Multiparameter molecular biology genetic testing (Novaintermed-Fujirebio)
12:00-12:50 Poster Session & Sponsors Stands
12:50-13:00 Votes Collection & Poster Awards
13:00-13:10 Concluding Remarks of RSBMB Conference 2017
13:10-13:20 Closing Ceremony
17:00-21:00 Visits at Izvin Stud Farm and Recas Winery

ORAL PRESENTATION FOR SECTION:

CORRELATION STRUCTURE–PROPERTIES IN BIOLOGICAL MODELS – DRUG DESIGN

Chairpersons: Andrei PETRESCU and Adriana ISVORAN

S1_OP01

14:30-14:45 – Petrescu Andrei J., Department of Bioinformatics and Structural Biochemistry, IBAR - Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Computational Assisted Research in Molecular Life Sciences*

S1_OP02

14:45-15:00 –Milac Adina-Luminița, Department of Bioinformatics and Structural Biochemistry, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *In silico investigation of protein-ligand interaction*

S1_OP03

15:00-15:15 – Adriana ISVORAN, Department of Biology-Chemistry, West University of Timisoara, Timisoara, Romania, *Chemoinformatics of xenobiotics*

S1_OP04

16:00-16:15 – Spiridon Laurentiu, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Using Robotics to Enhance Molecular Sampling*

POSTERS FOR SECTION:

CORRELATION STRUCTURE–PROPERTIES IN BIOLOGICAL MODELS – DRUG DESIGN

S1_P01

Surleac D. Marius, Department of Bioinformatics, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Structural insights into the functional divergence of human Topoisomerase IIα and IIβ on the decatenation checkpoint*

S1_P02

Bumbăcilă Bogdan, Laboratory of Computational and Structural Physical-Chemistry for Nanosciences and QSAR, Department of Biology-Chemistry, Faculty of Chemistry, Biology, Geography at West University of Timișoara, Timișoara, Romania, *Towards cubic clef method in QSAR*

S1_P03

Icriverzi Madalina, Department of Ligand-Receptor Interaction, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Design and molecular docking study of the noncleavable antibiotic hybrids; theoretical studies*

S1_P04

Gridan Mădălin, Department of Biology-Chemistry, West University of Timișoara, Timișoara, Romania, *Toxicity profiles and predicted biological effects on humans and environment of some pesticides used for wheat crops*

S1_P05

Roman Marin, "Victor Babeş" University of Medicine and Pharmacy of Timișoara, Romania, *Computational assessment of the biological effects of synthetic anabolic steroids on humans and environment*

S1_P06

Șisu Eugen, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Luminescent Nanoparticles Obtained by Thermal Decomposition of Coordination Compounds*

S1_P07

Șisu Eugen, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Luminescent TLC and Densitometric Analysis of Methanol Extracts from Marine Algae*

S1_P08

Șisu Eugen, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Long Bonds Involved in EI-MS Fragmentation of Aldose Derivatives Predicted using Advanced Basis Sets*

S1_P09

Surleac Marius D, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Roles of the C-terminal Domains of Topoisomerase IIα and Topoisomerase IIβ in Regulation of the Decatenation Checkpoint*

ORAL PRESENTATION FOR SECTION:

BIOCHEMISTRY AND MOLECULAR BIOLOGY IN CLINICAL MEDICINE AND PHARMACY

Chairpersons: Adrian ANGHEL and Ioan-Ovidiu SÎRBU

S2_OP01

14:30-14:45 – Marija GAVROVIĆ-JANKULOVIĆ, Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia, *Allergen interactions with intestinal epithelial cells can contribute to the sensitization process in food allergy*

S2_OP02

14:45-15:00 – Ioan-Ovidiu SÎRBU, Department of Biochemistry, University of Medicine and Pharmacy, Timisoara, Romania, *Plasma microRNAs in Parkinson's disease*

S2_OP03

15:00-15:15 – Manuela BANCIU, Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, "Babes-Bolyai University, Cluj-Napoca, Romania, *Improving the efficacy of tumor-targeted therapies using tumor-associated macrophages re-education strategies*

S2_OP04

15:15-15:30 – Denise-Minerva DROTAR, Department of Molecular Biology and Biotechnology, Babes-Bolyai University, Cluj-Napoca, Romania, *Liposomal prednisolone phosphate enhanced the antitumor activity of liposomal doxorubicin in B16.F10 murine melanoma in vivo*

S2_OP05

15:30-15:45 – Emilia LICARETE, Department of Molecular Biology and Biotechnology, Babes-Bolyai University, Cluj-Napoca, Romania, *A novel combination therapy for the treatment of melanoma by targeting tumor cell proliferation and angiogenesis*

S2_OP06

15:45-16:00 – Valentin-Florian RAUCA, Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babes-Bolyai University, Cluj-Napoca, Romania, *Enhanced antitumor efficacy induced by the coadministration of Simvastatin and DMXAA on an in vitro melanoma inflammation model*

S2_OP07

16:00-16:15 – Lavinia LUPUȚ, Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babes-Bolyai University, Cluj-Napoca, Romania, *Efficacy of the liposomal simvastatin in the colon carcinoma treatment approach*

S2_OP08

16:15-16:30 – Laura PATRAS, Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babes-Bolyai University, Cluj-Napoca, Romania and Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano-Sciences, Babes-Bolyai University, Cluj-Napoca, Romania, *Combination therapy of liposomal prednisolone and 5-flurouracil exerts strong anti-angiogenic and anti-inflammatory effects on C26 colon carcinoma*

S2_OP09

16:30-16:45 – Costin Ioan POPESCU, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Phosphoinositide 3-kinases class II are regulating Hepatitis C Virus life cycle*

POSTERS FOR SECTION:

BIOCHEMISTRY AND MOLECULAR BIOLOGY IN CLINICAL MEDICINE AND PHARMACY

S2_P01

Liana-Gabriela GRUESCU, Department of Biochemistry, University of Medicine and Pharmacology Timisoara, Romania, *miRNAs as diagnostic biomarkers of acute myocardial infarction*

S2_P02

Mădălina ICRIVERZI, Department of Ligand-Receptor Interaction, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Mesenchymal stem cell behavior on polymeric surfaces*

S2_P03

Mădălina ICRIVERZI, Department of Ligand-Receptor Interaction, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *In vitro biocompatibility testing of Cloisite-based nanocomposites for chemotherapeutic drug delivery systems*

S2_P04

Florian PAULA, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Novel lactoferrin-derived peptides with anti- hepatitis B virus capacity*

S2_P05

Florian PAULA, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Physical-chemical characterization and biological assessment of bovine derived hydroxyapatite thin films synthesized by pulsed laser deposition for a new generation of metallic implants*

S2_P06

Maria SALA-CÎRTOG, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania, *Plant microRNA identification & transfer – A new dimension to herbal medicine*

S2_P07

Marian DIANA, Department of Biochemistry, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania, *The influence of vitamin D serum level on periodontal parameters*

S2_P08

Andreea-Anda ALEXA, Department of Biochemistry, University of Medicine and Pharmacology Timisoara, Romania, *Short term activation of canonical WNT signaling pathway inhibits proliferation of LNCaP cells*

S2_P09

Anca MARCU, Department of Biochemistry, University of Medicine and Pharmacology Timisoara, Romania, *Differentially expressed long non-coding RNAs related to dietary interventions in a mouse model*

S2_P10

Andrei ANGHEL, Department of Biochemistry, University of Medicine and Pharmacology Timisoara, Romania, *RNA-markers in endometrial cancer*

S2_P11

Ioana POPA, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *New tools for discovering inhibitors of the receptor for advanced glycation end products*

S2_P12

Nevena MARKOVIĆ, Center of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Serbia, *Omega-transaminase in synthesis of potential pharmaceutical active ingredients*

S2_P13

Simona GHENEA, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Differential requirement for EDEM proteins in Endoplasmic Reticulum Associated Degradation (ERAD) in a living organism*

S2_P14

Cristina BEJINAR, Department of Biochemistry, University of Medicine and Pharmacy, Timisoara, Romania, *Plasma microRNAs in Parkinson's disease*

S2_P15

Florentina PENA, Institute of Biochemistry, Department of Molecular Cell Biology, Bucharest, Romania, *P5 – an endoplasmic reticulum protein with multiple functions*

S2_P16

Florin PASTRAMĂ, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Optimization of protein-protein interaction protocols for Hepatitis C Virus protein interactome determination*

S2_P17

Ștefana-Gheorghina DEZSI, West University of Timişoara, Department of Biology-Chemistry, Timişoara, Romania, *Selenium compounds and the Oxidative Stress*

S2_P18

David PATRICHE, Department of Viral Glycoproteins, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Selection of phosphoinositide 3-kinases class II depleted cell lines for functional studies in Hepatitis C Virus life cycle*

S2_P19

Gabriela CHIRIȚOIU, Department of Molecular Cell Biology, Institute of Biochemistry, Bucharest, Romania, *Role of Iba1 in amyloid peptide clearance by murine microglia*

S2_P20

Cristian MUNTEANU, Department of Bioinformatics & Structural Biochemistry, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *An integrated workflow for the identification of an ERAD mannosidase potential substrates*

S2_P21

Gabriela CHIRIȚOIU, Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Tyrosinase N-glycosylation site occupancy analysis using mass spectrometry*

S2_P22

Andrei JUNCU, Department of Viral Glycoproteins, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Identification of inhibitors of Hepatitis C Virus NS2 cysteine protease activity using high throughput screening*

S2_P23

Anda Cornelia VIZITIU, PhD student, Victor Babes University of Medicine and Pharmacy Timisoara Doctoral School, Timisoara, Romania, *qRT-PCR evaluation of selected microRNAs' expression in amniotic fluid and chorionic villus samples from Down syndrome pregnancies*

S2_P24

Alina SESARMAN, Department of Molecular Biology and Biotechnology, "Babes-Bolyai" University, Cluj-Napoca, Romania, *Cytotoxicity of long-circulating liposomes co-encapsulating curcumin and doxorubicin on C26 murine colon cancer cells*

S2_P25

Rodica BADEA, Department of Enzymology, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Identification of a protein tyrosine phosphatase that dephosphorylates PLCγ2*

S2_P26

Mariana VOICESCU, Romanian Academy, Institute of Physical Chemistry "Ilie Murgulescu", Bucharest, Romania, *Secondary Structure of Human Serum Albumin in Riboflavin loaded Myrj52-Silver Nanoparticles*

S2_P27

Georgiana MANICA, Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *EDEM3 and its function in ERAD*

S2_P28

Cristina POPESCU, Arad County Hospital, Clinic of Urology Arad, Romania, *Clinical Relevance Of Global DNA Methylation In White Blood Cells Of Prostate Cancer Patients*

S2_P29

Elena GANEA, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Protein Folding, and the Ages Efect on Amyloid Formation. Advanced Mass Spectrometrical Analysis of Glycated BSA, and Comparative Proteomic Screening of Temporal and Hyppocampal Healthy, Old Brain Tissue*

ORAL PRESENTATION FOR SECTION:

BIOCHEMISTRY AND MOLECULAR BIOLOGY IN PLANT AND ANIMAL MODELS

Chairpersons: Angel RAYA-CHAMORRO and Carmen SOCACIU

S3_OP01

14:30-14:50 – Carmen SOCACIU, Department of AgriFood Biochemistry, University of Agriculture Sciences and Veterinary Medicine Cluj-Napoca, Romania, *High-throughput omics technology applied to fingerprint and elucidate metabolic profiles and pathways*.

S3_OP02

14:50-15:10 – Adela PINTEA, Department of Chemistry and Biochemistry, University of Agriculture Sciences and Veterinary Medicine Cluj-Napoca, Romania, *Biological activity of polyphenols - evidence from in vitro tests*.

S3_OP03

15:10-15:30 – Lucian HRITCU, "Alexandru Ioan Cuza" University from Iasi, Romania, *Anxiolytic and antidepressant profile of the 6-hydroxy-L-nicotine in a rat model of chlorisondamine.*

S3_OP04

15:30-15:50 –Ioan HUŢU, Department of Animal Science, Faculty of Veterinary Medicine, Banat University of Agricultural Science and Veterinary Medicine "Michael I of Romania", Timisoara, Romania, *Tips and Facts for Animal Study Proposal in "Horia Cernescu" Experimental Units.*

S3_OP05

15:50-16:10 – Sofia POPESCU, Faculty of Food Processing Technologies, Banat University of Agricultural Science and Veterinary Medicine "Michael I of Romania", Timisoara, Romania, *Chemical and biological active compounds in plants and alcoholic extracts belonging to Lamiaceae family*.

S3_OP06

16:10-16:30 – Sofia POPESCU, Faculty of Food Processing Technologies, Banat University of Agricultural Science and Veterinary Medicine "Michael I of Romania", Timisoara, Romania, *Physical-chemical Characterization of some Local Fruits in the Banat Area Proposed for Harness.*

POSTERS FOR SECTION:

BIOCHEMISTRY AND MOLECULAR BIOLOGY IN PLANT AND ANIMAL MODELS

S3_P01

Gabriela BUNU, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *Gerontomics: a multi-omics prediction system for prioritization of gerontological interventions*

S3_P02

Simona MARC, Department of Reproduction, Obstetrics and Veterinary Gynecology, Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timisoara, Romania, *In vitro maturation of domestic cat oocyte in medium supplemented with antioxidants through different time interval*

S3_P03

Alexandru PETRUTA, Department of Molecular Cell Biology, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *The role of ERAD pathway in insulin synthesis and secretion in pancreatic β-cells and diabetes mellitus*

S3_P04

Alin CIOBICA, Department of Molecular and Experimental Biology, "Alexandru Ioan Cuza" University, Iasi, Romania, *Ten days intraperitoneally oxytocin administration results in antioxidant effects in Wistar rats*

S3_P05

Alin CIOBICA, Department of Molecular and Experimental Biology, "Alexandru Ioan Cuza" University, Iasi, Romania, *The effects of branchial and tegmental exposure of zebrafish to oxytocin on the oxidative stress status*

S3_P06

Alin CIOBICA, Department of Molecular and Experimental Biology, "Alexandru Ioan Cuza" University, Iasi, Romania, *Intraperitoneal administration of two doses of oxytocin in Carassius auratus exerts antioxidant effects*

S3_P07

Alin CIOBICA, Department of Molecular and Experimental Biology, "Alexandru Ioan Cuza" University, Iasi, Romania, *No clear effects of oxytocin in a rat epigenetic model of schizophrenia based on methionine administration*

S3_P08

Alin CIOBICA, Department of Molecular and Experimental Biology, "Alexandru Ioan Cuza" University, Iasi, Romania, *Oxytocin administration is reducing lipid peroxidation levels in aged rats*

S3_P09

Olena KUCHMENKO, National Pedagogical Dragomanov University, Kyiv, Ukraine, *Protective effect of ubiquinone-10 and complex of precursors and modulator of its biosynthesis on heart under doxorubicin treatment*

S3_P10

Mirela AHMADI, Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Romania, *Hematological Investigations after Ferrous Gluconate Administration in Animal Model*

S3_P11

Ștefan HULEA, Health, Wellness & Fitness, Toronto, Canada, *Plants and microorganisms may prove to be the best source of essential fatty acids for humans*

S3_P12

Ioan GOGOAȘĂ, Banat's University of Agricultural Science and Veterinary Medicine" King Mihai I of Romania", Timisoara, Romania, *Preliminary researchs on bioaccessible content of Ca and Mg from instant coffee*

S3_P13

Cornelia MILOVANOV, Faculty of Veterinary Medicine, Banat's University of Agricultural Science and Veterinary Medicine" King Mihai I of Romania", Timisoara, Romania, *Oviduct-specific glycoprotein role and effect on IVF embryos development*

S3_P14

Cornelia MILOVANOV, Faculty of Veterinary Medicine, Banat's University of Agricultural Science and Veterinary Medicine" King Mihai I of Romania", Timisoara, Romania, *Stem Cells Therapies – perspectives*

S3_P15

Sorina POPESCU, Faculty of Horticulture and Forestry, Banat University of Agricultural Sciences and Veterinary Medicine "King Mihai I of Romania" Timisoara, Romania, *Traceability of genetically modified (GM) DNA from farm to fork*

S3_P16

Oana-Maria BOLDURA, Faculty of Veterinary Medicine, Banat's University of Agricultural Science and Veterinary Medicine" King Mihai I of Romania", Timisoara, Romania, *Evaluation of Plant Variability Based on Molecular Markers Technology*

S3_P17

Camelia TULCAN, Faculty of Veterinary Medicine, Banat's University of Agricultural Science and Veterinary Medicine" King Mihai I of Romania", Timisoara, Romania, *Preliminary Study Regarding Biochemical Composition of Pikeperch (Sander lucioperca) Fresh Semen*

S3_P18

Gabriel BARLABAN, Faculty of Veterinary Medicine, Banat's University of Agricultural Science and Veterinary Medicine" King Mihai I of Romania", Timisoara, Romania, *Hematological modifications induced by enzootic bovine leukemia Retrovirus*

S3_P19

Alexandru RINOVETZ, Faculty of Food Processing Technologies, Banat's University of Agricultural Science and Veterinary Medicine" King Mihai I of Romania", Timisoara, Romania, *PCA Integration in the Effectiveness Interpretation of Dry Fractionation Operation in Centrifugal Field on a Natural Lipid Mix*

S3_P20

Narcisa MEDERLE, Faculty of Food Processing Technologies, Banat's University of Agricultural Science and Veterinary Medicine" King Mihai I of Romania", Timisoara, Romania, *The diagnostic value of hematology and blood biochemistry in piodemodicosis*

S3_P21

Iuliana POPESCU, Department of Chemistry, Banat University of Agricultural Science and Veterinary Medicine King Michael I of Romania , Timisoara, Romania, *Fatty acids profile of Argan oils*

S3_P22

Alexandru RINOVETZ, Faculty of Food Processing Technologies, Banat's University of Agricultural Science and Veterinary Medicine" King Mihai I of Romania", Timisoara, Romania, *Technical-economic study of natural storage for vegetal raw material – Red onion*

S3-P23

Monica DRAGOMIRESCU, Faculty of Animal Science and Biotechnologies,, Banat's University of Agricultural Science and Veterinary Medicine" King Mihai I of Romania" *Controlled release of enzymes from porous matrices*

S3-P24

RUGINA Dumitrita, Department of Chemistry and Biochemistry, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania, *Resveratrol attenuates hyperglycemia-induced stress in human retinal cells*

ORAL PRESENTATION FOR SECTION:

BIOTECHNOLOGIES AND BIOCHEMICAL INTERACTIONS IN ENVIRONMENTAL SCIENCES

Chairpersons: Rainer FISCHER and Radivoje PRODANOVIC

S4_OP01

14:30-14:45 – Ostafe Raluca, Department of Molecular Biotechnology, RWTH Aachen University, Aachen, Germany, *Ultra-high-throughput Screening Systems Based on Flow Cytometry and Microfluidic Devices for Directed Evolution of Different Enzyme Classes*

S4_OP02

14:45-15:00 – Prodanovic Radivoje, Faculty of Chemistry, University of Belgrade, Studentski trg 12, Belgrade, Serbia, *Development of ultrahigh-throughput screening platforms for directed evolution of glucose oxidase*

S4_OP03

15:00-15:15 – Cristea Adorján, Department of Molecular Biology and Biotechnology, Babeș-Bolyai University, Cluj-Napoca, Romania, *Physical and chemical detection of polyhydroxybutyrate from Halomonas elongata strain 2FF*

S4_OP04

15:15-15:30 – Boros Bianca-Vanesa, Department of Biology-Chemistry, West University of Timisoara, Timisoara, Romania, *Optimization of the ninhydrin reaction method for the estimation of acetylation degree of chitosan*

S4_OP05

15:30-15:45 – Vulpe Bianca, Department of Biology-Chemistry, West University of Timisoara, Timisoara, Romania, *Use of polyacrylamide gel electrophoresis for estimation of degree of polymerization of chitosan*

S4_OP06

15:45-16:00 – Roman Diana Larisa, Department of Biology-Chemistry, West University of Timisoara, Timisoara, Romania, *Structural bioinformatics characterization of chitin deacetylases*

S4_OP07

16:00-16:15 – Boros Bianca-Vanesa, Department of Biology-Chemistry, West University of Timisoara, Timisoara, Romania, *Preliminary results of an enzymatic test battery development for the assessment of toxicity of biopolymers*

S4_OP08

16:15-16:30 – Matica Adina, Department of Biology-Chemistry, West University of Timisoara, Timisoara, Romania, *3,5-Dinitrosalicylic acid method: an evaluation of enzymatic hydrolysis of chitosan*

S4_OP09

16:30-16:45 – Matica Adina, Department of Biology-Chemistry, West University of Timisoara, Timisoara, Romania, *Natural polymers used as wound dressings and their effect on microbial growth*

S4_OP10

16:45-17:00 – Isvoran Adriana, Department of Biology-Chemistry, West University of Timişoara, Timişoara, Romania, *Computational assessment of the pharmacokinetics properties of some water soluble chitosan derivatives*

S4_OP11

17:00-17:15 – Vlad-Oros Beatrice, Department of Biology-Chemistry, West University of Timisoara, Romania, *Environmental and clinical applications of chitosan*

POSTERS FOR SECTION:

BIOTECHNOLOGIES AND BIOCHEMICAL INTERACTIONS IN ENVIRONMENTAL SCIENCES

S4_P01

Blazic Marija, Faculty of Chemistry,University of Belgrade,Studentski trg 12, Belgrade, Serbia, *Directed evolution of the cellobiose dehydrogenase from Phanerochaete chrysosporium in yeast Saccharomyces cerevisiae*

S4_P02

Pahomi Alexandru, Department of Biology-Chemistry, West University of Timisoara, Timisoara, Romania, *Preliminary studies on the physicochemical properties of chitosan membranes*

S4_P03

Duda-Seiman Corina, Department of Biology-Chemistry, West University of Timisoara, Timisoara, Romania, *Extraction and antibacterial activity of natural compounds from walnuts (Juglans regia)*

S4_P04

Martin Eliza, Department of Bioinformatics, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania, *De novo Peptide Design for Enhanced Heavy Metal Accumulation*

S4_P05

Boiangiu Razvan-Stefan, Faculty of Biology, Alexandru Ioan Cuza University of Iasi, Romania, *Assessing the presence of nic-genes in Arthrobacter sp. AK-YN10*

S4_P06

Zelenović Nevena, Faculty of Chemistry,University of Belgrade,Studentski trg 12, Belgrade, Serbia, *Cellobiose dehydrogenase based screening system for directed evolution of cellulase from Trichoderma reesei*

S4_P07

Balaž Ana Marija, Center of Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, 11000 Belgrade, Belgrade, *Semi rational design of cellobiose dehydrogenase from Phanerochaete chrysosporium for increased oxidative stability*

S4_P08

Nešić Andrijana, Department of Biochemistry, University of Belgrade, Serbia, *Cloning, expression and purification of recombinant β-1,3-glucanase, allergen from banana, in the prokaryotic expression system*

S4_P09

Spasojević Milica, Innovative Center of the Faculty of Chemistry, University of Belgrade, Belgrade, Serbia, *Preparation of macroporous particles based on glycidyl methacrylate and ethylene glycol dimethacrylate for the enzyme immobilization*

S4_P10

Popović Nikolina, Faculty of Chemistry, University of Belgrade, 11000 Belgrade, Serbia, *Modification of carboxymethylcellulose with phenols for peroxidase induced hydrogels formation and electrospinning*

S4_P11

Mrkic Ivan, Faculty of Chemistry, University of Belgrade, Serbia, *Evaluation of specific immune response after immunization of mice with Der p 2 and Der p 2-hemagglutinin chimera conjugated to gold nanoparticles*

S4_P12

Preda Gabriela, Department of Biology-Chemistry, West University of Timisoara, Timisoara, Romania, *Bioremediation of waters polluted with heavy metals*

The Annual International Conference
Romanian Society for Biochemistry & Molecular Biology
8 – 9 June 2017, Timișoara

Editorial

Welcome Future by Biochemistry

New Front. Chem. is pleased to host this Special Issue dedicated to the second time in the decade of the Annual International Conference of the Romanian Society of Biochemistry & Molecular Biology at Timișoara (7-9 June 2017)!

As the XXI Century is more oriented to the living resources, paralleling the unprecedented human spreading and with more and more demands in every day life, the natural sciences in general and the biochemistry in special, are challenged to offer the key for long living, better living, and green living alike. To this aim, international conferences as the present one, belonging to a prestigious series, aim to report but also triggering new findings, new opportunities and new threats to be faced towards finding the molecular-biomolecular dynamic equilibrium, growing equilibrium with the environment, sustainable equilibrium with the future. Highly reputed professors and senior scientists working and communicating and interacting with younger scientists and scholars are gathered by this conference in the inspiring town of Timisoara. The actual Special Issue is just a glimpse of what the effervesce of the conferences unfolded, yet offering in a nutshell the relevant abstract letters for the selected presentations and posters, yet with a compendium value for the diversity and trans-disciplinarily biochemistry and molecular biology are based on. Smart enjoy the present! Wise welcome the future!

Editorial Board

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LATEST DEVELOPMENTS IN MOLECULAR FARMING - PRODUCTION OF BIOPHARMACEUTICALS IN PLANTS

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The development of recombinant antibodies and vaccines has allowed us to treat and prevent a large number of life-threatening diseases. However, as things stand in 2017, the speed, capacity and scalability of current production systems is beginning to place limitations on this crucial technology. The large-scale production of antibodies, vaccines and other pharmaceutical recombinant proteins is restricted by the industry's current reliance on fermenter technology, particularly the culture of mammalian cells. This expensive and time-consuming production platform is preventing the distribution of recombinant protein drugs to those most in need. One way in which the above limitations can be addressed is through the use of plants and plant-based expression systems for rapid recombinant pharmaceutical protein production.

The economic production of plant-based pharmaceuticals depends on satisfactory yields and product quality. This presentation will discuss the latest development in antibody and vaccine development and their production by molecular farming, focusing particularly on strategies to maximize protein yields during upstream production and optimize protein recovery in the downstream processing steps. Such strategies often involve careful consideration of how the protein is expressed and targeted within the plant cell, a factor which affects yield, stability, quality and ease of isolation. Our long-term objective is to ensure that next generation of plant-based production systems for recombinant proteins will create the opportunity to deliver antibodies, vaccines and other biopharmaceuticals beyond the industrialized nations and into the developing world. Several case studies will be presented: HIV antibodies were chosen to undergo fast-track development, including risk assessment, expression in tobacco, scale-up, downstream processing and regulatory development, with the aim of performing clinical trials. In addition use of engineered plant cells for human vaccine candidates will be discussed.

Pharma-Planta is an EU Sixth Framework Integrated Project whose primary goal is to develop an approved production pipeline for plant-derived pharmaceutical proteins (PDPs). Although previous research has provided proof of the PDP concept, Pharma-Planta aims to develop an entire production chain by taking candidate pharmaceutical

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molecules from the expression platform through all stages of production and processing, ultimately to initiate phase I human trials in Europe. The Pharma-Planta Consortium comprised 40 interacting groups representing 33 public institutes and SMEs from 11 European Member States and South Africa.

At the beginning of the project, eight target molecules were chosen representing four key indication areas including HIV. From these molecules, two HIV antibodies were chosen to undergo fast-track development, which would include risk assessment, cloning, expression and optimization of production in plants, scale-up, downstream processing and regulatory development, with the aim of submitting at least one of them for clinical trials within the five years of the program. Two HIV neutralizing antibodies have been expressed successfully in the two main production crops being developed within the consortium – maize and tobacco. One of these antibodies, 2G12, has been expressed at levels greater 100 mg per kg of plant material. The plant-derived antibodies remain stable and functional and retain their neutralizing activity. The consortium has investigated novel upscaling and downstream processing strategies to provide multiple grams clinical grade antibody material for human clinical trials. Preclinical trials in rabbits have been completed successfully and we also conducted successfully a phase I clinical trial in the UK. This work will now be moved forward for a phase IIa clinical trial which is funded by an Advanced ERC grant from the European Commission.

We have also developed an interesting multi-stage malaria vaccine and neutralizing rabies antibody candidates and will discuss how these products have matured over the years both in performance and in manufacturing with the aim in mind to bring these two products into translational research within the next months.

Finally state of the art technology developments to accelerate the development and production of PMPs as well as regulatory issues will be discussed. Along this line an innovative new manufacturing concept using LED lighting in a vertical farm concept have been developed.

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USE OF MASS SPECTROMETRY FOR PROTEIN CHARACTERIZATION AND BIOMARKER DISCOVERY

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Proteomics is an emerging field that focuses on the study of proteins. The workhorse of proteomic research is largely mass spectrometry (MS), which can be used to provide unbiased assessment of the protein components of a biological sample. MS can also be used to study other "omics" such as glycomics, lipidomics, metabolomics, interactomics and others. Here we describe MS and its applications for protein characterization and biomarker discovery. Examples of protein characterization that will be presented include identification of stable and transient protein-protein interactions, MS-based structural biology and protein post-translational modifications such as disulfide bridges, N-glycan analysis, 4-hydroxynonenal (HNE) modifications and experimentally-borne alkylations. We will then focus on a particular protein, Tumor Differentiation Factor using a variety of approaches including molecular biology, cell biology, biochemistry, MS and bioinformatics. For biomarker discovery, we show examples of application of MS on identification of a biomarker signature for obstructive sleep apnea using atrial tissue, early detection of autism spectrum disorder (ASD) using serum and saliva and identification of a biomarker signature for early detection and for prediction of the onset of breast cancer (BC) using human breast milk as a biological matrix.

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COMPUTATIONAL ASSISTED RESEARCH IN MOLECULAR LIFE SCIENCES

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Intertwining experiment with computer modelling of biomolecular structures, dynamics and interactions strongly increase the pace of discovery in molecular life sciences by allowing a more refined insight into the functioning of biological systems at molecular level.

On one hand experimental derived constraints allows a stern reduction of the complexity of the solution searching space, which significantly reflects on model accuracy.

On the other hand more accurate molecular models allow us to infer more precisely on systems properties or their response to changes; inferences can be then tested experimentally in the lab, in a model guided experimental approach rather than the less focused pure empirical, blind research.

Over the past decade the Department of Bioinformatics and Structural Biochemistry of IBAR has aimed to systematically develop such combined strategies and use them in tackling complex problems in structural biology and various fields of applied molecular life sciences. We present here some basics of our approach and some more exciting recent examples related to very remote protein homology modeling, protein posttranslational modifications, protein-protein and protein-DNA interactions.

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IN SILICO INVESTIGATION OF PROTEIN-LIGAND INTERACTION

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Proteins are the main regulators of multiple cellular functions (in normal or pathological states), through interaction with partners ranging from small chemicals to huge molecular complexes. A thorough understanding of the molecular mechanisms governing protein-ligand interactions is crucial for developing novel medical and biotechnological applications. Despite considerable recent progress in experimental methods, drug development is still a time-consuming process involving substantial costs. Computational approaches (generally termed “Computer-Aided Drug Design”, CADD) can bring a major contribution towards increasing drug discovery effectiveness. The specific methods used mainly depend on the amount of structural knowledge of target protein and this presentation will give detailed examples from each situation. When 3D structure of target protein is available, structure-based methods such as structural analysis, molecular dynamics simulations [1] and docking [2] are used to reveal structural features and conformational changes that affect ligand binding and affinity. In the absence of target structure, remaining options are ligand-based drug design methods making use of statistical knowledge on already known ligands: quantitative structure–affinity relationship (QSAR) [3] or artificial intelligence-based methods [4].

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CHEMOINFORMATICS OF XENOBIOTICS

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Humans are widely exposed to xenobiotics: drugs, food additives and food dyes, pesticides, phthalates, cosmetic preservatives, etc. Most of data concerning the biological effects of xenobiotics are obtained in vitro or in vivo using animal models, but their effects on humans are not well understood. Scientific literature becomes abundant in promoting computational tools able to predict the pharmacokinetics of molecules proposed as new drugs, their molecular targets and their biological effects. These tools provided to be also suitable for testing the chemical compounds that may have biological effects, such as different kinds of xenobiotics.

This presentation describes the theoretical basis and the features of some computational algorithms used for predicting the absorption, metabolization, distribution, excretion and toxicity (ADME-Tox) profiles, the oral bioavailability, the skin permeation, the inhibition of the human cytochromes P450 enzymes (CYPs), the molecular targets and the biological effects of different kinds of xenobiotics: food additives and food dyes, phthalates, parabens and pesticides.

For humans, the biggest part of xenobiotics is undergone to metabolic transformations by CYPs, usually the families 1-3, their induction or inhibition by xenobiotics disturbing the metabolism of some endogenous compounds and/or important drugs and, consequently, affecting the human health. This presentation also illustrates the assessments of the predicted interactions of xenobiotics with CYPs by using molecular docking studies implemented under SwissDock computational tool. Some of considered xenobiotics are able to bind to the catalytic sites of one or more CYPs and it reflects their inhibitory potential on the enzymes activities. Taking into account that CYPs are involved in about 80% of the total drugs metabolism, our findings are important as they underline the possible synergistic effects due to xenobiotics exposure and drugs administration.

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USING ROBOTICS TO ENHANCE MOLECULAR SAMPLING

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Simulations of large molecules, like proteins and DNA, are crucial forunderstanding chemical processes, relevant for medicinal and biochemical applications. However, achieving ergodic sampling from their proper Boltzmann distribution, has proven challenging even with the recent increase in the computational power. One of the strategies used so far was to coarsen the details of molecular interactions but this has the disadvantage of decreasing reliability. As an alternative to coarsening, by using robotics, we are now at the bringe of simulating larger molecules without losing molecular interaction details. Using rigid bodies and generalized coordinates to simulate macromolecules we can reproduce the Boltzmann distribution by drawing highly uncorrelated samples. Mixing fully flexible and generalized coordinates dynamics, we are able to achieve ergodicity and reproduce free energy landscapes that directly reflect lab results.

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STRUCTURAL INSIGHTS INTO THE FUNCTIONAL DIVERGENCE OF HUMAN TOPOISOMERASE IIα AND IIβ ON THE DECATENATION CHECKPOINT

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Topoisomerase (topo) IIα and II β enzymes are involved in regulation of essential cellular processes and are targets for diverse anti-tumor drugs. These enzymes maintain genome stability by relaxing or unwinding negatively or positively supercoiled DNA molecules. One essential cellular process is the decatenation checkpoint, which delays the progression of G2 cells into mitosis, and is the most efficiently regulated when both Topo IIα and II β enzymes are present. Topo IIα and IIβ are large enzymes (~1500 aminoacids long) composed of three big structural domains: an ATP binding domain, a DNA binding domain & a C-terminal domain (CTD). Although these two isoforms are highly similar in the ATP and DNA binding domains and display similar catalytic activities, their functional activities are different and this may be due to their divergent CTD sequences (22% identity).

The regulation of the decatenation checkpoint is influenced by a conserved non-catalytic tyrosine (Y640 in topo IIα and Y656 in topo IIβ) placed in the central DNA binding domain, and by the CTD (whose tridimensional structure is not yet determined).

We use here homology modelling and various prediction methods in combination with experiment to explain: (*-a*) the differences between the two enzymes in the structural proximity of the mentioned tyrosines; and (*-b*) the differential impact of CTD on the function of the topo enzymes.

Work supported by Romanian Academy programs 1 & 3 of IBAR; and UEFISCDI grant PN-II-ID-PCE-2011-3-0342.

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TOWARDS CUBIC CLEF METHOD IN QSAR

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A novel method (The Clef Method) for assessing the enzyme-inhibitory activity of some organophosphate pesticides, their toxic potential and their reactivity (on-site binding) is proposed in our study. [1] The molecules are wrapped up in a cubic system respecting the principles of the graph theory – all non-Hydrogen atoms are considered to have the same contribution in space – they represent one node in the graph. The interatomic distances are not correlated with the length of the edges between nodes in the cubic systems but their adjacency is respected. The homogeneous cubic system represents the chemical organic space ("cube"), filled with Carbon atoms, and the system with a molecule packed represents the inhomogeneous organic space ("molecule in the cube"). A residual difference can be described (the space in the cube around the packed molecule) – the potential space.

These cubic systems, the "molecule-filled" nodes in the cube and the "potentials" (differences between the Carbon cubic systems and the "molecule in cube" fragments for the organophosphate molecules are characterized with a well known topological index – Wiener Index and correlations with some physical-chemical features of the molecules, like logP are made. [2] Further, logP can be correlated with the toxicological profile of the molecule, so direct correlations between the topology of the cubic systems and the toxicology and pesticide activity of the compounds can be proven.

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DESIGN AND MOLECULAR DOCKING STUDY OF THE NONCLEAVABLE ANTIBIOTIC HYBRIDS; THEORETICAL STUDIES

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Recent studies have been focused on the development of noncleavable dual-action molecules with antimicrobial activity. One of the noncleavable antibiotic hybrids is cadazolid, composed by a fluoroquinolone and an oxazolidinone core via a stable linker [1]. Regarding the mode of action, it was reported that cadazolid is acting as an oxazolidinone molecule but fails to demonstrate a substantial contribution from the fluoroquinolone function. Actually, cadazolid behaves like a more potent linezolid with a low systemic exposure and a high local concentration in the gastrointestinal tract [1]. Our theoretical studies focused on the characteristics, molecular properties and molecular docking simulations to identify and visualize the most likely interactions between ligands - cadazolid, linezolid, quinolone - and the receptor protein (Staphylococcus aureus ribosomal subunit, PDB ID: 4WFA). A restricted hybrid HF-DFT calculation was performed in order to obtain the most stable conformer of each ligand and a series of DFT calculations using the B3LYP levels with 6-31G* basis set [2] has been conducted. The docking studies have been carried out using CLC Drug Discovery Workbench Software in order to predict the most possible type of interaction, the binding affinities and the orientation of the docked ligand at the active site of Staphylococcus aureus ribosomal subunit [3].

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TOXICITY PROFILES AND PREDICTED BIOLOGICAL EFFECTS ON HUMANS AND ENVIRONMENT OF SOME PESTICIDES USED FOR WHEAT CROPS

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Pesticides are the only toxic substances introduced voluntarily into the environment with the aim of prevention, destruction and protection against pests, growth and defoliation of plants and for stabilization of nitrogen. The purpose of this paper is to present the possible biological effects of pesticides used in Romania for the control of pests on the wheat crops on human health and on environment.

In order to predict the toxicity and biological effects of these pesticides on humans we use the following computational tools: FAFDrugs to produce the absorption, distribution, metabolism, excretion and toxicity (ADME-Tox) profiles, SwissADME to compute the pharmacokinetics of pesticides and PASS to predict their toxic and/or side effects. For predicting environmental toxicology, the logarithm of inverse of the concentration of a compound that provides 50% growth impairment of *Tetrahymena pyriforms* in aqueous environment, (log[IGC50-1]), is computed using Ochem tool.

Ours study reveals the followings: (i) most of the active substances of pesticides used for wheat crops are easily absorbed by ingestion and respiration and have a high toxicity on humans; (ii) these substances are able to penetrate easily through the skin, to penetrate the blood-brain barrier and thereby to affect the central nervous system; (iv) many of them inhibit the enzymes of the cytochrome P450 involved in drugs metabolism; (v) all the pesticides considered in this study produce various adverse effects on humans. All of these observations are especially important for workers handling these substances and who are frequently subject to their action.

The active compounds from the considered pesticides are water soluble and reflect environmental toxicity.

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COMPUTATIONAL ASSESSMENT OF THE BIOLOGICAL EFFECTS OF SYNTHETIC ANABOLIC STEROIDS ON HUMANS AND ENVIRONMENT

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Anabolic androgen steroids (AAS) are synthetic drugs derived from testosterone that can be used under medical prescription for treating diseases resulting from steroid hormone deficiency or from the loss of muscle mass. They are used to develop the male sexual characteristics (the androgenic effect) or/and to promote skeletal muscle growing (the anabolic effect) and are controlled substances in many countries. Administration of AAS provided to have dose dependent side effects manifested especially when they were used in high doses and for a long period of time.

To the best of our knowledge, some of AAS have been tested and approved as drugs used for humans or animals, but other AAS are under control/evaluation and their metabolism, effects and side effects are not well understood. More than it, literature data concerning the androgenic, anabolic and side effects of AAS are often discordant, the targets of AAS in the human body are not well known and characterized and molecular mechanisms of AAS actions are also poorly understood. In addition, the administrated doses by both athletes and non-athletes are often higher than those used in controlled studies conducting to unknown or more pronounced side effects than what is reported in scientific literature. Not at last, veterinary drugs are often used by humans to enhance their physical performances.

The aim of this study is to predict the absorption, distribution, metabolisation, excretion and toxicity (ADME-Tox) profiles, the biological activity spectra, the molecular targets, the molecular pathways and the toxicological and side effects of some AAS both on human organisms and environment and to correlate these predictions with available literature data concerning the toxicological effects of synthetic AAS.

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LUMINESCENT NANOPARTICLES OBTAINED BY THERMAL DECOMPOSITION OF COORDINATION COMPOUNDS

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Complex compounds composed of metal ions and organic ligands [1] can be used as precursors for micro- and nanoparticles with interesting properties, which include fluorescence and phosphorescence that are important for the imagistic identification of various medical pathologies. In particular, the magnesium–aluminum and strontium–aluminum polyoxalates are convenient precursors for the $MgAl_2O_4$ and, respectively, $SrAl_2O_4$ spinels [2]. Furthermore, lanthanide-doped spinels show promising optical properties, which make them valid candidates for biomedical applications. In this work, we present a preparation method for such nanoparticles, which is based on the thermal decomposition of heteropolynuclear coordination compounds. The thus-prepared nanopowders were characterized by X-ray diffraction, transmission electron microscopy, energy-dispersive X-ray spectroscopy and Fourier transform infrared spectroscopy. The visible green light emission of the obtained erbium-doped spinels under ultraviolet pumping was confirmed by spectrofluorimetry.

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TLC AND DENSITOMETRIC ANALYSIS OF METHANOL EXTRACTS FROM MARINE ALGAE

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The biological compounds extracted from algae constitute a valuable and highly available resource for the pharmaceutical field. A method of separation and analysis of photosynthetic pigments from two algae species, namely Padina pavonica and Codium fragile, collected from the Mediterranean Sea, was developed. The applied protocol consists in the Thin-layer chromatography (TLC) separation of these pigments, using silica gel polar phase, followed by UV-VIS densitometry analysis. The optimal conditions for the extraction of photosynthetic pigments and the densitometry characterization parameters are detailed in this paper.

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LONG BONDS INVOLVED IN EI-MS FRAGMENTATION OF ALDOSE DERIVATIVES PREDICTED USING ADVANCED BASIS SETS

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Computational methods frequently predict long bonds in radical cations of vicinal diols, like sugars [1,2]. Although semi-empirical methods offer lengths for such bonds that are likely to be exaggerated [1], the more advanced DFT methods can give better results. In this work we have performed DFT computations with large basis sets to calculate the length of the C4-C5 bond in radical cations of acetalized monosaccharides. The results correlate well with our previous studies regarding the position of the long bond. The lengths of these bonds are slightly shorter when compared with the values given by semi-empirical methods, like RM1 and PM7 [1].

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ALLERGEN INTERACTIONS WITH INTESTINAL EPITHELIAL CELLS CAN CONTRIBUTE TO THE SENSITIZATION PROCESS IN FOOD ALLERGY

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Prevalence of food allergies are on the exponential increase in the industrialized countries and understanding the pathomechanism of the sensitization process can enhance search for therapeutic approaches that can change the natural history of disease.

The epithelial cell layer plays an important role in the process of sensing insults from the environment and modulates both innate and adaptive immunity by producing functional molecules (cytokines, chemokines) and by physical interactions with cells of the immune system. Recent evidences that allergens can interact with the components of the innate immunity and promote Th2 response provide a novel pathway for investigation of the allergic sensitization process.

It is well known that inhalant allergens with intrinsic proteolytic activity can impair the epithelial barrier by direct activity against the tight junction proteins. Interactions of some clinically relevant food allergens with proteolytic activity (i.e. cysteine and serine proteases) on the epithelial layer of the gastrointestinal tract are not well understood. In addition, immunomodulatory effects of food allergens with epithelial cells are still ill defined.

We studied effects of food allergens on the integrity of epithelial cells by measuring trans-epithelial resistance and protein transport across the monolayer. Integrity of the monolayer was also assessed by confocal microscopy. Activation of innate immunity was assessed by measuring up-regulation of pro-inflammatory cytokines.

Our findings indicate that allergen interactions with the epithelial cells can contribute to the sensitization process in food allergy.

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PLASMA MICRORNAS IN PARKINSON'S DISEASE

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Parkinson's Disease (PD) is a progressive degenerative disorder of the elderly, characterized by a progressive loss of the dopaminergic neurons in the substantia nigra pars compacta. PD has a complex etiology, which involves both environmental and genetic factors (responsible for up to 10% of the cases)

The diagnostic of PD is based on specific clinical signs like tremor, rigidity, akinesia, postural change and a positive response to therapy (Levo-dopa +/- peripheral decarboxylase inhibitor). Currently there are no specific tests or biological markers to diagnose PD. Furthermore, the prognosis of PD progression is also based only on clinical features (initial tremor at presentation, older age, gender), and currently there are no biological markers able to predict the course of the disease.

MicroRNAs are newcomers in the PD field and, despite their remarkable stability in various biological fluids, the relatively few data gathered up to now are largely non-overlapping (when not straightforwardly conflicting), mainly due to differences in the study designs and the analytical platforms used (microarrays, qRT-PCR and qRT-PCR array, Next Generation Sequencing).

We have used a two-step approach based on qRT-PCR array (discovery step) and individual TaqMan qRT-PCR assays (validation step) to screen for plasma microRNAs in the plasma of PD patients recruited from the Neurology Clinic, County Hospital, Timisoara.

We further describe two microRNAs found to be specifically associated to dopaminergic neurons degeneration and discuss their relevance as putative PD diagnostic and prognostic biomarkers.

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IMPROVING THE EFFICACY OF TUMOR-TARGETED THERAPIES USING TUMOR-ASSOCIATED MACROPHAGES RE-EDUCATION STRATEGIES

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Among all tumor microenvironmental cell types, tumor-associated macrophages (TAM) seem most important in supporting tumor growth via coordination of angiogenesis, inflammation, oxidative stress, invasion, and metastatic capacity of tumors. Moreover taking the advantage of the high phenotypic plasticity of macrophages, TAM can be re-programmed or „re-educated" to treat cancer via therapeutic strategies that convert TAM to antitumor macrophages. In this view, our research investigated the antitumor efficacies of different combined long-circulating liposomal drug therapies that used TAM re-education strategies (via inhibition of TAM-mediated angiogenesis by using liposomal prednisolone phosphate or oxidative stress suppression by using liposomal simvastatin) together with either certain cytotoxic (liposomal doxorubicin or liposomal 5-fluorouracil) or anti-angiogenic (using administration of liposomal 5,6-dimethylxanthenone-4-acetic acid) tumor-targeted therapies [1-4]. These studies were performed in both in vitro and in vivo models consisting of C26 murine colon carcinoma and B16.F10 murine melanoma animal models. Our data indicated that both TAM re-education strategies emphasized the antitumor activities of the combined tumor-targeted treatments tested offering valuable promises for future anticancer therapies.

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LIPOSOMAL PREDNISOLONE PHOSPHATE ENHANCED THE ANTITUMOR ACTIVITY OF LIPOSOMAL DOXORUBICIN IN B16.F10 MURINE MELANOMA *IN VIVO*

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Amongst the skin-cancer types, melanoma is the most aggressive form causing 75% of the skin cancer-related deaths [1]. Its invasiveness and resistance are the main causes of unsuccessful systemic therapies [2]. Given the high heterogeneity of the tumor milieu, targeting cells that support tumor development and progression should improve the anti-tumor efficacy of conventional cytotoxic therapies. The liposomal formulation of prednisolone inhibits tumor growth by passively targeting tumor-associated macrophages (TAM), present in the tumor microenvironment [3]. In this study, we propose a novel combined liposomal drug therapy based on the simultaneous administration of long-circulating liposome-encapsulated prednisolone phosphate (LCL-PLP) (to target TAM) and long-circulating liposome-encapsulated doxorubicin (LCL-DOX) (to target tumor cells) (LCL-PLP+LCL-DOX).

Our results demonstrated that combined liposomal drug therapy tested (10 mg/kg of LCL-PLP and 2 mg/kg of LCL-DOX) inhibited almost totally the growth of B16.F10 melanoma in vivo. Moreover, LCL-PLP+LCL-DOX inhibited melanoma growth more efficiently than each single liposomal drug therapy tested. Thus, inhibition of tumor growth was about 85% after LCL-PLP+LCL-DOX, 70% after LCL-PLP and 56% after LCL-DOX. Our data also revealed a significant suppression of intratumor oxidative stress that might be responsible for the tumor development deceleration noted.

It might be possible that targeting TAM by LCL-PLP enhanced the sensitivity of cancer cells to LCL-DOX, by modulating the oxidative stress levels as well as inflammation and angiogenesis in tumor microenvironment. Further studies are needed to determine the molecular mechanisms involved in the antitumor efficacy of the combined targeted therapy proposed in this research.

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A NOVEL COMBINATION THERAPY FOR THE TREATMENT OF MELANOMA BY TARGETING TUMOR CELL PROLIFERATION AND ANGIOGENESIS

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Malignant melanoma is one of the most frequent forms of skin cancer being characterized by a high capacity of invasion and metastasis. Despite advances in the field, in a growing fraction of patients the melanoma progress to advance disease which has a poor prognosis. Recent advances in the understanding of how melanoma cells evade the immune system as well as identification of the key molecular mutations leading to malignant melanoma resulted in the approval of novel therapeutic agents for metastatic melanoma. However, suboptimal benefit, low rate response, high toxicity as well as therapy resistance are important limitations of the newly approved therapeutic agents. Therefore, in the present study we aimed to develop an effective combination therapy for melanoma by administration of a low dose of doxorubicin, a cytotoxic drug which targets tumor cells proliferation, concomitant with prednisolone phosphate, a substance with anti-inflammatory and anti-angiogenic properties which can modulate the tumor microenvironment (1). Our results have shown that the combination therapy induced an enhanced cytotoxic effect in melanoma cells co-cultured with murine macrophages via strong inhibition of cell proliferation as well as of the production of angiogenic/inflammatory proteins. Also, doxorubicin administrated concomitant with prednisolone phosphate induced a higher rate of apoptosis in tumor cells compared to both therapies administrated alone. Additionally, administration of a low dose of doxorubicin was associated with reduced oxidative stress, thus overcoming one of the main adverse effects of this cytotoxic drug.

Thus, this combination therapy might be a promising strategy for the treatment of melanoma by targeting different tumorigenic processes: tumor cell proliferation, inflammation and angiogenesis.

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ENHANCED ANTITUMOR EFFICACY INDUCED BY THE COADMINISTRATION OF SIMVASTATIN AND DMXAA ON AN *IN VITRO* MELANOMA INFLAMMATION MODEL

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Tumor-associated macrophages (TAMs) are the tumor microenvironmental cells with one of the most contrasting and controversial roles in angiogenesis – a crucial process for B16.F10 murine melanoma development. When the tumor is able to evade immunosurveillance, the macrophages recruited to the tumor site can be reprogrammed by the microenvironment to become corrupted, supporting cancer growth. Moreover, hypoxia is present in most tumors due to the abnormal vascularization, promoting invasiveness, metastasis, suppression of apoptosis via pro-survival anabolic gene expression and also local immunosuppression [1]. Therefore, a treatment based on the combined administration of two anti-angiogenic drugs, simvastatin (SIM) and 5,6-dimethylxanthenone-4-acetic acid (DMXAA) might lead to the obtaining of a successful therapy for melanoma. SIM belongs to statins – a class of drugs used in the therapy of cardiovascular diseases, but with known anticancer actions via enhancing antitumor properties of TAMs [2]. DMXAA is one of the most powerful tumor-vascular disrupting agents. Thus, the aim of this research was to investigate the cytotoxicity as well as the anticancer activity mechanisms of the combined administration of SIM and DMXAA on the in vitro model for melanoma microenvironment.

Our data suggested that high cytotoxic effects of the combined administration of SIM and DMXAA on the on the hypoxic co-culture of B16.F10 melanoma cells and macrophages were exerted via suppression of angiogenic properties of both cell types as well as reduction of the invasion potential of the tumor cells. In addition, proapoptotic effects were noted at higher concentrations of SIM in combination with DMXAA. Overall, the combined administration of SIM and DMXAA indicated a potentially synergistic drug combination for targeted melanoma therapy.

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EFFICACY OF THE LIPOSOMAL SIMVASTATIN IN THE COLON CARCINOMA TREATMENT APPROACH

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Chemoresistance and low therapeutic index are major limitations for colorectal cancer treatment. Tumor-targeted therapy with new therapeutic agents, such as simvastatin, a lipophilic statin recently displayed as an effective anticancer drug, could be a solution to overcome these drawbacks. Therefore, the aim of this study was to investigate the antitumor activity and the main antitumor mechanisms of long-circulating liposomal simvastatin (LCL-SIM) on C26 murine colon carcinoma-bearing mice.

To achieve this goal we used an in vivo colon carcinoma tumor model generated by subcutaneous inoculation of C26 cells in the right flank of 6-8 weeks-old male Balb/c mice. After free and liposomal SIM treatment, we evaluated the effects each therapy on tumor growth, proliferation and apoptosis through PCNA and BAX immunostaining of tumor tissues, angiogenesis via protein array analysis of pro- and anti-angiogenic proteins, expression levels of the transcription factor NF-kB by Western Blot analysis and the production level of malondialdehyde – an oxidative stress marker via HPLC.

Our results showed a strong inhibition of tumor growth after LCL-SIM treatment compared with control group, but not after free drug administration. LCL-SIM treatment apart from free SIM, correlates negatively with mitotic index and positively with apoptotic index. LCL-SIM therapy determined the overall reduction of pro-angiogenic proteins and inhibited the activation of NF-kB transcription factor.

In conclusion our study showed that LCL-SIM inhibited C26 colon carcinoma growth mainly via suppression of tumor angiogenesis and inflammation as well as through direct cytotoxic effects on C26 colon carcinoma cells.

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COMBINATION THERAPY OF LIPOSOMAL PREDNISOLONE AND 5-FLUROURACIL EXERTS STRONG ANTI-ANGIOGENIC AND ANTI-INFLAMMATORY EFFECTS ON C26 COLON CARCINOMA

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One of the main approaches for the treatment of advanced colorectal cancer (CRC) is based on 5-fluorouracil (5-FU) monotherapy. Nonetheless, the clinical applications of this drug are greatly limited due to the low therapeutic index and the resistance acquired by tumor cells after 5-FU administration. Hence, to increase 5-FU efficacy, we tested a novel tumor-targeted combination therapy of long-circulating liposomes encapsulating prednisolone phosphate (LCL-PLP) - with anti-angiogenic effects - and LCL-5-FU for the treatment of C26 colon carcinoma-bearing mice. To this end, tumors were induced into BALB/c syngeneic mice by subcutaneous injection of 1x106 C26 colon carcinoma cells. Liposomal formulations of LCL-5-FU (1.2 mg/kg) and LCL-PLP (20 mg/kg) were prepared by lipid film hydration method and administered alone or in combination by i.v. injection at days 8 and 11 after tumor induction.

We evaluated the effects of the proposed liposomal combination therapy on C26 colon carcinoma growth in vivo as well as on the main processes that support tumor development, such as tumor inflammation, angiogenesis, and oxidative stress via screening of angiogenic/inflammatory proteins, western blot analysis of key transcription factors, malondialdehyde measurement and immunohistochemistry staining. Our results revealed that concurrent administration of liposomal 5-FU and PLP exerted stronger tumor growth inhibitory effects than the single liposomal therapies, mainly through the suppression of angiogenesis and inflammation. Accordingly, our study emphasizes the main advantage of combination therapy over single agent therapy, that is, the enhancement of 5-FU cytotoxicity when adminstered together with PLP and displays an effective therapeutic approach that should be furthermore exploited.

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PHOSPHOINOSITIDE 3-KINASES KLASS II ARE REGULATING HEPATITIS C VIRUS LIFE CYCLE

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Hepatitis C Virus infection determines liver pathology which ranges from steatosis and fibrosis to cirrhosis and hepatocellular carcinoma. Lipid metabolism is central to HCV induced liver pathogenesis and it is deeply involved in every step of the viral life cycle. The host factors involved in HCV induced pathogenesis are poorly understood. In order to identify potential liver disease biomarkers and to understand the HCV assembly process, protein interactomics was used to determine the interactomes of three viral proteins (NS2, p7 and NS5A) essential for the HCV assembly process. Affinity purification (AP) suitable tags were fused to the viral proteins in the context of infectious recombinant viruses and AP followed by mass spectrometry was performed in infectious virions producing cells. To determine the function of different hits, loss of function and mutant rescue assays were used. Class II phosphoinositide 3-kinase α (PI3KC2 α) was identified as an interactor of the NS2 complex. PIK3C2 isoforms positively regulated the entry and the replication steps at different extent. While isoforms α and γ depletion stimulated virion production, the isoform β downregulation inhibited the infectious particle production. The N-terminal domain of the PI3KC2α was shown to be involved in virion production inhibition and to be a determinant of PI3KC2α interaction with the HCV NS2 complex. A role of the endocytic pathway in HCV assembly process is discussed.

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miRNAs as Diagnostic Biomarkers of Acute Myocardial Infarction

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Cardiovascular diseases are the first cause of death (over 30%), with the highest percentage represented by acute coronary syndromes [1]. Hospital mortality in patients with AMI varies between 6% and 14% [1]. Several recent studies have shown a decrease in mortality of acute and long-term after acute myocardial infarction, in parallel with the increased use of reperfusion therapy, primary percutaneous coronary intervention coronary, antithrombotic therapy and secondary prevention drugs.

Cardiac troponin T and I and creatine kinase isoenzyme MB (CK-MB) are currently the most commonly used biomarkers to determine myocardial necrosis. However, the diagnostic value of these classical cardiac biomarkers is limited, due to the fact that circulating levels fluctuate considerably in the early period after AMI and are easily influenced by hepatorenal function [2]. Also elevated levels of these biomarkers have been associated with other morbidities such as cardiac contusion or trauma, in heart failure, aortic valve disease, hypertrophic cardiomyopathy, tachycardia or bradyarrhythmia, rhabdomyolysis with cardiac injury, pulmonary embolism or severe pulmonary hypertension, metabolic diseases amyloidosis, hemochromatosis, sarcoidosis, inflammatory diseases, excessive effort [2].

Therefore, the identification of novel biomarkers for early and correct diagnosis of AMI is needed. Recent studies provide growing evidence that exosomal miRNAs can be used as biomarkers in cardiovascular diseases [3].

miRNA miR-1 levels in circulation were significantly elevated in patients with acute myocardial infarction compared to non-acute myocardial infarction controls. Furthermore, other studies demonstrated a strong correlation of miR-1 levels with CK-MB levels as well as with cardiac TnI levels. Additional miRNAs involved in myocardial infarction include miR-133, miR-208, miR-499, and miR-214 [4].

Identifying plasma microRNAs directly involved in AMI, can help develop a new generation of diagnostic and prognostic biomarkers.

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MESENCHYMAL STEM CELL BEHAVIOR ON POLYMERIC SURFACES

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Poly N-isopropylacrylamide (pNIPAm) is of great interest especially for tissue engineering applications [1,2]. Low research on pNIPAm coated surfaces cytotoxicity effect on cells was conducted till now. In this study bone marrow human mesenchymal stem cells (hMSC) behavior on pNIPAm and its derivatives based surfaces was monitored. pNIPAm thin films have been obtained using Matrix Assisted Pulsed Laser Evaporation (MAPLE) method. The effect of thin films surface structure/properties on hMSC responses like adhesion, morphology, viability and proliferation was assessed by quantitative and quantitative cell-based methods.

In vitro detection of cell viability using colorimetric assay based on conversion of MTS to formazan by metabolically active cells showed similar proliferation rate of hMSC cells on all tested materials. Results have been confirmed by live/dead cell viability/cytotoxicity test which measures dual parameters, intracellular esterase activity and plasma membrane integrity of the cells. Thus, cytometric analysis based on ethidium homodimer-1 quantification revealed that different surfaces were not cytotoxic to the hMSC cultured for 72 h; more than 95% of the cells retained their viability. SEM, phase-contrast microscopy investigations and fluorescent immunostaining of cytoskeleton proteins showed that surface modification can induce morphology changes and alter initial steps of cell attachment and expansion. Microscopy explorations revealed attachment and shape differences at 3h post seeding of osteoprogenitor cells according to surface properties. Long-term experiments showed no significant differences in hMSC cell morphology and spreading after 72h of cultivation on coated surfaces, compared with control.

All these findings proved that polymeric coated surfaces support cell proliferation and exhibit no cytotoxic effect on hMSC cells, thus pNIPAm-modified thin films designed by laser method could be used as potential substrates for regenerative medicine.

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IN VITRO BIOCOMPATIBILITY TESTING OF CLOISITE-BASED NANOCOMPOSITES FOR CHEMOTHERAPEUTIC DRUG DELIVERY SYSTEMS

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Encapsulation of different chemotherapeutic molecules is an attractive approach for improving delivery efficiency and specificity of anti-cancer drugs. Incorporation of nanoclays into polymeric systems seems to modulate drug release [1]. Potential cloisite candidate for polymer interpenetrated networks development and the assembled networks were evaluated for their cytotoxic effects against two different mammalian cell lines.

Commercially available natural montmorillonite, Cloisite®Na+ and the organically modified ones, Cloisite®30B and Cloisite®93A were tested in vitro at different concentration up to 48 hours on normal MDBK and colon adenocarcinoma HT29 cells. Using an automated live microscopy imaging system, cell morphology was examined. The effect of nanoclays on cell viability and proliferation was assessed by a colorimetric assay based on cellular metabolic activity. Results revealed that among the various clay nanoparticle tested, Cloisite®93A at low concentration is the best candidate for incorporation into poly (methyl) methacrylate (PMMA) polymeric network. Three different PMMA-cloisite-based constructs were designed and further employed for in vitro analysis. All biopolymer-modified clays did not affect cell viability, proliferation and morphology after 24 and 48 hours of direct exposure on either cell lines.

Overall the in vitro results show the possibility of PMMA- Cloisite®93A based novel structures to be used as a promising type material with adequate properties for efficient drug release in cancer therapy.

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NOVEL LACTOFERRIN-DERIVED PEPTIDES WITH ANTI-HEPATITIS B VIRUS CAPACITY

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Lactoferrin (Lf), a glycoprotein from the transferrin family, features antiviral activity against both naked and enveloped viruses at an early phase of infection. Previously it was reported that two glycosaminoglycans (GAGs) binding sites from the N-terminal (N-t) region of Lf are important for its antiviral activity, one of them being a cationic cluster (GRRRR).

We have demonstrated that four human Lf (HLf)-derived peptides (HLPs), from the N-t domain of the native protein (1-47 amino acids sequence), were able to inhibit hepatitis B virus (HBV) infection by 40 to 80%. Peptide HLP1-23 containing the GRRRR cluster was the most potent inhibitor at 250 μM, preventing HBV infection by neutralizing the viral particles.

We aimed to improve the antiviral capacity of the HLPs by investigating other new peptides corresponding to N-t domain of HLf: a short peptide with increased overall positive charge containing the first GAGs binding site of HLf (HLP1-9), thus supposedly displaying improved affinity for hepatic cell, and a longer peptide with increased number of aromatic amino acids and increased stability through supplementary aromatic stacking interactions and containing the second GAGs binding site of HLf (HLP20-45).

The new HLPs were assayed for potential cytotoxicity and infectivity tests in HepaRG and HepG2.2.2.15 cell infection systems. The data revealed that both peptides exhibited inhibition of HBV infection at an early step. However, HLP1-9 demonstrated only modest inhibition of HBV infection (30 %) at 500 μM in HepaRG cells, while HLP20-45 inhibited HBV infection by 45 % at 250 μM. Experiments performed in HepG2.2.2.15 HBV replication system revealed that the peptides are not viral replications inhibitors. Further studies in search of a more robust inhibition of HBV infection by other HLPs are under investigation.

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PHYSICAL-CHEMICAL CHARACTERIZATION AND BIOLOGICAL ASSESSMENT OF BOVINE DERIVED HYDROXYAPATITE THIN FILMS SYNTHESIZED BY PULSED LASER DEPOSITION FOR A NEW GENERATION OF METALLIC IMPLANTS

FLORIAN Paula E. [1], POPESCU Andrei C. [2], STAN George E. [3], POPESCU-PELIN Gianina [2], ENCULESCU Marius I. [3], ZGURA Irina[3], OKTAR Faik N.[4,5], SIMA Livia E. [1], ROSEANU Anca [1], DUTA Liviu M.[2*]

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We report on the synthesis by Pulsed Laser Deposition technique of hydroxyapatite (HA) thin films from renewable biological (bovine bones) sources. The role of reinforcement agents (e.g., MgF2, Li3PO4, Li2O, or Li2CO3) on the morphology, structure, bonding strength and cytocompatibility of the films was investigated.

The morphological, structural, compositional, and mechanical characteristics of the films were evidenced by Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-Ray Spectroscopy (EDS), Atomic Force Microscopy (AFM), X-Ray Diffraction (XRD), Fourier Transformed Infrared (FTIR) Spectroscopy, and pull-out tests, respectively. In vitro tests were performed to evaluate the viability of human mesenchymal stem cells (hMSC) grown on thin films, using calcein AM/ethidium homodimer method.

SEM investigations of the films evidenced a surface morphology consisting of particulates with diameters of (2-3) μm. The roughness of the surface, inferred from AFM measurements, was in the range of (3-8) nm. XRD analyses demonstrated that the synthesized structures consisted of a pure HA phase, with different degrees of crystallinity mainly influenced by the reinforcement agents. FTIR spectra showed a growth of a biomimetic HA layer after three days of immersion in Simulated Body Fluids, proving a good bioactivity of the films. Besides the main constituents of the mineral bone part, EDS spectra indicated the presence of typical natural bone doping elements. Importantly, the calculated bonding strength values of HA structures were superior to the ones imposed by International Standards. In vitro viability tests revealed that high concentrations of Li2O within HA thin films were very toxic for hMSC, whilst deposition of Li2CO3, Li3PO4 or MgF2 promoted the cell growth on all thin film surfaces.

Taking into consideration the characteristics of the thin films, the low fabrication cost from renewable resources and the good biocompatibility, these reinforced materials could represent a prospective candidate for a new generation of metallic implants.

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PLANT MICRORNA IDENTIFICATION & TRANSFER– A NEW DIMENSION TO HERBAL MEDICINE

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Introduction: MicroRNAs (miRNAs) are a group of small, noncoding endogenous RNA (21-25 nucleotides long) with an important role in gene expression regulation by targeting specific mRNAs in plants, animals and humans. To date, no miRNAs from marigold (Calendula officinalis), one of the best known medicinal plants, have been identified.

Objective: Identification of plant microRNAs that survive the passage through gastrointestinal tract following digestion in mice

Materials and Methods - Plant small RNA isolation and extraction
- Sequencing data analysis and plant miRNA identification
-Animal experiment

Results: The cDNA libraries of two tissues from Calendula (petals and inflorescence) were prepared and small RNA-seq were conducted according to Ion Torrent sequencing protocols. A total of 4 miRNAs, with 0 mismatches, (ath-mir166a, osa-mir166h, ppt-mir894 and ath-mir8175), were identified based on their sequence complementarities.

Conclusion: The potentially conserved miRNAs from Calendula were identified only in the inflorescence, which is the part used for medicinal purposes. This could lead to a better understanding of the relationship between plant exogenous genetic material and the changes in mammal upon oral ingestion.

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THE INFLUENCE OF VITAMIN D SERUM LEVEL ON PERIODONTAL PARAMETERS

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Thirty years ago, vitamin D has been studied only for its involvement in bone metabolism. In our days, the active form of vitamin D—1,25(OH)2D—has also been investigated for its role in inflammatory, infectious and autoimmune diseases. The low level of vitamin D serum has been associated with a multitude of diseases such as osteoarticular, metabolic, cardiovascular, digestive, endocrine, neurological and psychiatric diseases.

This case-control study included 56 patients with chronic periodontitis and 56 healthy periodontally patients, selected from the Department of Periodontology of "Victor Babeş" University of Medicine and Pharmacy in Timisoara, between 2014 and 2016. The diagnosis of chronic periodontitis was established based on the criteria listed by the International Workshop for a Classification of Periodontal Diseases and Conditions (IWCP) in 1999.

The measured periodontal parameters were: plaque index, periodontal probing depth, bleeding on probing, periodontal attachment level and the number of absent teeth. Also 2 ml of venous blood were collected from each examined patient in a K2-EDTA tube and were sent to the Department of Biochemistry of "Victor Babes" University of Medicine and Pharmacy from Timisoara to determine the serum level of vitamin D.

Almost all the considered clinical parameters were highly positively correlated with each other and negatively correlated with the vitamin D level. The vitamin D serum level of patients (13.01±5.10 ng/ml) was significantly lower than in the control group (22.10±5.63 ng/ml) ($p < 0.001$).

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SHORT TERM ACTIVATION OF CANONICAL WNT SIGNALING PATHWAY INHIBITS PROLIFERATION OF LNCaP CELLS

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The canonical Wnt pathway (or Wnt/β-catenin pathway) plays crucial roles in proliferation, pluripotency and cell fate during differentiation and development [1]. Upon Wnt stimulation, displacement of GSK-3β from the destruction complex inhibits β-catenin degradation and leads to translocation of β-catenin in the nucleus with consecutive activation of TCF/LEF target genes which promotes proliferation [2]. Activation of canonical Wnt-signaling has been involved in the biology of various cancers and proliferation of cancer stem cells [3].

Here we have investigated the effect on cell proliferation of canonical Wnt signaling activation through GSK3β inhibition by 6-[[2-[[4-(2,4-Dichlorophenyl)-5-(5-methyl-1H-imidazol-2-yl)-2-pyrimidinyl]amino]ethyl]amino]-3-pyridinecarbonitrile (CHIR 99021) on human LNCaP prostatic cancer cells. We show that short-term stimulation of canonical-Wnt signaling in LNCaP cells by treatment with CHIR 10μM determined a reduction in the number of cells, due to an inhibition of cell proliferation. Cell cycle analyses depicted significant changes in the distribution among G0/G1/S/G2/M phases, arguing for a possible effect at the level of the cell cycle check points.

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DIFFERENTIALLY EXPRESSED LONG NON-CODING RNAS RELATED TO DIETARY INTERVENTIONS IN A MOUSE MODEL

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Long non-coding RNAs (linc) have received widespread attention in recent years due to their involvement in various physiological and pathological conditions. The aim of the current study was to investigate the effect of soy enriched diet modifications on the expression of linc species in mice target tissues. Mixed genetic background 3-months old male mice were fed for 4 weeks a soy enriched diet, and liver and intestine tissues were collected upon dissection and preserved in RNAlater solution. A similar lot of mice were fed their usual diet as controls. Total RNA was extracted with Trizol and transcriptome analysis was performed using the Agilent G3 mouse gene expression v.2 arrays containing 4578 linc species. Data analysis was performed using an R/limma package, taking the median expression signal, quantile normalized, after background correction. Benjamini-Hochberg correction for multiple comparisons was also performed.

Five putative linc sequences mapped to 4 non-overlapping genetic positions on chromosomes 1, 9, 15 and 17 were found to be at least 1.5 times differentially expressed in the liver of soy-treated mice compared to controls, at an adjusted $p<0.05$. The lincs on chromosomes 9 and 15 were up-regulated, and the lincs on chromosomes 1 and 17 were down-regulated. However, blast interrogation of lncrna database (http://www.lncrnadb.org) showed partial identity to linc1257/Linc-Myo1g and B2 SINE RNA only.

Nine putative linc species mapped to 7 non-overlapping chromosomal positions (chromosomes 3, 5, 6 8, 11, 17, 19) were found to be at least 1.5 times differentially expressed in the intestine of soy treated mice compared to controls, at an adjusted $p<0.05$. The lincs on chromosomes 5, 17 and one from chromosome 6 were down-regulated, and the lincs on chromosomes 3, 8, 11, 19 and one from chromosome 6 were upregulated. Blast interrogation of lncrna database (http://www.lncrnadb.org) showed partial identity to linc1257/Linc-Myo1g, Jpx/Enox, MascRNA/Malat1 and Beta-MHC antisense intergenic beta MHC transcripts; however, four of the genomic locations interrogated did not render any lncrnadb outputs. Of note, the sequence mapped to chr17:22335458-22466521_R and putatively orthologue of linc1257/Linc-Myo1g was significantly downregulated in both the soy-treated intestine and the liver, with fold ratios of -3.09 ($p=0.034$) and -2.89 ($p=0.013$), respectively.

The soy enriched diet led to modification of expression of several linc species in a tissue specific manner, one putative linc being significantly downregulated in both intestine and liver.

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RNA-MARKERS IN ENDOMETRIAL CANCER

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Endometrial cancer is one of the most common neoplasms in women, occupying the 4th place after breast cancer, colon cancer and lung cancer. However, there are few biomarkers useful for early diagnosis, prognostic evaluation and therapeutic monitoring.

MicroARNs are small molecules of non-coding RNA (21-25 nucleotides) that modulate the expression of target genes by post-transcriptinal mechanisms. Long noncoding RNAs are RNA molecules of over 200 nucleotides that modulate the transcription of the target genes at both transcriptional and post-transcriptional levels. Recent data suggest that microARNs and long non-coding RNAs directly interact to regulate expression of target genes at all levels of gene control: pre-transcriptional, transcriptional and posttrascriptional but also translational. In this way, this vast family of non-coding RNA affects virtually all aspects of normal and cancer cell biology: cell division, cell death and apoptosis, cell differentiation and migration, etc.

Existing research shows that lncRNAs exerted biological functions in various tumors via participating in both oncogenic and tumor suppressing pathways. The previous studies indicated that lncRNA taurine upregulated 1 (TUG1) play important roles in the initiation and progression of malignancies. Through a series of experiments, the results demonstrated that lncRNA-TUG1 enhances the evolution and progression of EC through inhibiting miR-299 and miR-34a-5p.(1)

Long non-coding RNAs (lncRNAs) are a novel class of non-protein coding molecules that have recently been implicated in the pathogenesis of many types of cancer including gynaecological tumours. Although they play critical physiological roles in cellular metabolism, their expression and function are deregulated in EC compared with paired normal tissue, indicating that they may also participate in tumour initiation and progression (2).

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NEW TOOLS FOR DISCOVERING INHIBITORS OF THE RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS

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The receptor for advanced glycation end products (RAGE) is a cell surface molecule of the immunoglobulin superfamily. Alternatively spliced variants lacking either only the intracellular domain or both the intracellular and the transmembrane domains are also expressed in some tissues. RAGE functions in development and inflammation. It interacts with multiple ligands including members of the S100 protein family, high mobility group box-1 protein (HMGB1), and advanced glycation end products. The receptor and some of its ligands are overexpressed in various cancers, diabetes, rheumatoid arthritis, and neurodegenerative diseases. Targeting the receptor or its ligands with neutralizing antibodies resulted in positive effects in several experimental pathologies.

We aim to discover new molecules that block the interaction of the receptor with its pathological ligands. To this end we designed an in vitro high-throughput screening protocol.

We report here the cloning, expression and purification of several tagged constructs of the ligands and the receptor, as well as preliminary data of their interactions. The full length S100B, S100P, and HMGB1, and the extracellular domain of RAGE (soluble RAGE) were expressed in bacteria as GST or His-tagged proteins. In pull-down experiments with the purified proteins we found that GST-sRAGE was able to interact with His-HMGB1, but not with the S100 proteins, suggesting that the large GST tag fused at the N-terminal end of RAGE prevents its binding to some partners. This was confirmed by the finding that a His-tagged sRAGE was able to interact with the GST-S100B and GST-S100P. Other constructs consisting of ligands fused to EGFP and RAGE with a C-terminal GST or His tag are under investigation.

The pairs of receptor-ligand found to interact will be further fluorescently labeled and used for high-throughput screening of compound libraries.

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OMEGA-TRANSAMINASE IN SYNTHESIS OF POTENTIAL PHARMACEUTICAL ACTIVE INGREDIENTS

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Transaminases (EC 2.6.1.X) are enzymes which catalyze reversible transfer of amino group from amino acids to α-keto acids by using pyridoxal-5'-phosphate as a coenzyme. There is a huge interest for the application of ω-transaminases in industrial production of chiral amines and alkaloids since those compounds are extensively used in pharmaceutical, agricultural, and chemical industries. Application of ω-transaminases in asymmetric synthesis of these compounds enables efficient production of biologically active amines, due to their catalytic properties for synthesis with a high level of enantioselectivity, substrate promiscuity (they are capable to aminate keto acids, aldehydes and ketones), high turnover number, no requirement for regeneration of external cofactors, and among other cheaper, simpler and green process of production. We are developing biocatalytic route for the synthesis of amino steroids by using ω-transaminase, (R)-selective, ATA-117 enzyme variant from *Arthrobacter sp*. Enzyme expression was done in *Escherichia coli* BL21 D3 pLysS, and HPLC analysis of enzyme activity and specificity toward 15 structurally different steroid compounds was performed. (R)-methylbenzylamine was used as amino group donor and pyridoxal-5'-phosphate as cofactor. Activity of the enzyme was also measured in bacterial lysate based on the absorbance of acetophenone, which is formed during the transamination reaction of (R)-methylbenzylamine. Based on the results, we have selected four steroid compounds for which enzyme showed highest activity and with a potential for biological activity. The next step was optimization of the reaction conditions with a low cost amino donor isopropyl amine, and isolation and characterization of pure amino steroid products. Until now we have managed to enzymatically synthesize and purify one amino steroid which should be further analyzed by spectral characterization and its biological activity will be determined.

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DIFFERENTIAL REQUIREMENT FOR EDEM PROTEINS IN ENDOPLASMIC RETICULUM ASSOCIATED DEGRADATION (ERAD) IN A LIVING ORGANISM

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ERAD is the major pathway for disposal of misfolded proteins from ER that contributes to the maintenance of cellular homeostasis. In eukaryotes, disruption of ER homeostasis impairs cellular processes such as protein secretion, Ca2+ signaling, cellular signaling and so far, nearly 70 human diseases have been linked to ERAD malfunction. However, how misfolded proteins from ER are processed and the cellular significance of this process in response to ERAD disruption in vivo is still not clear. The ERAD machinery is well conserved in Caenorhabditis elegans and in this study we used genetic analysis to investigate the mechanism that couples ERAD defects to cellular responses in a living organism. EDEM-1, EDEM-2 and EDEM-3 belong to a conserved mannosidase-like family of proteins in all eukaryotes that accelerate ER disposal and proteasomal degradation of ERAD substrates in cells in culture. In this study we specifically investigated the role of C. elegans EDEM-1, EDEM-2 and EDEM-3 in animal development under both physiological and ER stress conditions.

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PLASMA MICRORNAS IN PARKINSON'S DISEASE

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Parkinson's Disease (PD) is the second most common neurodegenerative disease, which manifests clinically when the majority of the patients' dopaminergic neurons are already affected. Both the diagnostic and prognosis of PD are entirely clinic, based on specific signs and on the positive response to therapy; currently there are no reliable biological markers associated to PD pathology. The lack of appropriate, specific and sensitive biomarkers hinders the diagnostic, prognosis and therapeutic management of PD, and impinges on the development of effective prevention studies and clinical trials.

MiroRNAs are short non-coding RNAs known to post-transcriptionally modulate gene expression by binding to complementary sequences in the 3'-UTR of target mRNAs, which further alters on their stability and translatability. Of note, microRNAs are renowned for their exceptional stability in various biological milieus, which qualifies them as prospective biomarkers.

Here we have used a two-step biomarker analysis procedure based on qRT-PCR array (discovery step) and individual TaqMan qRT-PCR assays (validation step), and show data arguing for the usefulness of this approach in the analysis of plasma microRNA associated to PD. We describe two plasma microRNAs as associated to Parkinson's Disease, and show preliminary data for their validation as diagnostic biomarkers in a cohort of patients diagnosed with sporadic PD.

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P5 – AN ENDOPLASMIC RETICULUM PROTEIN WITH MULTIPLE FUNCTIONS

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P5 is a member of the PDI (protein-disulfide isomerase) family. PDI proteins are localized in the endoplasmic reticulum (ER) and are responsible with introduction and/or isomerization of disulfide bonds into the structure of secretory proteins. So far we know more than 20 family members of the PDI family. The exact function of each PDI protein is largely unknown. They share common structural features, indicating that they may share same substrates. Nevertheless, the fact that so many PDI coexpress within the same cell rather indicates that each PDI protein have unique function. P5 (PDIA6) role in the ER is far from being completely elucidated. Only recently it was suggested that P5 plays a role in modulating the unfolded protein response (UPR). Using mass spectrometry we created a list of proteins that interact with P5. We made use of HT1080 derived cell lines that overexpress V5-tagged P5, either wild-type (WT) or P5 substrate-trapping mutant and further analyzed the proteins that eluate together with P5 after immunoprecipitations. After a careful analysis of mass spectrometry data we selected several proteins for further evaluation. Aiming to understand P5 role we created using CRISPR/Cas9 technology a P5 knockout cell line and another cell line where we reintroduced P5 (rescue cell line). Cells were stressed with tunicamycin and we monitor the kinetics of the unfolded protein response (UPR). We found that UPR kinetics is dependent on P5 levels. Furthermore we found that P5 is involved in assisting folding of secretory proteins. Several proteins that participate to the formation of the extracellular matrix maturate with a slower pace in the absence of P5. Thus we proved that the cell lines we created constitute a very useful tool for investigating P5 function. We demonstrated that P5 has a dual role in the ER, modulating UPR and also assisting several secretory proteins to fold properly.

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OPTIMIZATION OF PROTEIN-PROTEIN INTERACTION PROTOCOLS FOR HEPATITIS C VIRUS PROTEIN INTERACTOME DETERMINATION

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Hepatitis C Virus represents a major health issue with more than 170 million people being infected worldwide. The majority of infections become chronic inflicting liver disease. The recent clinical use of direct acting antivirals (DAAs) revolutionized the HCV standard of care reaching sustained virological response (SVR) in more than 90% of the treated patients. However, the ongoing liver pathogenesis process is not always reversible. Thus, it is important to undestand the molecular mechanisms which govern the HCV-host interaction in order to find potential useful biomarker and liver disease therapy targets.

To investigate host-virus interaction, we took a protein interactomics approach using affinity purification followed by mass spectrometry analysis (AP-MS). Foreign tags were introduced in infectious viral genomes in positions which did not affect viral infectivity. Following the AP-MS protocol optimization, HCV NS2, p7 and NS5A viral proteins interactomes were determined in HCV cell culture system. The hit lists were ranked and validated. Previously identified viral protein interactors and host factors reported in HCV life cycle loss of function screens were confirmed. Some of the confirmed hits were further validated by co-immunoprecipitaion followed by western blot.

In conclusion, AP-MS protocols were optimized to identify viral protein interactomes in a biological relevant system. The presented data constitues the basis for ongoing functional studies which are characterizing the role of the identified hits in HCV life cycle.

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SELENIUM COMPOUNDS AND THE OXIDATIVE STRESS

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Selenium is a vital element for the proper development of the human body [1]. It constitutes the active center of the selenoproteins, selenoenzymes and other compounds with smaller molecular weight, with important roles in numerous biological functions [2]. Se compounds with small molecular weight are absorbed in the body and then they are integrated in enzymes and proteins through specific pathways [3]. One of the most important Se compound is SeCys, also called "the 21st amino acid". It is a tripeptide located in the enzyme in a catalytic position, and it represents an important residue of the selenoenzymes and selenoproteins. Until now, 30 selenoproteins have been identified, out of which 25 are encoded by the human genome and some of their functions are not known [4]. The oxidative stress (OS) is expressed as an imbalance between ROS/RNS and the antioxidant systems in the body, participating in the destruction of the intracellular balances. The first line of defence against oxidative stress are the endogenous antioxidants, such as the Se containing compounds [5]. It has been discovered that the SeCys containing compounds have a strong antioxidant effect. The most important ones are the glutathione peroxidases (GPx), thioredoxin reductases TrxR2, but so are selenoproteins (SeIR, protein to be precise) and smaller selenium compounds [6]. The limits between deficiency, normal and excess values of Se in human body are very close [7]. From the studies we made we concluded that Se is a very important micronutrient, but the physiological concentrations must be carefully established for it can easily become toxic for the body.

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SELECTION OF PHOSPHOINOSITIDE 3-KINASES CLASS II DEPLETED CELL LINES FOR FUNCTIONAL STUDIES IN HEPATITIS C VIRUS LIFE CYCLE

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Hepatitis C Virus (HCV) represents a major global health problem. 80% of the infections evolve to chronicity determining liver pathogenesis starting with steatosis and fibrosis and ending with cirrhosis and hepatocellular carcinoma. Despite the unprecedented success of the new direct acting antivirals based regimen, the HCV induced liver pathology is not always reversible. Thus, understanding the interaction between host and virus is crucial for identifying strategies to treat the viral induced pathology. By a proteomic approach, we identified class II phosphoinositide 3-kinases as potential regulators of HCV life cycle. Class II phosphoinositide 3-kinases (PI3KC2) are known to be involved in various cellular signaling pathways. The human genome has three isoforms: PI3K-C2α, PI3K-C2β, and PI3K-C2γ. To study the role of PI3KC2 in HCV life cycle, we aimed to select partial or total knockout cell lines to be further used in functional studies. For that, a CRISPR/CAS9 genome editing strategy was employed. Different single guide RNA sequences were selected and cloned in CRISPR/CAS9 plasmids. To minimize the non-specific genome editing effects, CRISPR/CAS9D10A nickase plasmids were also used. Despite numerous attempts, we could not select cell lines using the CAS9D10A based system and the cell line selection continued with the CAS9 based system. Cell clones were selected by limited dilution and they were screened for the target protein presence by western blot.Finally, viable cellular clones with partial or total knockout of PI3K-C2α and PI3K-C2β, respectively were selected. Some cell clones were evaluated for their capacity to produce infectious viral particles. In conclusion, a CRISPR/CAS9 genome editing protocol was optimized which is routinely used to select knockout cell lines for various targets with potential functional role in HCV life cycle.

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ROLE OF IBA1 IN AMYLOID PEPTIDE CLEARANCE BY MURINE MICROGLIA

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The most frequently encountered neurodegenerative disorder is Alzheimer's disease (AD), characterized by extracellular accumulation of fibrillar deposits of the amyloid-β peptide (Aβ) in senile plaques, intraneuronal neurofibrillary tangles of abnormally hyperphosphorylated tau protein, oxidative stress, synaptic loss and neuronal degeneration. A major challenge of research in this field is to understand the onset events leading to disease progression to develop targeted and more efficient therapies.

Microglia are macrophages resident of the brain that play an important role in neurodegenerative disorders by engulfing foreign molecules like amyloid peptide, secreting signalling molecules like cytokines and maintaining tissue homeostasis. Iba1 (ionized calcium-binding adapter molecule 1) is a specific marker for microglia and macrophages, actin cross-linking protein involved in phagocytosis and pro-inflamatory phenotype. A hyperactivation of microglia, correlated with elevated levels of Iba1, was associated with neurophatic pain and more recently with neurodegeneration. We aim to investigate whether silencing of Iba1 affects microglia activation in the context of Alzheimer's disease, using as model a mouse microglia cell line.

Our preliminary results show that cultured microglia possesses the capacity to incorporate exogenous Aβ peptide that is trafficked through the endocytic pathway and eventually degraded. Moreover, a higher amount of Aβ peptide was found co-localizing with late-endosomal markers after stimulation with ATP of the purinergic receptors. This suggests that the process kinetics is accelerated in this case and the cells store the up-taken peptide in endosomal structures that will eventually be degraded. We are further interested to investigate the Aβ internalization and trafficking upon silencing of Iba1 and evaluate the effect of the silencing over the pro-inflammatory response of microglia upon exposure to amyloid peptide.

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AN INTEGRATED WORKFLOW FOR THE IDENTIFICATION OF AN ERAD MENNOSIDASE POTENTIAL SUBSTRATES

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One of the most aggressive types of skin cancer with increased incidence in the world is melanoma, which is currently considered a key area of intervention in immunotherapy. In this aspect, a major source of melanoma epitopes is the degradation of misfolded proteins in Endoplasmic-Reticulum Associated Degradation, (ERAD) pathway [1]. Recently, it was shown that EDEM2 (ER degradation-enhancing alpha-mannosidase-like protein 2) initiates the degradation of misfolded glycoproteins from ER by catalyzing the conversion of Man9GlcNAc2 to Man8GlcNAc2 of the protein glycan core [2].

Although it is currently accepted the mannosidase role of EDEM2 in N-glycoprotein ERAD (NgpERAD), the identity of its endogenous substrates still remains unknown. The importance of this aspect becomes even clearer in the context of melanoma, as these substrates could constitute a source of melanoma epitopes. Here, we describe an integrative approach for the identification of such proteins in A375 melanoma cells stably expressing EDEM2. By combining distinct proteases with lectin enrichment we extended the glycoproteome coverage resulting in the identification of more than 1000 potential N-glycosylation sites from this melanoma cell line. Of these, more than 300 sites were identified with high confidence in at least two analytical replicates and were found to be Endo H sensitive.

Approximately 5 % of the identified peptides containing N-glycosylation sites were found as differentially expressed between EDEM2 and the CTRL cell line. This workflow will be further used for the confirmation of EDEM2 substrates, in correlation with other experiments.

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TYROSINASE N-GLYCOSYLATION SITE OCCUPANCY ANALYSIS USING MASS SPECTROMETRY

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Secretory and membrane proteins follow the ER-Golgi route for synthesis, folding and post-translational modifications prior to delivery to destination. One important post-translational modification (PTM) for secretory proteins is N-glycosylation. The modification is critical for a variety of cellular processes such as protein-ligand interaction, cell attachment or for the biosynthetic-secretory pathway, where it is the basis of a quality control mechanism of the molecules trafficked from the ER to the Golgi and beyond. Thus, the identification of glycosylation sites and their occupancy is of high interest in the biological field.

The high-mannose N-glycans attached to the newly synthesized polypeptides within the ER are processed to complex structures in the Golgi apparatus. In vitro, the sugars moieties can be detached from the polypeptide chains either by peptide-N-Glycosidase F (PNGase F) transforming asparagine to aspartic acid, or by EndoH, which removes only the high-mannose and hybrid N- glycans leaving a HexNAc modified asparagine. In both cases, the removal of the glycan chain results in mass shifts amenable to mass spectrometry detection.

Using this approach combined with mass spectrometry analysis, we characterized the glycosylation site occupancy of human tyrosinase, a protein able to generate antigenic peptides in melanoma. Using specific inclusion lists that target peptides containing all the predicted glycosylation sites we confirm all the glycosylation sites. Even more we show that not all glycosylation sites are fully occupied in melanoma cells.

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IDENTIFICATION OF INHIBITORS OF HEPATITIS C VIRUS NS2 CYSTEINE PROTEASE ACTIVITY USING HIGH THROUGHPUT SCREENING

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Hepatitis C Virus (HCV) infects more than 170 million people worldwide. The recent clinical use of interferon free direct acting antiviral (DAAs) regimes profoundly inproved the standard of care. A high rate of sustained virologic responses in achieved across all genotypes and liver conditions.[1] However, the drug resistance and genetic variability of the virus are still issues to be considered. The DAA cocktail in the clinic is targeting three viral proteins: the NS3 protease, multifunctional protein NS5A and the NS5B polymerasse.[2]

HCV NS2, the first viral encoded protease, was validated as an attractive drug target being responsible for polyprotein processing and virion assembly, but no drug like compound was identified to inhibit its activity. The difficulty to identify NS2 cysteine protease inhibitors is explained by the lack of an automatable assay. A cell based assay for NS2 cysteine protease activity was developed, miniaturized and automatized for the 384 well plate format for high throughput screening. Using „in silico" docking techniques, we selected a targeted library of 2000 potential cysteine protease inhibitors which were screened. To eliminate false positives a counter screen was performed followed by a secondary screen using the HCV subgenomic replicon system. The identified hits were finally validated in the HCV cell culture system. New chemical scaffolds were identified with IC50s in the low micromolar range.

Further studies will determine the mode of action of the validated hits confirming HCV NS2 as a druggable target.

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QRT-PCR EVALUATION OF SELECTED MICRORNAS' EXPRESSION IN AMNIOTIC FLUID AND CHORIONIC VILLUS SAMPLES FROM DOWN SYNDROME PREGNANCIES

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Trisomy 21, responsible for 95% of Down Syndrome (DS) cases, is the most common viable aneuploidy, the prenatal diagnosis of which is based on genetic tests run on samples obtained by invasive procedures. Here we show data on the expression of hsa-miR-371-3 placenta specific cluster and of 5 microRNAs mapped on chromosome 21 (hsa-miR-99a, hsa-let-7c, hsa-miR-125b-2, hsa-miR-155, hsa-miR-802) in biological samples obtained by amniocentesis (AF) and chorionic villous (CV) sampling.

The AF and CVS biological samples obtained as part of the screening and diagnostic procedures for DS were stored in RNAlater at -80^0 C until further use. MicroRNA quantification was performed using dedicated TaqMan assays on cDNAs synthesized using a two-step Cells-to-Ct kit procedure. The changes in microRNA expression were calculated using the ΔΔCt method.

We found a statistically significant change in the expression of let-7c in chorionic villous DS-samples compared to euploid ones. Data regarding the transcriptional profiles of primary let-7c are provided and a putative mechanism explaining the results is discussed. We performed network-inferred GATHER target prediction and pathway enrichment analysis in order to evaluate the impact of let-7c deregulation on placental signaling pathways.

We provide evidence that let-7c stability is compromised in CV samples and provide a putative explanatory mechanisms. Our data highlight the putative impact on DS placental transcriptome of let-7c down-regulation and open new research avenues in DS placenta physiology and pathology.

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CYTOTOXICITY OF LONG-CIRCULATING LIPOSOMES CO-ENCAPSULATING CURCUMIN AND DOXORUBICIN ON C26 MURINE COLON CANCER CELLS

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Co-administration of conventional anti-cancer agents with naturally occurring drugs, may overcome chemotherapy limitations. The aim of this study was to assess the effects of PEGylated long-circulating liposomes (LCL) co-delivering curcumin (CURC) and doxorubicin (DOX) on murine colon carcinoma cells (C26) and to investigate the pro-tumor processes that might be affected as a consequence of the inhibition of cell proliferation, such as tumor associated-inflammation, angiogenesis, and oxidative stress. Our results indicated that PEGylated LCL-CURC-DOX exerted antiproliferative effects towards C26 cells, to a similar extent as free CURC-DOX, but higher than either agent administered alone in their free form. These effects obtained for LCL-CURC-DOX were due to inhibition of the production of angiogenic/inflammatory proteins in a NF-κB -dependent manner, but independent of ROS production or AP-1 c-Jun activation. Although both co-delivery of CURC and DOX in free as well as in LCL form determined similar cytotoxic effects on these cancer cells, the anti-angiogenic actions of LCL-CURC-DOX appeared to be much stronger than those induced by co-administration of CURC and DOX in their free form. Our findings suggest that co-delivery of CURC and DOX in PEGylated LCL might be of great potential for the therapy of colon cancer.

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IDENTIFICATION OF A PROTEIN TYROSINE PHOSPHATASE THAT DEPHOSPHORYLATES PLCγ2

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Phospholipase C gamma 2 (PLCγ2) is a multimodular protein playing a critical role in the initiation and propagation of B cell signaling in response to membrane-bound antigens. The binding of antigen to B cell receptor (BCR) leads to the assembly of a signaling complex composed initially by the immunoglobulin α and β subunits of BCR, kinases: Lyn, Syk and Btk and the adaptor protein B cell linker (BLNK). Subsequently, PLCγ2 is recruited to the complex by BLNK through its N-terminal SH2 domain (nSH2) and is activated by phosphorylation by Btk [1]. Normally, the C-terminal SH2 domain (cSH2) interacts with the surface of PLCγ2 core masking the active site and keeping the enzyme in an inactive form. Phosphorylation of the tyrosine Y759 in PLCγ2 in the linker region between the cSH2 domain and the SH3 domain enables the active site of the catalytic domain to gain access to the membrane substrate phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] [2]. At present, dephosphorylation of PLCγ2 causing return to its inactive state is poorly understood and the protein tyrosine phosphatase (PTP) responsible for that was not identified, yet.

In this study, we provide evidence that a member of the Eyes absent family of PTPs, Eya3 specifically binds and dephosphorylates PLCγ2. First, we showed by immunoprecipitation experiments that PLCγ2 interacts with substrate trapping mutants of Eya3 when they are co-expressed in HEK293T cells, in either presence or absence of protein tyrosine kinases. Next, we expressed in E. coli and purified a recombinant fragment of PLCγ2 comprising both SH2 domains and the linker region between cSH2 and SH3 domains, (SH2-SH2)γ2 and demonstrated using microscale thermophoresis that the phosphorylated (SH2-SH2)γ2 interacts with wild type (WT) as well as different substrate trapping mutants of Eya3. Also, we showed that Eya3 WT dephosphorylates both (SH2-SH2)γ2 and PLCγ2 full length, by in vitro experiments.

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SECONDARY STRUCTURE OF HUMAN SERUM ALBUMIN IN RIBOFLAVIN LOADED MYRJ52-SILVER NANOPARTICLES

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Fluorescence and dichroism circular studies of Human Serum Albumin (HSA) in riboflavin (RF) loaded Myrj52-silver nanoparticles (SNPs), were performed. Transmission Electron Microscopy showed the formation of colloidal spherical SNPs with the average size of ~12 nm. The structure, stability, dynamics and conformation of HSA binding RF on SNPs, have been studied. The influence of RF on the secondary structure of HSA in the presence of SNPs showed a predominant α-helix structure (61.7 %). No changes induced by RF binding to HSA, were observed. The effect of temperature on the HSA fluorescence in the SNPs / RF-HSA systems, has been also studied. The results are relevant as regard the secondary structure of proteins in the nanoparticles based systems.

Keywords: silver nanoparticles, polyethylene glycol, riboflavin, proteins, secondary structure, spectroscopy

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EDEM3 AND ITS FUNCTION IN ERAD

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The endoplasmic reticulum (ER) located processes of protein synthesis and protein quality control are highly regulated in the eukaryotic cell. However, not all proteins attain their native conformation, subsequently becoming clients of the ER-associated degradation pathway (ERAD). A large number of chaperones and other ER resident proteins were proposed to assist in the selection, transport and dislocation of the misfolded cargo. Among them, ER-degradation enhancing alpha-mannosidase-like protein 3 (EDEM3) is thought to possess mannosidase activity and increase the rate of degradation of ERAD substrates.

In order to elucidate the correlation between EDEM3 structure and function, a series of truncated mutants of the protein were investigated. To monitor the effect of EDEM3 mutants we have generated an EDEM3 knockout cell line using the CRISP-R/Cas9 technology. We further co-expressed the mutants with a canonic ERAD substrate, the null Hong Kong variant of alpha1-antitrypsin (NHK). Overexpressed mutants had different effects upon NHK accumulation in the knockout cells as compared to wild type EDEM3.

Together, our preliminary results suggest the relative function of each conformational domain of EDEM3. Thus, the characterization of the truncated mutants makes a novel contribution to the understanding of EDEM3 role in the quality control of misfolded proteins.

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CLINICAL RELEVANCE OF GLOBAL DNA METHYLATION IN WHITE BLOOD CELLS OF PROSTATE CANCER PATIENTS

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Development of prostate cancer (PC) biomarkers to guide terapeutic decission at the time of diagnosis has been extensively studied at the tissue level but less is known about the clinical relevance of epigenetic biomarkers in biofluids. Monitoring these patients over time is difficult but esential in the view of high rates of overtreatment and clinical repalse. Epidemiologic studies have reported association between global white blood cells (WBC) methylation and several cancers. Our study aimed to evaluate the discriminating power of noninvasive WBC global methylation in newly diagnosed prostate cancer patients in conjunction with well-established clinical parameters in prostate cancer.

This preliminary study involved 50 newly diagnosed (T0) PC patients, 60 males with benign prostatic hyperplasia (BPH), and 10 healthy individuals from Arad. 20% of these patients were reevaluated 3 (T1), 6 (T2), 9 (T3) and 12 moths (T4) after initial diagnosis. All patients were examined clinically and the PC occurrence was confirmed by PSA and Gleason scores. The Ethic Commission approved the study and all patients signed the informed consent. Peripheral blood, serum/plasma, and urine samples were collected. DNA was extracted from blood using Qiagen kit. The global 5mC levels were assessed by Quest DNA ELISA (ZymoResearch) kit. Global methylation was also determined by direct digestion of DNA using EpiJET DNA Methylation Kit (ThermoFisher). GSTP1 methylation was analyzed by MS-PCR. The data were first square root transformed. Then we applied a Levene's test to check if the measured values satisfy the preconditions of homoscedasticity (equal variance among groups), followed by application of ANOVA with Newman-Keuls tests as post hoc analysis when appropriate.

Subjects were stratified by age and PSA level. Data were analyzed at two layers: differences among different categories of patients at the same time point; differences among the patients from the same category at different time points. Then, we assessed the effect of age on 5mC levels. We found a similar variance in 5mC percentages among the groups investigated ($p = 0.790$). Application of post-hoc analysis showed significantly higher 5mC levels for PC patients than for BPH ($p = 0.011$). At 6 moths monitoring, the 5mC levels measured in studied groups were homoscedastic ($p = 0.448$), with significant differences among groups (Anova, $p = 0.004$), whereas post hoc testing revealed a significant decrease in 5mC content in CP and BPH patients as compared to controls (PC patients: $p = 0.011$; BPH patients: $p = 0.023$). In controls the measured values at different time points were similar (Anova, $p = 0.487$). Correlation of PSA with 5mC level in groups is also shown.

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PROTEIN FOLDING, AND THE AGES EFECT ON AMYLOID FORMATION. ADVANCED MASS SPECTROMETRICAL ANALYSIS OF GLYCATED BSA, AND COMPARATIVE PROTEOMIC SCREENING OF TEMPORAL AND HYPPOCAMPAL HEALTHY, OLD BRAIN TISSUE

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Most of the biological functions in the cell are performed by proteins, which must fold into three-dimensional structure. Protein unfolding, induced by posttranslational modifications, such as glycation, aldehyde binding, etc. leads to protein aggregation and amyloid fibril formation, associated with various neurodegen¬erative disorders.

In the present work we prepared and characterized amyloid-like fibrils by extensive glycation of BSA and investigated the way to inhibit the fibril formation. The formation of fibrilar structures with amyoid-like properties were visualized by fluorescence microscopy.Glycated BSA in the presence of pyruvate showed a reduced number and size of fibrils, indicating that pyruvate can prevent formation of However, a very strong interaction has been found between the brain tissue and BSA-AGEs, incubated together. The presence of the receptor RAGEs could contribut to this result.

Another aspect studied was RAGE - AGEs interaction in the presence of AGEs inhibitors . Immunofluorescence microscopy showed that AGEs formed in the presence of the inhibitors, retain the ability to interact with RAGE.. Mass spectrometry (MS) has been used for the structural analysis of the AGEs-modified proteins in plasma and tissues of patients with diabetes mellitus, cataract, uremia, and other diseases. Matrix-assisted laser desorbtion/ionization (MALDI)-MS was one of the most popular methods applied for the direct analysis of AGEs-modified proteins such as albumin and IgG. Unfortunately, the low peaks quality sometime obtained, could hinder the analysis of intact AGEs-modified proteins. Some new, improved MS methods for certain aspects of the study are suggested. In the present work we studied the structural modifications of BSA induced by the glycation with ribose for 4 weeks, analysing the trypsin digested protein by liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS/MS). Using the SequestHT search algorithm on the basis of the MS/MS sequence ion data we identified 17 glycated peptides. Identification of specific glycation sites in the glycated BSA has been also realized. Some of the original methods proposed above, as well as the BSA results have been used by us to the analysis of AGE-modified proteins from brain tissue. Comparative proteomic analysis by ESI/MS of hippocampal and temporal regions from an old, normal human brain has been performed and the results corroborated withWestern blot analysis of the brain regions using anti-AGEs immune serum. We believe the present work could contribute to demonstrate the important role of protein-linked AGEs in several diseases, being useful in diagnosis and therapeutic control.

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HIGH-THROUGHPUT OMICS TECHNOLOGY APPLIED TO FINGERPRINT AND ELUCIDATE METABOLIC PROFILES AND PATHWAYS

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High Throughput technologies are based on analysis automation and large datasets of biological or biochemical assays to determine large numbers of metabolites, proteins or genes. Such technology aims different applications, from diversity oriented synthesis of key molecules, drug discovery from natural products collections, disease diagnosis in early stages, treatments monitoring, etc. The omics' technology involves the the near global analysis of such molecules. Metabolomics, the "apogee" of the omics trilogy [1,2] proved to provide a useful readout of cellular biochemistry and metabolic pathways involving small molecule metabolites (< 1500 Daltons, including carbohydrates, amino acids, lipids, nucleotides). The metabolome is the complete set of metabolites within a cell, tissue or biological sample at any given time point.

Using emerging analytical techniques like ultraperformance liquid chromatography (UPLC) coupled with mass spectrometry (MS), Magnetic Resonance, either from a plant/foodmatrix or from a biofluid (blood, urine, saliva). Untargeted metabolomics complemented with targeted analysis can provide high level information regarding the key-biomarkers for a specific fingerprinting and recognition of a plant or food [3-5], or for a specific diagnosis (early or progression-related to a specific disease.

New insights and discoveries are related to plant biodiversity, food authenticity and traceability, human and animal cellular metabolism, in vitro tests on normal and tumor cells, cancer diagnosis and prognosis, as well different biological processes. Metabolomics reflect the "personalized" picture for a plant or food product under different influencing factors like environmental stress or processing, or a specific medical fingerprint of a human/animal body fluid, as a noninvasive personalized profile of human or animal samples (by identifying and quantifying specific biomarkers) [6,7]. Updated biostatistics tools (e.g. Profile Analysis, Metaboanalyst) and databases of metabolome and metabolic biomarkers (Lipid Gateway, Human Metabolomic Data Base, KEGG) are available and increase exponentially every year in all domains (plant/food/nutritional/medical/pharmaceutical/toxicological research). The personalized medicine based on specific metabolic fingerprints, related to age, physiology and pathology, is ready to demonstrate beside the genetic background, the nutritional and "lifestyle" influence, providing solutions to modulate metabolic disturbances. Specific case studies will be presented to demonstrate the large impact of metabolomics involvement in systems biology, across all possible physiological and pathological stages of a living organism.

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BIOLOGICAL ACTIVITY OF POLYPHENOLS – EVIDENCE FROM IN VITRO TESTS

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Polyphenols represent one of the largest and most important group of natural compounds, displaying a wide range of biological effects. Beside the well-know antioxidant properties, polyphenols are able to modulate metabolic and cell signaling pathways, having anti-inflammatory, anti-proliferative, pro-apoptotic and anti-angiogenic properties [1,2]. Here we present an overview of the results obtained in our laboratory during in-vitro studies of polyphenols in different experimental models.

Retinal Pigmented Epithelial cells were used as model for Age-Related Macular Degeneration and for Diabetic Retinopathy, in conditions that mimic the major sources of oxidative stress in human retina: generation of hydrogen peroxide during phagocytosis, hyperglycemia and the presence of photosensitizers. Resveratrol and some phenolic acids were found to protect retinal pigment epithelial cells by direct inactivation of reactive oxygen species or by enhancing the level of reduced glutathione or the activity of antioxidant enzymes. A novel strategy to increase the bioavailability of natural compounds is to develop new formulations, such as multilayer polyelectrolyte microcapsules (PEM). Microcapsules dopped with phenolic compounds (resveratrol, anthocyanins) were succesfully produced using biocompatible materials. Resveratrol and anthocyanins containing PEM were not cytotoxic in a large range of concentrations and were internalized by RPE cells, as demonstrated by confocal microscopy, TEM and SEM.

Anthocyanin fractions from rich sources (blueberries, chokeberries, elderberries), purified and characterized by powerfull analytical techniques (HPLC/MS), were tested in various cell culture models, using normal or tumour cell lines. Besides the well known antioxidant properties, anthocyanins from chokeberries showed antidiabetic properties by stimulation of insulin secretion in β-TC3 murine pancreatic cells. The antiproliferative and proapoptotic properties of anthocyanin-rich extracts were demonstrated in B16-F10 murine melanoma cells and HeLa human tumor cervical cells for blueberries and elderberries extracts.

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ANXIOLYTIC AND ANTIDEPRESSANT PROFILE OF THE 6-HYDROXY-L-NICOTINE IN A RAT MODEL OF CHLORISONDAMINE

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6-hydroxy-L-nicotine (6HLN), a nicotine derivative from nicotine degradation by Arthrobacter nicotinovorans pAO1 strain was found to improve behavioral deficits and to reverse oxidative stress in the rat brain [1]. We designed to evaluate the anxiolytic and antidepressant effects of nicotine (0.3 mg/kg) and 6HLN (0.3 mg/kg) using a chlorisondamine (CHL) rat model. The anxiolytic effect was evaluated by elevated plus maze test, while the antidepressant effect was investigated using the forced swimming test. Both nicotine and 6HLN improved cognition related behaviors in anxiety and depression effectively induced by CHL in the laboratory rats.

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TIPS AND FACTS FOR ANIMAL STUDY PROPOSAL IN "HORIA CERNESCU" EXPERIMENTAL UNITS

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Study project hypothesis must translate into statistical hypothesis, and research projects using animals in procedures must be done under Authorization of the research project proposal (mandatory starting with January 2018) in authorized and highly specialized units, in order to grant the necessary level of biosecurity.

In Experimental Units of Horia Cernescu research infrastructure, the projects developed must follow the sequence of steps further detailed.

1. Obtaining the Administrative Availability Agreement, based on unit capacity and on microbiological unit characteristics.
2. Scientific evaluation of projects, by the Bioethics Commission, in view of getting the Assessment Certificate.
3. The authorization (renewal or withdrawal) of the project authorization, involving the Veterinary Competent Authority, is made through the Application for authorization of the research project. Besides the Application, the researcher team will send: i) Assessment certificate, ii) Nontechnical resume and iii) A self-declared statement that the project has not been completed, in order to avoid undue duplication of procedures, as per Veterinary Order no. 97 of September 1, 2015.

The Animal Study Proposal (ASP) is a confidential and optional document, and a helpful tool for all actors involved in Authorization of the research project proposal. There are no rules for the ASP contents; normally, though, the entries are as further detailed: administrative data; animal requirements; study objectives; procedure narrative; rationale for animal use made of; description of experimental design and animal procedures; field studies and personnel training.

The content of ASP will be detailed enough so as to satisfy the demands in different stages of the evaluation process, in order to obtain the research project Authorization.

Present project is supported by Contract no. 6584 din 19.10.2015 supported by Horia Cernescu Research Unit, established by infrastructure project POSCCE SMIS 2669.

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1. Veterinary Order no. 97 of September 1, 2015.
2. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

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CHEMICAL AND BIOLOGICAL ACTIVE COMPOUNDS IN PLANTS AND ALCOHOLIC EXTRACTS BELONGING TO *LAMIACEAE* FAMILY

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The aim of this paper was to characterize medicinal plants belonging to *Lamiaceae* family in terms of proximate composition and biological active compounds. Also, from these plants were obtained alcoholic extracts which been characterized in terms of active principles content with possible role in antifungal action. The plants studied were: *Mentha piperita, Melissa officinalis, Satureja montana, Lavandula angustifolia, Thymus vulgaris* and *Origanum vulgare*. Plants were purchased from private producers from Western Romania. Alcoholic herbal extracts were obtained by macerating matrices in 70% ethyl alcohol in a ratio of 1:10 (m/v), dried and ground and then the extracts were filtered and stored at 2-40°C until antioxidant activity was tested. The characterization of the plant matrices was carried out in the laboratories of the Interdisciplinary Research Platform of USAMVB Timisoara by determining the proximate composition (moisture, protein content, lipid, mineral substances, macro and microelements content) using the official AOAC methods and atomic absorption spectrometry (SAA). Alcoholic extracts have been characterized in terms of total polyphenols (TP) content using Folin-Ciocalteu method and the main compounds responsible for the biologically active action of alcoholic extracts were determined by LC-MS.

The results regarding the proximate composition of the studied plants reveal that the moisture content of the samples, previously free-dried, varies between 4-8% depending on the species, the lowest value of protein content was registered in *Melissa officinalis,* sample and the highest were found in *Satureja montana* and *Lavandula angustifolia*. The amount of ash, which reflects the mineral content of the samples, was maximum in *Satureja montana* sample. The level of macro elements in plants depends on the preferential absorption of plants and the characteristics of soil. *Lavandula angustifolia* and *Melissa officinalis,* registered the highest values in terms of microelements. *Satureja montana* recorded the highest content of macroelements as K, Ca and Mg.

Regarding the (TP) from alcoholic extracts, Melissa o. extract registered the highest content. Polyphenols were individually determined from alcoholic extracts by LC-MS, identifying 11 major compounds. Rosmarinic acid was found in highest amount in alcoholic extracts of *Lamiaceae* family medicinal plants, the maximum value being registered in Thymus vulgaris, followed by *Melissa officinalis,* and *Satureja montana. Lavandula angustifolia* recorded the highest level of Caffeic Ferulic and cumaric acids. Rutin, Kaempherol and Quercitin had maximum values in *Lavandula angustifolia* extract and minimum values in *Satureja montana* extract. Due to the high content of macro and microelements, as well as the intake of polyphenols with antioxidant activity, the plants belonging to *Lamiaceae* family can be considered as an important source of bioactive compounds with a role in the body's function.

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PHYSICAL-CHEMICAL CHARACTERIZATION OF SOME LOCAL FRUITS IN THE BANAT AREA PROPOSED FOR HARNESS

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Our study have the aim to establish the chemical composition of white (*Morus alba* L.), red (*Morus rubra* L.) and black (*Morus nigra* L.) mulberry fruits grown in the West Region of Romania. The mulberry belongs to the Morus genus of the *Moraceae* family. Mulberry is an economically important plant being used for sericulture, for its foliage, to feed the silkworm (*Bombyx mori* L.).

Mineral compositions, fat content, total antioxidant capacity, total polyphenols content, vitamin C (ascorbic acid), pH, total soluble solids content was investigated in this study. *Morus nigra* displayed the highest total phenolic contents, while in White mulberry the highest total fat content were observed. The ascorbic acid content was less in red mulberry. The interaction between fruit color and sampling location showed significant effect on antioxidant capacity. The total polyphenols content was evaluated by Folin Ciocalteu method and the total antioxidant capacity was analyzed by using the spectrophotometric method – CUPRAC method.

Differences of phenolic compounds in the fruits depend on many factors, such as the maturity degree at harvest, genetic differences, and environmental conditions during fruit development.

When analysing these data, one may conclude that mulberries are a rich source of phenolics, with high levels in *M. nigra* and low content in *M. alba*.

Our results indicate that all the studied mulberry fruits are a valuable horticultural product, based on their rich and beneficial nutrient composition and may be useful in a balanced diet. Their nutritive and phytomedical potentials are increased by their higher phenolic contents with antioxidant activity

The data was statistically analyzed by using the statistical program (Origin Version 7.1). One way analysis of variance (ANOVA), with multiple ranges significant difference (LSD) test ($p < 0.05$).

Keywords: mulberry species, minerals, fat content, total polyphenols, antioxidant capacities, CUPRAC method.

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GERONTOMICS: A MULTI-OMICS PREDICTION SYSTEM FOR PRIORITIZATION OF GERONTOLOGICAL INTERVENTIONS

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Organismal aging is a biological process defined by a progressive loss of viability and an exponential increase in fragility; also, it is the main risk factor for many diseases. Understanding the process and the mechanisms underlying it is one of the major biological and biomedical challenges of our century. With the current advances in high-throughput technologies many of the molecular aspects of aging can now be easily screened at various “omics” levels, using a wide range of models and starting from various hypotheses (e.g.: analysis of “normal” aging, long- and short-lived mutants, calorically restricted animals, etc).

Gerontomics will use a systems biology approach to combine genomics, transcriptomics and epigenomics data from various models of aging in order to create a large-scale data-driven analysis and prediction platform for experimental interventions in gerontology. This multi-omics platform will be used to analyze the data in a holistic way, using bioinformatics techniques, in order to identify common and complementary patterns and signatures of aging.

Next, we will use machine learning algorithms to learn features about longevity-associated genes and to predict novel combinations of genes that may modulate lifespan and healthspan. To test the predictive capacity of the system, we will perform in vivo genetic interventions on C. elegans, either from early life stages or post developmentally.

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IN VITRO MATURATION OF DOMESTIC CAT OOCYTE IN MEDIUM SUPPLEMENTED WITH ANTIOXIDANTS THROUGH DIFFERENT TIME INTERVAL

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Assisted reproductive technologies (ART) in feline specie are used with multiple purposes but the results are less conclusive then in other species. Some of the reasons are difficulty to estimate optimal time for oocyte in vitro maturation and the higher level of reactive oxygen species due to higher external oxygen concentration during in vitro condition, conditions that can affect the oocyte competence to sustain early embryonic development. In the present study we observed the nuclear maturation status of cats oocytes at 24, 30 and 40h in medium supplemented with/without antioxidants.

Only COC grades I were cultured in maturation medium (TCM-199 Earl's medium supplemented with 25mM HEPES, 4 mg/ml bovine serum albumin, 2,20 mg/ml NaHCO3, 50 µg/ml gentamicin sulfate, 0.2 mM sodium pyruvate, 10 µg/ml FSH, 1.06 UI/ml HCG). Antioxidants used were 0.13 mmol/L cysteine and 0.5 mmol/L ascorbic acid. Nuclear status of the oocyte was evaluated by Hoechst 33342 staining.

At 24 h after IVM, 23.71% COC were with cumulus expanded and 30.92% at 40h. When the medium was without antioxidants at 24 h after IVM, 17.74% COC were with cumulus expanded and 33.87% at 40h. At 30 h after IVM, 14.28% were with cumulus expanded. Examination of nuclear status revealed only in oocytes cultured for 30h 5.71 % of them were in GVBD and 2.85 % in M II, the rest of the oocytes examinated contained unidentifiable chromatin in all groups.

With these inconclusive results and based on our experience with culture interval of sow and cow oocytes, we will try to determine the expression of apoptotic-related genes (BAX and BCL-2) in cat oocytes cultured in medium supplemented with antioxidants at various time interval.

Aknowlegdment: The research was carried in the IVF (In Vitro Fertilization) laboratory from the Horia Cernescu Research Unit equiped through POSCCE 2669 program

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THE ROLE OF ERAD PATHWAY IN INSULIN SYNTHESIS AND SECRETION IN PANCREATIC β-CELLS AND DIABETES MELLITUS

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Plasma glucose is maintained within a narrow range by mature insulin which is produced and secreted from pancreatic beta cells. It is well known that improperly folded proteins in the ER are degraded by the Endoplasmic-reticulum-associated protein degradation (ERAD) system. Here, we investigate the functional significance of ER stress proteins and ERAD components on insulin secretion in both pancreatic β-cells and diabetic rat model.

To understand the role of ERAD on proinsulin folding and processing we developed stable cell lines overexpressing ERAD components and we determined their effect on insulin synthesis and secretion. Some of the ERAD components increased glucose-stimulated insulin secretion (GSIS) in both first-phase and second-phase of insulin secretion from pancreatic beta cells. We also observed that the opposite was true when we silenced some of the ERAD components, which led to a decrease in proinsulin and insulin levels. In addition, pulse chase and immunoprecipitation experiments revealed that overexpression of ERAD components increased the stability of the proinsulin and insulin secretion. Subcellular fractionation for insulin secretory granules revealed rapid traffic of proinsulin from ER to the mature secretory granules. Moreover, we found that overexpression of ERAD components protect pancreatic beta cells to prolonged exposure to high glucose levels and ER stress. We also investigated the physiological role of ERAD components in diabetic rats and the results showed regeneration of pancreatic beta cells, increased serum insulin and normal level of blood sugar.

Our data indicates that ERAD components are critically involved in proinsulin processing and insulin secretion from pancreatic beta cells and therefore may represent promising targets for novel therapies aiming to increase insulin secretion and decrease glucose level.

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TEN DAYS INTRAPERITONEALLY OXYTOCIN ADMINISTRATION RESULTS IN ANTIOXIDANT EFFECTS IN WISTAR RATS

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There are very few studies regarding the influence of oxytocin on the oxidative stress status modifications. Even more, the very few studies that exist in this area of research are suggesting controversial results, with reports stating antioxidant, pro-oxidant or sometimes no modifications at all for the specific oxidative stress determined [1,2]. In this context, in the current study we decided to preliminary study the relevance of 10 days intraperitoneally oxytocin administration on one antioxidant enzyme (glutathione peroxidase-GPX) and one lipid peroxidation marker (malondialdehyde-MDA) in Wistar rats total serum, as compared to a control group of rats which received saline. Thus, we report here a significant antioxidant activity for the administration of oxytocin, as demonstrated by a significant increase of the specific enzymatic activity of GPX and an decrease of MDA concentration in the serum of Wistar rats. This could be relevant in the context of the increased awareness regarding the relevance of oxytocin in the treatment of the main neuropsychiatric disorders, especially when administrated through the intranasal route, considering also that oxidative stress represent an important landmark in these deficiencies.

This work is supported by PN-II-RU-TE-2014-4-1886 grant, number 120 from 01/10/2015.

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THE EFFECTS OF BRANCHIAL AND TEGMENTAL EXPOSURE OF ZEBRAFISH TO OXYTOCIN ON THE OXIDATIVE STRESS STATUS

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As mentioned before, the very few studies that exist regards the effects of oxytocin on the oxidative stress status are displaying controversial results, with reports stating both antioxidant and pro-oxidant effects of this peptide [1,2]. In this context, in this study we decided to preliminary determine the relevance of two different doses of oxytocin (33,3 ng/ml; and 66,6 ng/ml) administration in the water for one minute and a half, on the specific activity of two antioxidant enzymes (superoxide dismutase-SOD and glutathione peroxidase-GPX) and one lipid peroxidation marker (malondialdehyde-MDA) in zebrafish (Danio rerio), as compared to a control group that received saline. Thus, we report here a possible antioxidant activity for the administration of oxytocin, as demonstrated especially by a significant increase of the specific enzymatic activity of GPX. This could be relevant in the further use of zebrafish for a variety of neuropsychiatric disorders models existing for this specie, especially considering that lately intranasal oxytocin represent an important management aspect in these deficiencies [3] and also the well known relevance of the oxidative stress status in these disorders [4].

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INTRAPERITONEAL ADMINISTRATION OF TWO DOSES OF OXYTOCIN IN CARASSIUS AURATUS EXERTS ANTIOXIDANT EFFECTS

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As mentioned before, there are very few studies regarding the influence of oxytocin on the oxidative stress status metabolism. Even more, these studies resulted in controversial reports, describing both pro-oxidant and antioxidant actions of oxytocin [1,2]. Thus, we decided here to study the effects of two very different doses of oxytocin (10 ng/kg and 5000 ! ng/kg) intraperitoneal administration on the oxidative stress metabolism of Carassius auratus, by seeing the specific activity of two antioxidant enzymes (superoxide dismutase-SOD and glutathione peroxidase-GPX) and one lipid peroxidation marker (malondialdehyde-MDA), as compared to a control group that received saline. Our results mostly suggested a trend for the antioxidant activity of oxytocin, as demonstrated by a significant increase of the specific enzymatic activity of SOD and GPX. As already mentioned, this could exert some relevance in the future use of zebrafish for some neuropsychiatric disorders models existing for this specie, considering that in the modern literature intranasal oxytocin is increasingly cited as a fundamental player for possibly treating these deficiencies [3] and the fact that oxidative stress is one of the most important and commune modification characterizing these neuropsychiatric disorders [4].

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NO CLEAR EFFECTS OF OXYTOCIN IN A RAT EPIGENETIC MODEL OF SCHIZOPHRENIA BASED ON METHIONINE ADMINISTRATION

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We present some of our preliminary data regarding the administration of oxytocin for 9 days in some specific behavioural tasks used to assess working memory and anxiety behaviour in a methionine-induced rat model of schizophrenia. Male Wistar (n=21) rats were used and divided in 3 groups: control, methionine and methionine+oxytocin group. The model of schizophrenia was induced in methionine and methionine+oxytocin group through the subcutaneous administration of methionine for 2 weeks (5.2 mmol/kg). After that, oxytocin was intraperitoneally injected in the methionine+oxytocin group in a dose of 10 mg/kg/body weight for 9 consecutive days. The oxidative stress markers determined were represented by the specific activity of two antioxidant enzymes (superoxide dismutase-SOD and glutathione peroxidase-GPX) and one lipid peroxidation marker (malondialdehyde-MDA). Our initial data is showing facilitatory no clear effects of intraperitoneal oxytocin administration on the main oxidative stress markers we determine mentioned above.

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OXYTOCIN ADMINISTRATION IS REDUCING LIPID PEROXIDATION LEVELS IN AGED RATS

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Lately, there is increased interest in understanding the roles of oxytocin in the main neuropsychiatric disorders, such as Alzheimer's disease, anxiety, depression, schizophrenia or autism and the variety of behaviours exhibited by both the administration of intranasal or peripheral oxytocin on the developed animal models for the aforementioned disorders (1,2). As oxidative stress in an accepted mechanism in these disorders, the effects of oxytocin administration on oxidative stress should be also be relevant (3). In this way, here we present some of our preliminary research regarding the administration of intraperitoneally oxytocin for 12 days and its effects on some oxidative stress markers in aged Wistar rats. Aged (two years old) male Wistar rats (n=15) were divided in 3 groups: control, aged and aged+oxytocin. Oxytocin was intraperitoneally injected in the aged+oxytocin group in a dose of 10 mg/kg/body weight for 12 consecutive days. The oxidative stress markers determined were represented by the specific activity of two antioxidant enzymes (superoxide dismutase-SOD and glutathione peroxidase-GPX) and one lipid peroxidation marker (malondialdehyde-MDA). Our initial are suggesting some possible antioxidant effects of oxytocin administration, as we could observe a significant decrease of MDA concentration as a result of oxytocin treatment, when compared to aged alone group of rats.

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PROTECTIVE EFFECT OF UBIQUINONE-10 AND COMPLEX OF PRECURSORS AND MODULATOR OF ITS BIOSYNTHESIS ON HEART UNDER DOXORUBICIN TREATMENT

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Doxorubicin is a potent anticarcinogenic drug that is widely used in practical oncology. Its use is accompanied by cardiotoxicity and toxicity to other tissues. Ubiquinone (CoQ) is an important electron and proton carrier, antioxidant, regulator of genes expression. Its biosynthesis may be inhibited under number of pathologies. The aim was to study the state of mitochondrial electron transport chain (ETC) components, CoQ content and redox state, superoxide anion radicals and NO production rates, and matrix metalloproteinase-2 and -9 activities in rat heart tissues under treatment with doxorubicin, CoQ10 medical, and complex preparation of precursors and modulator of CoQ biosynthesis.

Doxorubicin was administered intraperitoneally in dose of 2.2 mg/kg daily for 8 days. Experimental rats in addition to doxorubicin received per os α-tocopherol acetate, 4-hydroxybenzoic acid, and methionine (EPM complex) and CoQ10. Under doxorubicin treatment heart weight to body weight ratio decreased.

Treatment with EPM complex and CoQ10 in addition to doxorubicin administration exerts a protective effect on heart cells' mitochondria, evidenced by restoration of electron transport in electron transport chain, which is expressed as decreased nitrile complexes formation with Fe-S-proteins and increased ubisemiquinone content. It should be stressed that the protective effects of EPM complex on mitochondrial electron transport chain under doxorubicin administration is on par with those of CoQ10.

EPM complex and CoQ10 administration leads to normalization of NO content, superoxide-anion radicals generation, free radical lipid (conjugated dienes, TBA-reactive products) and protein peroxidation and activity of antioxidant enzyme systems (catalase, superoxidedismutase) under doxorubicin treatment.

Concurrently, matrix metalloproteinase-2 and -9 activities are decreased, which gives evidence of lessened extracellular matrix destruction. CoQ10 appeared to be more efficient at this if compared to EPM complex.

The expiremental data obtained may become the basis of development of approaches to correction of adverse effects of doxorubicin. These data may be used to substantiate the application of these biologically active substances within frameworks of complex treatment of oncological and cardiovascular pathologies.

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HEMATOLOGICAL INVESTIGATIONS AFTER FERROUS GLUCONATE ADMINISTRATION IN ANIMAL MODEL

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Ferrous gluconate is an iron supplement used in animal and human treatment, to prevent or treat low sideremia. Iron is very important mineral, being involved in the structure of hemoglobin and red blood cells, and having a very important role in blood oxygen transport. But also high doses of iron lead to iron intoxication that can lead to kidney failure or can negatively affect other organs and tissues. Iron intoxication is more severe in children and baby animals because can lead to death [1]. Our experimental research was performed on German Lop Eared Rabbits – five weeks old, three experimental groups: one control group, one experimental group (E1) with administration of 10g Fe2+/kg body weight as ferrous gluconate and the second experimental group (E2) with administration of 15g Fe2+/kg body weight, for 43 days. The solution of iron was aqueous ferrous gluconate and the administration was intraperitoneally for experimental animals and for control group we administrated NaCl 0.9% (normal saline solution) also intraperitoneally to respect the same procedure for all animals. The diet of rabbits included fresh plants and seeds with high protective role: black seed, clover, parsley, leek, chives, coriander, rucolla, cucumber, radish, fenugreek, and carrots. The in vivo experiment assures very good conditions for rabbits according with specific good practice guidelines and animal protection lows [2, 3]. The hematological investigations consist in erythrocytes, leukocytes, platelets, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width. The results presented not significant increases of tested parameter for E1, but the rabbits from E2 presented significantly high concentration of hemoglobin, due to high iron doses administration during a short period of time. One protective role against iron intoxication was also assigned to fresh leaves, fruits and seeds form plants from rabbits diet.

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PLANTS AND MICROORGANISMS MAY PROVE TO BE THE BEST SOURCE OF ESSENTIAL FATTY ACIDS FOR HUMANS

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Polyunsaturated fatty acids (PUFA), linoleic acid and α-linolenic acid are essential fatty acids (EFA) because the human body cannot synthesize them. α-linolenic acid (ALA) is the precursor of long chain fatty acid eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Both n-3 and n-6 fatty acids (known as ω3 and ω6) are structural components of cell membranes and serve as precursors of lipid mediators involved in numerous physiological processes such as inflammation, cell signaling, vascular endothelial function, vision, neurological development as well as essential for growth and development. The n-6 series is metabolized into more pro-inflammatory PUFA (arachidonic-derived eicosanoids) whereas the n-3 series is turned into more anti-inflammatory PUFA (ALA-derived EPA & DHA) although the biological activity of these eicosanoids is intertwined as not all arachidonic acid-derived eicosanoids are pro-inflammatory and not all EPA-derived eicosanoids are anti-inflammatory. Unfortunately, humans are not able to make EPA and DHA to any significant level (< 0.5 %) because the two desaturation enzymes involved in the process are affected by low vitamin B6, magnesium and zinc status as well as inflammation, hyperglycemia and hypercholesterolemia. Matters are further complicated by the fact that modern Western diet is rich in n-6 PUFA at the expense of n-3 PUFA. The imbalance in the n-3/n-6 ratio has a negative impact on health such as increased tendency to pro-inflammatory reactions, increased blood viscosity and vasoconstriction. On the other hand, a higher intake of n-3 PUFA is beneficial in the prevention of hypertension, coronary heart disease, type two diabetes, low-grade systemic inflammation, ulcerative colitis, rheumatoid arthritis and cancer. Supplementation appears to be a good choice to n-3 PUFA deficit in the diet of many people. It is recommended that the daily intake of EPA/DHA should be in the 200-400 mg range. So far, most commercial EPA/DHA supplements come from processing marine organisms, in particular salmon. The best salmon is the wild type but supply is limited to certain geographic areas that are less polluted. Farmed Atlantic salmon has its own problems. Fish feed nowadays contains mostly plant protein and soybean oil and that translates in lower amounts of EPA/DHA in the salmon fillets. A recent study found that mice fed on farmed Atlantic salmon meal developed insulin resistance and hepatic fat accumulation. Epidemiological studies have shown conflicting results on omega-3 supplementation, some studies indicated that supplements were protective against CVD while others did not. Since humans can only make EPA and DHA to a limited extent and the supply of marine n-3 fatty acids cannot keep up with the demand it became imperative to look elsewhere to acquire these important fatty acids. Alternative sources of long-chain n-3 fatty acids are certain plants and herbs, microorganisms such as microalgae, bacteria, fungi and diatoms. Since plants do not possess the n-3 long-chain PUFA biosynthetic pathway several attempts were made to transfer algal, microbial and yeast genes involved in this pathway into higher plants. Thus, microbial genes encoding Δ6-desaturases, Δ6-elongase components and Δ5-desaturases have been expressed in plants such as the oilseed crop *Brassica juncea* and the small flowering weed thale cress or Arabidopsis. Both plants had EPA and DHA present in their seeds. Transgenic plants could be a huge asset as potential sources of n-3 LC-PUFA but so far the yield of these fatty acids in plants is quite low so we are still far from large scale commercial operations. Cheaper production costs could be achieved using microorganisms. Thus, DuPont has developed a clean and sustainable alternative source of omega-3 fatty acids through fermentation using a metabolically engineered strain of *Yarrowia lipolytica*. Specifically, desaturase and elongase genes have been introduced into the oleaginous yeast to utilize certain carbon sources to synthesize omega-3 fatty acids under fermentation conditions. The strain candidates were evaluated via culture tubes, shake flasks, and fermentation tanks experiments. The typical fermentation process consists of a nitrogen-rich growth phase and a nitrogen-starved oleaginous phase. Medium components (e.g., nitrogen source) and process conditions (e.g., pH control, base addition) were optimized to improve the omega-3 fatty acid production.

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PRELIMINARY RESEARCHES ON BIOACCESSIBLE CONTENT OF CA AND MG FROM INSTANT COFFEE

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The benefits of coffee consumption are mainly determined by the nature and proportion of the main nutrients presented in coffee, including some essential elements.

During and after consuming these beverages, the essential minerals are not used or fully accessible for use in the homeostatic metabolism. Therefore, knowing of the total mineral content is not sufficient to establish the mineral coffee content. In this study, the bioavailable mineral concentration is considered to be the concentration of mineral elements released from the coffee infusion matrix following gastrointestinal digestion, which becomes available for physiological functions of the body.

The present paper aims to determine the bioavailable concentrations of two essential macroelements in diffused instant coffee beverages, more often used for preparing coffee and assessing their mineral intake in the recommended daily diet.

Gastrointestinal digestion model "in vitro" determined the bioavailability of calcium and magnesium in various brands of instant coffee: J.K., J.V., N.B., and D.I., more often used in local consumption. The bioavailable concentrations of the coffee infusions were measured with ICP-MS spectrometry.

The preliminary results show that analyzed instant coffee samples contain about 2mg/L of calcium and 3.4 mg/L of magnesium in the conditions of our experiment. Estimating data regarding the mineral intake reveals that a consumption of 200 ml of coffee (the equivalent of two cups of coffee) assures only a small amount of Ca (about 1%) and Mg (about 5%) of daily requirement. Thus, in these conditions, we can conclude that infusions of instant coffee regarding our experiment is not relevant in terms of Ca and Mg intake, but the intake of calcium and magnesium from coffee have to be discussed referring also to the caffeine content.

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OVIDUCT-SPECIFIC GLYCOPROTEIN ROLE AND EFFECT ON IVF EMBRYOS DEVELOPMENT

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Oviductine or oviduct-specific glycoprotein is secreted in the oviduct by epithelial cells during the follicular stage of estrous cycle in mammals. Oviductine is part of oviductins – a subclass of oviduct secretory compounds, with specific implications in association with zona pellucida of mammal's ovulated oocytes and also with the early embryo. Oviductine (oviduct-specific glycoprotein) in the amino-terminal end shows similarity with the 18 glycosyl-hydrolases family, named chitinases, and also with a chitinase-like protein group with a biological role not yet fully understood. The similarity of the sequence with the chitinases would be responsible for the oviductine linkage with the zona pellucida, whereas common features with the mucins confer the oviductine a protective and anti-adhesive role. Oviduct-specific glycoprotein is a major glycoprotein in mammalian fluids secreted specifically by the non-tubular epithelial cells during the estrus. In rodents and ruminants, the oviductine binds to the zona pellucida and to the perivitelline space of the ovocyte, modulating the gametes interactions, diminishing polyspermia. According to other studies, the oviduct-specific glycoprotein may be involved in the metabolic substrate carrier (such as cations). In this study we tested in vitro the effect of oviductine on the bovine embryo development. The results present the oviductine effect on the embryo development rate for 7 days, in different conditions: in presence or absence of oviductine (4 g/L), and also in the presence of oviductine together with gonadotropin and oviduct cells. After 24 hours of culture, the presence of oviductine does not have rapid negative effect on the embryo, but there was a late negative effect observed after 7 days of culture. Our results suggest that addition of oviductine (4 g/L) may affect the embryo development. In our study, the embryo division rate is quite high, without significant differences compared to control. The blastocyst rate after 7 days negatively influenced the development of bovine embryos.

Key words: oviductine, mammals, embryo development.

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STEM CELLS THERAPIES – PERSPECTIVES

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Stem cells are the specific cells provided with self-renewal capacity and the ability to give birth to the daughter cells engaged in developmental stage, so finally they will eventually differentiate into specialized cells. Stem cells can be germinal stem cells, starting from the germinal crest where germination line differentiation occurs; and adult stem cells – hematopoietic cells that generate blood cells and immune system cells, and which are found in the bone marrow, although in the fetal phase they are found also in the liver and spleen. The characteristics of stem cells to be used in cellular and human transplant therapies must be: pluripotent or totipotent – ideal for generating any type of cell; immortal – to have unlimited proliferation capacity (self-renewal); stable, well-characterized molecular phenotype; without tumor potential; sensitive to genetic manipulation to allow accurate genetic modification, including the introduction of therapeutic genes. The principal therapeutic advantage of stem cells is that they can be used for cellular and tissue transplantation without the problem of allograft. Ideally, it would be the derivation of tissues with histological identity of the same patient for allograft. The use that keeps more attention would be the use of differentiated cells starting from embryonic stem cells for cellular therapies as well as reconstruction of damaged tissues. The security for long-term cellular therapies becomes a serious aspect, and one of the questions being debated is the biological age of the cells. Some researchers say they are talking about advanced age, and others say their own somatic nucleus transfer oversees cells and stimulates telomerase. It remains to clarify the possible implications of genetic imprinting on embryonic stem cells and their derivatives obtained after nuclear transfer. In adult stem cells, their unexpected flexibility for differentiation is demonstrated, including in cell lines that does not have the same origins. If these data are confirmed and if this fields are developed, a new possibility of cellular therapies will take place, without ethical problems for manipulation and destroying the human embryos. Even if they are artificial, they are obtained by somatic nucleus transfer to oocytes. These cell therapies represent a field of interdisciplinary cell engineering that applies the principles of engineering and life sciences to obtaining biological substitutes for restoring, maintaining or improving tissue function.

Key words: cellular therapy, embryo stem cells, adult stem cells.

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TRACEABILITY OF GENETICALLY MODIFIED (GM) DNA FROM FARM TO FORK

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Roundup Ready (RR) soybean is the principal biotech crop being tolerant to the glyphosate family of herbicides by expressing transgenic DNA that encodes 5-enolpyruvylshikamate-3-phosphate synthase (CP4 EPSPS). Most of the compound feed produced in the EU contains GM soybean. Since 2003 all foods and feeds containing or derived from GM products in amounts that pass 0.9%, are compulsory labeled. However, food products that derived from livestock fed with GM containing feeds are exempt from EU-labeling laws.

The safety of GMO regarding the introduced DNA and protein, is based on strong scientific and market regulatory assessments. Highly sensitive detection technologies such as PCR (Polymerase Chain Reaction) are used to assess the fate of foreign DNA in animal that are fed with GMO containing forage [1].

The digestive process includes the exposure of the gastrointestinal tract to foreign DNA. Ingested food is mechanically disrupted; the DNA is released and cleaved into small sequences and free nucleotides. The activity of various enzymes is further affecting the structural integrity of DNA. The digestive fate of transgenic DNA is continuously raising more issues about traceability and precision of detection. The actual trend is to detect small traces of transgenic DNA in different tissues of GM feed animals, in attempts to detect the position and the time until total degradation occurs [2].

The presence of GM sequence was emphasized in animal tissue samples that were fed with transgenic soybean, pointing out that the plant DNA is not totally destroyed during the digestion process. The obtained data suggest that the number of detected GM sequences is higher in muscle and liver compared to stomach. To extend the research a screening of different processed meat products from the market was performed, the GM sequences being detected in few of them neither one being labeled as GMO containing food.

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EVALUATION OF PLANT VARIABILITY BASED ON MOLECULAR MARKERS TECHNOLOGY

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Using of molecular markers on plant genome analysis allows the evaluation of the degree of relatedness between different genotypes, the establishment of the genetic fingerprint or even the lineage.

The aim of our research was to analyze and compare different types of molecular markers, each of them being distinctive for a specific part of the genome. Therefore the RAPD (Random Amplified Polymorphic DNA) recognized random sequences and amplified that DNA regions, ISSR (Inter Simple Sequence Repeats) are specific for the microsatellites sequences and amplify the fragment between two neighboring regions and DAMD (Directly Amplified Minisatellite DNA) primers amplify the regions between two minisatellites []1,2].

The DNA was isolated from fresh leaf tissue using modified CTAB method and the amplification reactions were performed according to the marker type. The PCR products were separated on 1.8% agarose gel and visualized under UV light in ethidium bromide presence. The obtained data were statistically evaluated based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) establishing the similarity coefficients and the sample clustering [3].

First, five ISSR primers were used to amplify the DNA extracted from five different Salix species. All the amplified fragments were analyzed, scoring with 1 the presence and with 0 the absence of the bands. Based on five ISSR markers 128 fragments were visualized, 114 being polymorphic (89.1%). The fifth DAMD markers emphasized 94 fragments, of what 87 were polymorphic (92.6%). and the RAPD markers generated a number of 103 fragments, 97 being polymorphic (94.2%).

For each type of markers the dendrograms were different pointing out the differences between the annealing sites for the specific primers. For this reason, it is recommended to use several categories of markers to increase the accuracy and specificity of the results.

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PRELIMINARY STUDY REGARDING BIOCHEMICAL COMPOSITION OF PIKEPERCH (*SANDER LUCIOPERCA*) FRESH SEMEN

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Continuous increasing of the demand for stocking material for both natural and intensive culture in Sander lucioperca is a challenge nowadays for reproduction and farming technology. The semen preservation in pikeperch has been in the last two decades an important issue among ichthyologists and important achievements in controlled pikeperch reproduction techniques has been noticed. In the validation of laboratory screening technology for male selection, the necessity to evaluate both the biochemical composition of the seminal material and the oxidative degrading processes at the spermatozoa's membrane level, processes which occurring during the semen preservation represent an important step in pikeperch reproduction biotechnology. The goal of this preliminary research is to evaluate biochemical composition of both seminal plasma and pikeperch spermatozoa. Twelve pikeperch (Sander lucioperca), males, 4-years-old, farmed in ponds in polyculture with carps, were used in our experiment. The milt collected from each male was placed in small sterile vessels and cooled at 4°C during the transport to laboratory. The sperm concentration of the milt and pH was determined from total semen. The evaluation of enzymatic (alkaline phosphatase, ALAT, ASAT) and non-enzymatic (glucose, total protein and cholesterol) contituents has been performed from both seminal plasma and spermatozoa extract obtained by ultrasonication.

Acknowledgments: The research has been conducted in the Aquaculture facility and Antioxidant Research Laboratory A1c - „Horia Cernescu" Research Laboratory Center from Banat's University of Agricultural Sciences and Veterinary Medicine „King Michael I of Romania" from Timișoara .

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HEMATOLOGICAL MODIFICATIONS INDUCED BY ENZOOTIC BOVINE LEUKEMIA RETROVIRUS

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The research was conducted on 82 blood samples from BLV(+) and BLV(-) cattle and aimed to underline the evolution of hematological modifications linked with the evolution of the bovine enzootic leukemia virus. The blood samples were collected from the jugular vein and analyzed with an MB-1830 automat hematological analyzer (Maysun Technology Co., Ltd., R.P. China), for human and veterinary use, device which allows the determination of the following hematological parameters: the total count of leukocytes, the lymphocyte, monocyte and granulocyte count, red blood cell count (RBC), the hematocrit (HCT), the hemoglobin concentration (HGB), the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), the mean corpuscular hemoglobin concentration (MCHC), the red blood cell volume distribution width (RDW), the platelet (PLT), the mean platelet volume (MPV).The results underline a significant rise of the total count of leucocytes and lymphocytes ($p \leq 0,05$) at BLV(+) cattle, compared with BLV(-) cattle. The others hematological parameters suffered some variations based on the positivity status for the retrovirus, but these modifications were statistically insignificant. Still, a slightly rise of the monocyte count and a slightly drop of the granulocyte count at the BLV(+) cattle could be mentioned.

Based on the leukocyte formula, the percentage increase of the lymphocytes could be a consequence of the significant reduction of the granulocyte proportion, especially the reduction of the neutrophyles. This "substitution" cannot be interpreted "as it is", because, comparing the BLV(+) and the BLV(-) cattle for the absolute values of lymphocytes (11500±6600/µl, respectively 4900±1600/µl) and for granulocytes (5700±2000/µl, respectively 6100±1700/µl), it can be underline the fact that is a real rise of lymphocyte count and just a slightly reduction, statistically insignificant, of the granulocytes, in general. Analyzing the two hematological parameters with statistically significant differences at BLV(+) cattle – the leukocyte count and the lymphocyte count – individual variations were found to be very high (CV = 55% for total leukocytes and CV = 57% for lymphocytes). In conclusion, it can be affirmed that from the evaluated hematological parameters, the leukocyte and the lymphocyte count was statistically significant higher at BLV(+) cattle compared to BLV(-) cattle. Also, the increase of lymphocyte count in persistent lymphocytosis VLB (+) cattle does not influence the granulocyte count.

Key words: retrovirus, leukocyte formula, bovine

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PCA INTEGRATION IN THE EFFECTIVENESS INTERPRETATION OF DRY FRACTIONATION OPERATION IN CENTRIFUGAL FIELD ON A NATURAL LIPID MIX

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Our study shows the efficacy of a dry fractionation operation in centrifugal field on a complex lipid mixture of pork lard. This was done using multivariate analysis and principal components analysis (PCA), namely the correlation between the operating parameters (time – temperature – centrifugal rotor speed) and the studied quality parameters. The recorded results highlight a well-defined group of the two fractions: liquid (olein) and solid (stearin). Changes induced to the lipid mixture and repeated fractional crystallization (olein / stearin) in accordance with operator parameters and correlated with the PCA interpretation demonstrate the correctness of the dry fractionation applied. Multivariate Statistical Analysis of PCA for pork lard and lipid (olein / stearin) fractions obtained by dry fractionation indicate a possible clear classification based on the type of lipid mixture obtained. Lipid fraction analysis was performed with GC Hewlett Packard HP 6890 Series, coupled with a Hewlett Packard 5973 Mass Selective Detector mass spectrometer. Thus, the more saturated solid fractions (FS - stearin) are better grouped. Responsible for this group is the mixture of stearic acid and palmitic acid with residual oleic inclusions, confirmed by the presence in the same area of the liquid fraction I (fraction FLI). This demonstrates the insufficient separation of saturated compounds from unsaturated fractions, highlighting the need for optimization of operating parameters (time, temperature, centrifugation speed). The liquid fractions – FL (oleins), FL II, III and IV are better grouped. Responsible for this group are the superior unsaturated organic acids (oleic and linoleic) presented in significant quantity.

Keywords: dry fractionation, fractionation crystallization, olein, stearin, PCA.

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THE DIAGNOSTIC VALUE OF HEMATOLOGY AND BLOOD BIOCHEMISTRY IN PIODEMODICOSIS

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Demodicosis is a serious ectoparasitosis produced by Demodex sp. which is localized in hair follicles in animals and humans, also. Complementary examinations are indicative in the diagnosis of parasitosis, but associated with a clinical and microscopic examination, they have a value useful the clinician [4]. There are bibliographic studies [2,3] which emphasize some importance in the diagnosis and treatment of demodicosis, paraclinical examinations. They are also authors [1] who support the irrelevance of these tests in adult dogs, with a slight reluctance when it comes to young dogs.

Based on these considerations, the aim of the present study was to determine the values of certain blood parameters in dogs diagnosed with piodemodicosis and to appreciate the importance of these exams in the diagnosis of acariosis.

The investigations were performed on a group of 37 dogs diagnosed with piodemodicosis (PD), aged 2-3 years and on a control group of 20 dogs, aged 2-3 years. Blood samples were processed and blood counts were determined: blood count, alkaline phosphatase (ALP), alanine aminotransferases (ALT), aspartate aminotransferases (AST), serum urea, serum creatinine, total proteins, albumin and globulin. The results were statistically interpreted by the Microsoft Excel program.

Haematological investigations results in studied group (PD) reveal values of erythrocytes, hemoglobin, hematocrit and platelets at the lower limit of the normal values; decreased red blood cell count and decreased hematocrit concentration. Leukocytosis is found: lymphocytes and granulocytes increase and monocytes decrease. This aspect reveals the response of the host to the parasite, but also to the bacterial suprainfection. Blood biochemical parameters ranged, as average values, within the normal reference range, with mild serum increasing on ALP, ALT, AST associated with decreased albumin and total serum protein. These values highlight either a non-parasitic insufficiency or are the result of the toxic action of Demodex mite.

We did not record changes in kidney parameters (creatinine and serum urea). In the control group, the results of haematological and biochemical investigations were within the reference limits.The results of haematological and blood biochemical investigations reveal changes from the normal values of the monitored parameters. In young dogs diagnosed with piodemodicosis, haematological investigations and blood biochemistry are important in the diagnosis of acariosis.

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FATTY ACIDS PROFILE OF ARGAN OILS

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Argan oil is one of the most precious vegetal oil, important for the rarity of its geographical source, for the uniqueness of its composition and for its prestige resulted for all those considerations. For those reasons, there is a great temptation for this oil to be adulterated, on the profile market.

The aim of our study was: (1) to evaluate the chemical composition of 7 argan oil samples, all originals from Maroccan manufactures, considered to be for cosmetic use, by using GC-MS method, (2) to compare those values with another sample of argan oil, sold as an alimentary oil, much less expensive than previous ones, (3) to evaluate and compare the chemical composition for a sample of cosmetic product, released on the cosmetic market by a multinational factory, product which has written on its label: *hair protection with argan oil*.

Analyzing obtained results, it was observed the differences between the authentic Maroccan argan oil samples and the 2 others samples, manly regarding the ratio between *oleic acid* and *linoleic acid*. Maroccan argan oil was rich in linoleic acid 41% and the ratio between *oleic acid* and *linoleic acid* was ½. These big differences raise the hypothesis of adding another oil, which could by organoleptically similar to argan oil (for exemple olive oil).

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TECHNICAL-ECONOMIC STUDY OF NATURAL STORAGE FOR VEGETAL RAW MATERIAL – RED ONION

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Onion (Allium Cepa), a very commonly used vegetable, ranks third in the world production of major vegetables. Apart from imparting the keys delicious pungency and flavor due to STI in many culinary preparations, medicinal Several Purposes Also it serves. Processing and preservation of onion by Suitable Means is a major thrust area since a long time.

In the first part of this paper here were carried out specialized studies on the use of onions in the daily food, chemical composition and its therapeutic effects.

We have conducted laboratory tests to identify the drying process of the vegetal material (red onion) during storage.

Time and storage conditions, both in warehouses with mechanized ventilation, as well as in cold storage, plant materials depend on their resistance to storage, chemical composition and structural-textural strength peculiarities. After harvesting these products the metabolic processes continues the action using its own enzymes, which involves directing careful microclimates factors, such as: temperature, relative air humidity, light. For red onions an environment which assures a temperature of -1 °...+1°C, with relative humidity of 75-80%, can be stored maximum 6-7 months.

By applying thermo-gravimetric analysis using thermo-balance Sartorius was monitored remove water during the process of storing the product analyzed by a continuous weighing. By applying mathematical modeling using Kernel density that is observed every 14 days, the plant material is dried.

Key words: red onion, storage, thermo-gravimetric analysis, dried plant

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CONTROLLED RELEASE OF ENZYMES FROM POROUS MATRICES

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Fragile or heat-sensitive biomolecules have been entrapped in porous matrices for controlled release biotechnological applications. The most methods are based on organic polymer structures and only a few controlled release systems have made using inorganic matrices. Sol-gel chemistry avoids synthesis of inorganic polymers with structure adapted to the physico-chemical properties of biomolecules. Controlled release of enzymes is important in an increasing number of industrial applications. The release system obtained by entrapment of enzyme in sol-gel matrices has some key advantages: sol- gel synthesis is enzyme friendly and conducted at the room temperature, the matrices porous size and the release rate can be controlled, the obtained silica gels are biocompatible with the enzyme molecules, the precursors are cheap and commercially available in high quantities [1,2].

The aim of our study was to obtain a controlled release system to be used in the hydrolysis reaction of starch by sol-gel entrapment of a microbial amylase from Aspergillus niger. The released studies were carried out with both swollen and dry xerogels loaded with amylase. The gels were obtained using tetramethyl orthosilicate precursor, in the presence of different additives. The structure of the xerogel and the release rate of enzyme molecules from the silica network were changed by the used additive and also by the characteristic of the release environment.

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RESVERATROL ATTENUATES HYPERGLYCEMIA-INDUCED STRESS IN HUMAN RETINAL CELLS

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One of the most important human senses that help us perceive the environment around us is the ability to see. Therefore, the sight loss reduces the quality of life thus affecting our daily activities. A severe ocular complication that causes visual impairment and even worse, blinding, due to a damage of the retina and the optic nerves is diabetic retinopathy (DR). The current therapies developed for DR include the anti-vascular endothelial growth factor (VEGF) therapies, the focal laser therapy and the steroids. During recent years, there was expressed a general interest in using resveratrol (RV) (3,5,4'-trihydroxystilbene) for prevention or complementary therapy for eye diseases. A desirable strategy to enhance the bioavailability of RV and to increase its solubility, stability and release is to develop an efficient delivery system for RV and target it directly towards retina. Our aim was to compare the efficiency of RV-free molecule and RV-loaded into polyelectrolyte multilayer microcapsules (PEM) in hyperglycemia-induce conditions in retina pigmented epithelial cells. The synthesis process of the RV-PEM complex is based on a simple layer by layer assembly approach. The RV-loaded PEM obtained by this procedure were characterized using spectroscopic and microscopic methods. Mimicking the hyperglycemia physiology which occurs in DR patients by using D407 cells as a platform for an in vitro experimental model for this eye disease, we assessed in vitro the therapeutic potential of RV-free molecule and RV-loaded into PEM. First, in order to determine the amount of therapeutic agent who reaches the target site the up-taken RV by D407 cells was assessed by HPLC-ESI-MS analysis. Moreover, confocal and scanning electron microscopy proved that RV-loaded PEM were internalized in RPE cells in normal and hyperglycemia-induced conditions. The present strategy can be used to have a dual effect, one from the laser, used in the current therapy, and the other from the therapeutic agent released only when the laser light reaches the cells.

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ULTRA-HIGH-THROUGHPUT SCREENING SYSTEMS BASED ON FLOW CYTOMETRY AND MICROFLUIDIC DEVICES FOR DIRECTED EVOLUTION OF DIFFERENT ENZYME CLASSES

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In the field of biotechnology, we are often employing enzymes to perform the tasks previously reserved for chemical catalysts. Enzymes have the advantages that they are environmentally friendly and are more specific compared to the chemical catalysts. However it is not always possible to find in nature an enzyme that performs the exact reaction that is needed for the industrial process or that is active in the required conditions. Therefore the natural properties of the enzymes often need to be tuned for specific process conditions by changing specificity, optimal reaction temperature, optimal pH or increasing activity.

Enzymatic properties can be changed by means of directed protein evolution. Similar to the Darwinian evolution, directed evolution comprises of iterative cycles of mutations and selection and screening but at an accelerated speed.

The main bottleneck in this type of experiments is the development of a screening method that can be used for the selection of an improved variant with the property of interest. The widespread used screening methods are based on micro-titer plates (MTPs) but they are expensive, laborious, time-consuming and only a small number of variants can be screened (103-106 in months). We have been focusing on establishing ultra-high-throughput screening (HTS) assays that can be used for screening mutant libraries using either fluorescence activated cell sorting or microfluidic devices. Using these improved methods more than 107 variants can be evaluated in much shorter timeframes (hours).

The developed screening systems were successfully applied for the directed evolution of different enzymes like glucose oxidase, cellulases, xylanases, chitinases and peroxidases. All the chosen catalysts have numerous industrial applications ranging from biomedicine to biofuel industry.

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DEVELOPMENT OF ULTRAHIGH-THROUGHPUT SCREENING PLATFORMS FOR DIRECTED EVOLUTION OF GLUCOSE OXIDASE

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Directed protein evolution is a powerful algorithm for improving enzyme properties in iterative cycles of diversity generation and screening. In order to be effective directed evolution requires screening of very large gene libraries. State of the art throughput technologies comprise display technologies (106-1010 variants; mostly used for evolving affinity/binding) and in vitro compartmentalization (IVC; 1010 reaction compartments per mL reaction volume). In IVC enzymatic reactions are performed inside water microdroplets dispersed in water-in-oil-in-water emulsions. The diameter of microdroplets ranges from 0.5-10 μm and enables in combination with flow cytometry sorting of these compartments at a very high speed (up to 107 droplets per hour). There are however two drawbacks of IVC technology based on double emulsions: high complexity and polydispersity of emulsion structure that is limiting quantitative analysis; and difficulty to modify content of droplets after gene/cell encapsulation. These limitations can be overcome by using droplet-based microfluidic systems that allow generation of highly monodisperse emulsions (water-in-oil), and fusion and splitting of droplets that were developed at Prof Dave Weitz lab and used for development of an ultrahigh-throughput screening platform enabling screening of 108 individual peroxidase reactions in only 10 h [1]. Still, the major challenge for IVC technology remains development of fluorescent assays that will reflect industrially relevant activity we are aiming to evolve, and that will fit to the complex (bio)chemical environment of a single cell enzyme measurement in microdroplets [2]. Here an overview of fluorescent assays for glucose oxidase will be given and a detailed presentation of the development process for the vanadium bromoperoxidase and fluorescein tyramide based screening systems for glucose oxidase based on flow cytometry that we have developed [3,4]. Application of these screening systems for directed evolution of glucose oxidase using flow cytometry and their adaptation for use on microfluidic lab on chip devices previously developed for peroxidases will be also presented. Using developed screening systems several mutants of glucose oxidase from Aspergillus niger with increased activity were found. Possible applications of these assays in high throughput screening methods in emulsion systems using flow cytometry and microfluidic lab on chip devices for cellobiose dehydrogenase, cellulases and peroxidases will be discussed.

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PHYSICAL AND CHEMICAL DETECTION OF POLYHYDROXYBUTYRATE FROM *HALOMONAS ELONGATA* STRAIN 2FFF

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Introduction: Polyhydroxyalkanoates (PHAs) are carbon and energy storage polymers accumulated intracellularly as round inclusions by numerous microorganisms during growth with excess of carbon and depletion of phosphorus and nitrogen sources [1]. Tree major types of PHAs are frequently produced by Bacteria and Archaea: polyhydroxybutyrate (PHB), polyhydroxyvalerate (PHV), and polyhydroxybutyrate-co-valerate (PHBV). PHA derivatives might be used as biodegradable, biocompatible, and thermoplastic polymers in packaging and biomedical applications.

The aim of present work was to investigate the capacity of PHA production by a halophilic bacterial strain grown under saline conditions.

Materials and methods: PHA accumulation in the *Halomonas elongata* strain 2FF (isolated from a salt lake located in Transylvanian Basin) was induced by growth in high-salt (10% NaCl, w/v) medium supplemented with 1% glucose as C-source and limiting P and N substrate concentrations. The biopolymer was extracted using the hypochlorite-chloroform method. Crotonic acid assay, elemental analysis followed by Fourier-Transform Infrared (FTIR) spectroscopy, Raman microspectroscopy, nuclear magnetic resonance (^{1}H-NMR), X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC) were employed for the physical and chemical analysis of the purified biopolymer.

Result: The peak recorded spectrophotometrically at 235 nm was characteristic for crotonic acid resulted from polymer. Elemental analysis indicated that the obtained C-H-O values were close to the theoretical value of standard PHB. FTIR analysis identified (C=O) and (C-O-C) vibrations whereas Raman spectroscopy evidenced the (C=O) and (C-C) vibrations seconded by (–CH) deformation. ^{1}H-NMR and XRPD revealed that the polymer was very likely PHB. The DSC indicated that the melting temperature of the polymer was 162.5oC.

Conclusions: The isolated extremely halotolerant *H. elongata* strain 2FF was able to produce significant amounts of PHB within 48-72 hours of growth under high salinity (10% NaCl) by using glucose as C-source. The polymer was successfully extracted, purified and further analyzed by physical and chemical techniques.

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OPTIMIZATION OF THE NINHYDRIN REACTION METHOD FOR THE ESTIMATION OF ACETYLATION DEGREE OF CHITOSAN

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Chitosan is a high molecular weight polysaccharide composed of randomly repeating units of N-acetyl-D-glucosamine and D-glucosamine, derived from chitin that occurs naturally in crustacean and insect exoskeletons, fungi cell walls and cephalopods [1]. The colorimetric quantitative assay of chitosan using ninhydrin was first used in 1993 by Curotto and Aros, using test tubes [2]. In this paper we present the adaptation to ELISA plate format of the method of reaction of ninhydrin with the amino groups of chitosan and optimization of some reaction parameter.

Using glucosamine as a standard compound, we studied the influence of several parameters such as the time of reaction, the stability in time of the final coloured product and the influence of the temperature. Also we obtained linear calibration curves for this chemical [3]. The results showed that this miniaturized method is sensitive and reproducible. The optimized miniaturized method was then used to determine the quantity of amino groups from alkaline deacetylated chitin and chitosan.

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USE OF POLYACRYLAMIDE GEL ELECTROPHORESIS FOR ESTIMATION OF DEGREE OF POLYMERIZATION OF CHITOSAN

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In this study an attempt was made to estimate the degree of polymerization of chitosan using polyacrylamide gel electrophoresis. Usually, the polyacrylamide gel is used for migration of negative charged polymers, but in the present work, this method was adapted for the migration of positive charged polymers In this case we used the chitosan as a polymer with a masses ranging from 10 to 103 kDa. The samples for electrophoresis were obtained from hydrolysis of chitosan with hydrochloric acid and with a mixture of some enzymes (chitinase, cellulase and α-amylase), or with these enzymes used alone. The first results have demonstrate that α – amylase and cellulase increased the hydrolytic activity of chitinase on the glycosidic bounds of monomers glucose-amine or N-acetyyl-glucoseIn this study an attempt was made to estimate the degree of polymerization of chitosan using polyacrylamide gel electrophoresis. Usually, the polyacrylamide gel is used for migration of negative charged polymers, but in the present work, this method was adapted for the migration of positive charged polymers In this case we used the chitosan as a polymer with a masses ranging from 10 to 103 kDa. The samples for electrophoresis were obtained from hydrolysis of chitosan with hydrochloric acid and with a mixture of some enzymes (chitinase, cellulase and α-amylase), or with these enzymes used alone. The first results have demonstrate that α – amylase and cellulase increased the hydrolytic activity of chitinase on the glycosidic bounds of monomers glucose-amine or N-acetyyl-glucose-amine from chitosan chain. The results of this study was shown that cellulase has a relatively small chitinolytic activity.

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STRUCTURAL BIOINFORMATICS CHARACTERIZATION OF CHITIN DEACETYLASES

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Chitin deacetylases are enzymes catalyzing the deacetylation of chitin, a structural biopolymer usually found in marine organisms. These enzymes regularly occur in marine bacteria, several fungi and a few insects. Within this study we have considered chitin deacetylases from some microorganisms having structures deposited in Protein Data Bank. *Colletotrichum lindemuthianum* chitin deacetylase is the most studied and its catalytic mechanism, biochemical properties and biological roles are well known. We have characterized structures of these proteins and compared their sequences and structures with those of the *Colletotrichum lindemuthianum* chitin deacetylase to detect similar regions and to transfer known information for the other chitin deacetylases. Investigated overall structures are quite dissimilar, but their catalytic sites reflect high similarity. The most similar structures are revealed by chitin deacetylases belonging to *Colletotrichum lindemuthianum* and *Aspergillus nidulans*, while the most dissimilar structures of chitin deacetylases belong to *Aspergillus nidulans* and *Vibrio cholerae*. This information is valuable for increasing our knowledge concerning the use of chitin deacetylases for the enzymatic conversion of chitin to chitosan, as the currently used chemical procedure is toxic for the environment.

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PRELIMINARY RESULTS OF AN ENZYMATIC TEST BATTERY DEVELOPMENT FOR THE ASSESSMENT OF TOXICITY OF BIOPOLYMERS

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Biopolymers are polymers produced by living organisms that have a wide range of applications, such as in wound healing (dressings, sutures, stents), drug delivery (micro- and nanoparticles, fibers, coatings), or cosmetics (scrubs, containers). Due to this, biopolymers can come into contact with the environment and the human body, where they can have different effects, possibly toxic. So it is necessary to test their environmental and human toxicity. Before testing the effect of chemicals on cells or whole organisms, some preliminary tests can be realized at molecular level, i.e. testing the influence on some enzyme activities [1]. The biopolymers tested were starch, cellulose and chitosan. The effect of possibly toxic chemicals on enzyme activity is conveniently and rapidly determined via simple enzymatic assays that can be miniaturized, automated, and measured using plate readers.

The development of the enzymatic test battery consisted in the selection of some key enzymes, from different classes and from different metabolic pathways [2]. The enzymes used in this work were alkaline phosphatase, trypsin and alpha amylase which are from the class of hydrolases; peroxidase, lactate dehydrogenase and catalase, from the class of oxidoreductases.

The tests showed that the assessed biopolymers had no significant inhibitor effect on the selected enzymes.

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3,5-DINITROSALICYLIC ACID METHOD: AN EVALUATION OF ENZYMATIC HYDROLYSIS OF CHITOSAN

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Chitin is a natural polysaccharide synthesized by numerous organisms. The main source of chitin comes from seafood processing industry. Chitosan is the main deacetylated derivative of chitin, used in various applications, mainly after the hydrolysis of the long chain of this polymer. The method used in this study was DNS (3,5-dinitrosalicylic acid) assay. The main disadvantage of the classical method is the heating step, which requires boiling in water and thus using glass test tubes.

In this work, an improved DNS method was performed in microtiter plates heated on a thermoblock. Furthermore this method was applied to the study of hydrolysis of chitosan with chitinase.

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NATURAL POLYMERS USED AS WOUND DRESSINGS AND THEIR EFFECT ON MICROBIAL GROWTH

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In all biomedical fields today, biopolymers are playing a very important role. Many natural polysaccharides such as , chitosan, alginate, cellulose, starch, and carrageenan are widely used as wound dressings. Due to its antimicrobial properties, biocompatibility, biodegradability, and low toxicity, chitosan is the most promising polymer used in wound healing processes. Various derivatives of chitosan have been proposed so far in multiple combinations with other polymers.

In this study, the effect on microbial growth, of several natural polymers used as wound dressings, was investigated. Susceptibility test were performed on *Escherichia coli*, *Pseudomonas aeruginosa, Staphilococcus aureus* and *Candida albicans* using solutions of chitosan, alginate, starch and carboxymethyl cellulose. The following methods were used to determine antimicrobial activity: agar well diffusion, Minimum Inhibitory Concentration and Minimum Bactericidal Concentration tests and the viability of the cells was tested using 2.3.5-Triphenyl tetrazolium chloride assay. The results showed that chitosan inhibits bacterial growth but does not affect yeast cells growth. On the contrary, we have observed that *Candida albicans* cells can metabolize chitosan. The rest of the polymers tested showed no inhibitory effects on microbial growth.

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COMPUTATIONAL ASSESSMENT OF THE PHARMACOKINETICS PROPERTIES OF SOME WATER SOLUBLE CHITOSAN DERIVATIVES

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Chitosan is a nontoxic and biodegradable polysaccharide used as a biomaterial for the production of drug delivery systems. Chitosan has a poor solubility in neutral or alkalinized media and it restricts its pharmaceutical and biomedical applications. To improve the solubility in aqueous media, different derivatives of chitosan are obtained and they may be used for producing biomaterials and in the development of biomedical nanodevices and controlled release drug formulations. Within this study we use SwissADME, Ochem and PASS online computational tools for predicting absorption, distribution, metabolism, excretion and toxicity (ADME-Tox), pharmacokinetics profiles, biological activity spectra, toxic/adverse effects and environmental toxicity of the some water soluble chitosan derivatives used to obtain chitosan-based nanomaterials related to pharmaceutical applications.

The pharmacokinetics profiles of these derivatives reveal poor gastrointestinal absorption and consequently, low oral bioavailability. Chitosan derivatives cannot pass the blood-brain barrier, which means they cannot affect the central nervous system. Also, chitosan derivatives illustrate low skin permeation and they are not able to inhibit the enzymes of the cytochrome P450 that are involved in the metabolism of xenobiotics. The main side effects of chitosan and its derivatives in humans are: weight loss, acidosis, gastrointestinal toxicity. They reflect different degrees of environmental toxicity.

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ENVIRONMENTAL AND CLINICAL APPLICATIONS OF CHITOSAN

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Chitosan is a biopolymer containing glucosamine and acetylglucosamine units that give its distinctive properties: cationic behaviour in acidic solutions and strong affinity for metal ions [1]. Other properties of chitosan are biocompatibility, high charge density, non-toxicity and mucoadhesion. Chitosan interacts with polyphosphates, sulphates and glutaraldehyde forming gel beads and films, property that allows its use in coating of pharmaceuticals and food products, gel entrapment of biochemicals, plant embryo, whole cells, microorganism and algae [2]. The physicochemical properties of chitosan depend considerably on its degree of deacetylation, molecular weight and crystallinity [1]. This overview of our work summarizes the influence of the chitosan molecular weight, the preparation method and properties of chitosan based biomaterials such as beads [3] and films [4] related to various practical applications. This analysis also includes factors that affect the drug *in vitro* release from membranes, loading capacity, adsorption efficiency and also the influence of the drug immobilization method, the pH of the environment and the initial drug loading [4].

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DIRECTED EVOLUTION OF THE CELLOBIOSE DEHYDROGENASE FROM *PHANEROCHAETE CHRYSOSPORIUM* IN YEAST *SACCHAROMYCES CEREVISIAE*

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The cellobiose dehydrogenase (CDH) gene from *Phanerochaete chrysosporium* has been cloned in pYES2 vector and expressed in yeast *S. cerevisiae*InvSc1. Expression of the construct was optimized and he was purifed by ion-exchange chromatography and ultrafiltration. Recombinant CDH produced in yeast had lower specific activity of 0.6 U/mg of pure protein than native CDH produced in *P.chrysosporium*. Recombinant enzyme showed similar substrate specificity for cellobiose and lactose. Optimal temperature and pH stability was slightly different compared to native CDH. The molecular weight of recombinant CDH was higher than molecular weight of native CDH (90 KDa) with a broad band on SDS electrophoresis gel at 120kDa that was result of hyperglycosylation. Results showed that CDH can be expressed in yeast *S.cerevisiae* which can be used in directed evolution experiments. CDH gene library was generated using error-prone PCR to create random mutations and obtained mutants were tested in microtiter plates for improved activity using adapted DCIP assay. Several mutants with increased activity were detected in microtiter plates. Mutants were purified and characterization showed increased activity and kinetics than that of a recombinant enzyme.

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PRELIMINARY STUDIES ON THE PHYSICOCHEMICAL PROPERTIES OF CHITOSAN MEMBRANES

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The main purpose of this study is to analyze the physicochemical properties of chitosan-based membranes. The cationic nature of chitosan is primarily responsible for electrostatic interactions with negatively charged molecules leading to his applications in environmental issues, tissue engineering, drug delivery systems, etc. [1]. Chitosan is a functional linear polymer derived from chitin consisting of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units link with (1–4) bonds. We used this natural polysaccharide because of its low cost, large-scale availability, antimicrobial activity, low toxicity, biodegradability and biocompatibility [2,3]. In this study we prepared, at different temperatures, two types of membranes, one containing 2% chitosan and the other chitosan and glycerol. In order to overcome some of the disadvantages of pure chitosan membranes, such as poor mechanical property, solubility in acidic conditions and severe shrinkage, we chose glycerol, one of the most used plasticizer [4]. In order to characterize membranes in certain conditions, we determined porosity and we also conducted swelling and degradation studies at different pH values. From the results obtained we noticed that the properties of the chitosan-glycerol membrane were slightly enhanced comparative with the chitosan membrane.

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EXTRACTION AND ANTIBACTERIAL ACTIVITY OF NATURAL COMPOUNDS FROM WALNUTS (*JUGLANS REGIA*)

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Introduction: Walnut (*Juglans regia*) is grown primarily for its fruits, which are consumed both fresh and processed. Kernel compounds containing a wide range of important natural compounds for human organism, the most important being polyphenols. This class of natural compounds have antibacterial activity and show a great medical interest especially in the field of pharmacy.

Methods: Extracting polyphenols from walnut kernels was done in absolute ethanol solvation. Extracted natural compounds in ethanol were tested to determine by microbiological techniques their antibacterial and antifungal activity.

Discussion: By measuring the diameter of the zone of inhibition around the discs loaded with the extract of *Juglans regia*, we found that the strongest effect antimicrobial proved the *Streptococcus pyogenes* (11 mm) and *Staphylococcus aureus* (10 mm), followed by *Proteus mirabilis* (9 mm) *Candida albicans* (9 mm) and *E. coli* (7 mm). The alcoholic extract of *Juglans regia* was completely ineffective at the tested concentration against *Pseudomonas aeruginosa* to other microbial strains.

Conclusions: Although antibiotics have excellent antibacterial effects, use widely in time lead to the emergence of resistant strains difficult to control. Therefore the use of herbal extracts with antibacterial effects can inhibit the multiplication of microorganisms that means a protective effect against infectious diseases.

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DE NOVO PEPTIDE DESIGN FOR ENHANCED HEAVY METAL ACCUMULATION

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Using computational and bioinformatical methods we investigate here the structural motifs and tridimensional architectures that favor stable chelation of metal ions, especially for 3d, 4d series transitional metal ions, and propose engineered peptides that enhance metal hyper accumulation.

In coordination with experiment this study is focused on achieving both a higher understanding of the structural basis involved in metal ion chelation processes, but also to questing for optimizing the design of engineered peptides and proteins able to hyper accumulate metal ions in yeast without affecting too aggressively their viability and provide augmented resistance for this type of environmental pollutants.

This computational driven experimental approach aims at improving existing technologies for bioremediation and bioextraction of contaminated soil and water using transgenic organisms that exhibit enhanced heavy metal accumulation behavior.

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ASSESSING THE PRESENCE OF *NIC*-GENES IN *ARTHROBACTER SP.* AK-YN10

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Introduction: The pAO1 megaplasmid of *Arthrobacter nicotinovorans* is responsible for the bacteria's ability to degrade nicotine due to an encoded *nic*-gene cluster. pAO1 shows only low levels of sequence similarity with other *Arthrobacter* plasmids, but shares most of its nic-genes with other *Arthrobacter* strains including *Arthrobacter sp.* AK-YN10. The objective of this study is to identify whether *Arthrobacter sp.* AK-YN10 strain contains any nic-genes and to establish the location of these genes. For this, plasmid DNA was isolated from both *Arthrobacter sp*. AK-YN10 and *A. nicotinovorans* pAO1 strains and the presence of a *nic*-genes was assayed by PCR.

Materials and Methods: *A. nicotinovorans* pAO1+ (strain ATCC 49919) and *Arthrobacter* AK-YN10 were grown on citrate medium supplemented with nicotine 0,05% on a rotary shaker at 280C/190 rpm. Total DNA was isolated from the cells of the two strains of *Arthrobacter* using a protocol based on phenol-chloroform extraction and isopropanol precipitation (Anderson & McKay, 1983). The isolated DNA was further separated by agarose gel electrophoresis and the plasmid DNA was extracted using a Zymoclean™ Gel DNA Recovery Kit. The presence of the 6hlno gene encoding a key enzyme in the nicotine degradation pathway of pAO1 was assayed by PCR using the following primers: HindIII6hlnofw (5'AAGAAGGAAAGCTTTGTATGACGCGA3'); KpnI6hlnorev (5'GTCTGCAGGTACCTACGAATGCAG3').

Results and Discussions: Total DNA preparations enriched in plasmid DNA were produced using the previously described protocol. A high molecular weight DNA band, corresponding to plasmids was identified by electrophoresis. Plasmid DNA was cut from the gel, purified and used as a template for PCR analysis. The 6hlno gene was successfully amplified by PCR suggesting that at least one gene involved in nicotine degradation pathway located on pAO1 is also located on *Arthrobacter* AK-YN10 plasmid.

Conclusions: *Arthrobacter sp*. AK-YN10 contains at least one plasmid on which is located the *6hlno nic*-gene.

Key words: nicotine, *Arthrobacter*, plasmid DNA

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CELLOBIOSE DEHYDROGENASE BASED SCREENING SYSTEM FOR DIRECTED EVOLUTION OF CELLULASE FROM *TRICHODERMA REESEI*

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Cellulase (EC 3.2.1.4) are important enzymes in food, paper, textile, detergent and biofuel industries. Most cellulases have low activity and stability. Improving these properties would have substantial impact on numerous industrial processes. Enzymatic properties can be improved by directed evolution, but the screening process is the limiting step. Coupled cellulase assay has been developed in order to improve the screening process. This method does not require boiling samples and allows rapid screening of mutants in a microtiter plate. The aim of this study was to establish enzyme coupled assay where cellulase first hydrolyzes carboxymethylcellulose (CMC), and cellobioses dehydrogenase (CBDH) and dichlorophenolindophenol (DCPIP) is used subsequently for detection of reducing ends.

Cellulase gene (wt) derived from *Trichoderma reesei* was cloned in the pESC-TRP vector, and expressed in the yeast *S. cerevisiae*. Obtained heterologous protein is used to optimize enzymatic assay conditions, including pH optimum, CMC concentration, and CBDH amount. Mutants were obtained using semi-rational design, and analyzed by previously optimized assay. Selected mutants showed increased cellulase activity. Cellulase gene (wt) has been recloned also in pCTcon2 vector and the library was created by introducing random mutations using error prone PCR. Gene library was scanned with above-mentioned assay and mutants with higher cellulase activity were selected.

Cellulase was successfully produced in *S. cerevisiae*, and both libraries yielded mutants with increased cellulase activity. Developed assay allowed us a quick and efficient way of scanning aforementioned gene libraries.

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SEMI RATIONAL DESIGN OF CELLOBIOSE DEHYDROGENASE FROM *PHANEROCHAETE CHRYSOSPORIUM* FOR INCREASED OXIDATIVE STABILITY

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Cellobiose dehydrogenase (CDH, EC 1.1.99.18) from *Phanerochaete chrysosporium* belongs to a group of oxidoreductases and has the ability to degrade different components of woody plants. CDH is secreted by wood degrading, phytopathogenic and saprotrophic fungi and this widespread appearance implies hers important function and makes her an important enzyme for applications in industrial and biotechnological processes, as well as biosensors and biofuel cells. Cellobiose dehydrogenase is also used in industry for bleaching cotton and in food industry for lactose detection. CDH is monomeric enzyme consisting of two domains, flavin domain containing FAD as cofactor and smaller hem b containing cytochrome domain, connected via flexible linker. Physiological role of CDH is reflected in the degradation of cellulose and lignin in cooperation with other cellulolytic enzymes, because CDH catalyzes oxidation of celobiose (Glc – β – 1,4 Glc) and other β – 1,4 – linked disaccharides and oligosaccharides to the corresponding lactons. Enzymes used in biosensors and for bleaching cotton should have high stability, especially toward reactive oxygen species. In order to improve oxidative stability of CDH, we have mutated CDH and tested its stability in presence of hydrogen peroxide. After successful cloning of the CDH gene in pYES2 vector, saturation mutagenesis was used to make library mutants where tree methionine residues were mutated. Residual activity of mutants was measured after the enzyme incubation in 0.3 M hydrogen peroxide for 0, 2 and 6h. After analysis of large number of mutants, it was observed that three mutants are showing higher oxidative stability compared to the wild – type enzyme. Residual activities of these mutants after 6 hour incubation in the hydrogen peroxide were over 50%, whereas wild-type has 30%. Selected mutants were expressed in *S.cerevisiae* and purified on DEAE column. Purity and activity of the enzymes were detected on the electrophoresis gel, oxidative stability of purified mutants was measured once again and characterization of these mutants was done. Mutants showing increased oxidative stability were sequenced and we have decided to combine these mutations with each other in order to make combined mutants that will be tested for oxidative stability.

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CLONING, EXPRESSION AND PURIFICATION OF RECOMBINANT B-1,3-GLUCANASE, ALLERGEN FROM BANANA, IN THE PROKARYOTIC EXPRESSION SYSTEM

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Banana (*Musa acuminata*) is an important fruit in human nutrition, and despite of positive effects, it has been identified as a food allergen source. There are six IgE reactive proteins, among them *β*-1,3-glucanase, which was identified as a candidate for the component-resolved allergy diagnosis. Replacement of food allergen extracts with a panel of recombinant molecules from a particular allergen source is a promising strategy for the improvement of allergy diagnosis.

The purpose of this study was to produce recombinant *β*-1,3-glucanase in *E. coli*. The strategy was to produce the protein with 6His tag at the C terminus. Gene for *β*-1,3-glucanase was cloned into pET-23b vector and protein was expressed in BL21 (DE3) *E. coli* cells. Recombinant protein was purified by metal affinity chromatography and ion exchange chromatography (patent P-2015/0783A1). Purified protein was characterized by CD spectroscopy, 2D PAGE, size exclusion chromatography, and complete amino acid sequence was confirmed by de novo sequencing using mass spectrometry. *β*-Glucanase activity was tested in a reducing sugar assay using laminarin as substrate.

Recombinant *β*-1,3-glucanase cDNA encodes a protein with molecular mass of 35 kDa and calculated pI 6.14. By mass analysis of in-gel tryptic digest, the complete cDNA sequence of recombinant *β*-1,3-glucanase was confirmed on the protein level. Experimentally obtained CD dataset was evaluated by K2D3 method for prediction of protein secondary structures which determined 77.2 % of α helix and 2.8 % of *β* strand in the recombinant *β*-1,3-glucanase. The CD spectrum is similar to the CD spectrum of barley β-1,3-glucanase.

Recombinant *β*-1,3-glucanase is a homogenous protein with the expression yield of about 20 mg per liter of the cell culture. Recombinant *β*-1,3-glucanase reveals glucanase activity (7.84 IU/mg) of protein indicating a correct protein folding. As a novel reagent it should be tested in the component-resolved allergy diagnosis of banana allergy.

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PREPARATION OF MACROPOROUS PARTICLES BASED ON GLYCIDYL METHACRYLATE AND ETHYLENE GLYCOL DIMETHACRYLATE FOR THE ENZYME IMMOBILIZATION

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Macroporous particles consisting of glycidyl methacrylate (GMA) crosslinked with ethylene glycol dimethacrylate (EGDMA) were synthetized by the dispersion polymerization. The prepared particles were 1.5 to 2.5 μm large. We investigated the influence of different reaction conditions on properties of the macrporous particles. Higher conversions and therefore, yields were obtained when the amount of crosslinker EGDMA in the initial reaction mixture was increased. Longer reaction times provide higher yields, bigger particles and more narrow particle size distributions. Porosity and the pore size are influenced by the reaction time as well, but also by the amount of EGDMA in the initial reaction mixture and the presence of water in the reaction medium. Higher porosity and larger pores were obtained with the increased amount of crosslinker in the reaction mixture, whereas the water content and reaction time have the opposite effect on the pore size and porosity: the porosity increases and the pore size decreases with the lower water content in the reaction medium and shorter reaction times. The obtained particles were modified by treating the epoxy group of glycidyl methacrylate with ammonia solution and gluteraldehyde. These modified particles can be used for the enzyme immobilization.

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MODIFICATION OF CARBOXYMETHYLCELLULOSE WITH PHENOLS FOR PEROXIDASE INDUCED HYDROGELS FORMATION AND ELECTROSPINNING

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Carboxymethylcellulose (CMC) is water-soluble cellulose ether which is used in food and cosmetics industry. It also has big potential for use in pharmaceutical products due to its high biocompatibility, biodegradibility, low immunogenicity and low price. Crosslinked CMC can absorb large amounts of water and swell to form hydrogels with great physical properties. The need for new biomaterials and hydrogels is growing daily, due to their use in tissue engineering, drug delivery and cell and enzyme immobilization studies. In this study we modified CMC, in order to get a crosslinkable polymer that can make hydrogels by chemical and enzymatic means. After periodate oxidation of CMC we obtained CMC with different degrees of oxidation: 2.5, 5, 10, 15 and 20 mol%. Further modification using reductive amination in the presence of different phenolic compounds like tyramine, was done. This modification of CMC was confirmed by UV–VIS and FT-IR spectroscopy, while concentration of phenol and ionizable groups was determined using absorbance at 275 nm and acid–base titration. All CMC-tyramines were able to form hydrogels after cross-linking with horse radish peroxidase (HRP) and hydrogen peroxide. CMC derivatives have been successfully electrospun and crosslinked afterwards. Due to the introduction of amino groups and decrease in molecular weight, they were significantly more soluble in water up to 30 % (w/w) compared to native polysaccharides and their electrospinability also improved. We aim to make nanofibers using tyramine-polysaccharides that will be more stable in cell culture media after cross-linking covalently and with calcium/barium ions. Diameter of nanofibers was determined by scanning electron microscopy (SEM). Cross-linked nanofibers that we obtained will be used for tissue engineering of blood vessels.

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EVALUATION OF SPECIFIC IMMUNE RESPONSE AFTER IMMUNIZATION OF MICE WITH DER P 2 AND DER P 2-HEMAGGLUTININ CHIMERA CONJUGATED TO GOLD NANOPARTICLES

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Allergen specific immunotherapy (ASIT) represents the only curative and specific way for the treatment of allergic diseases, which have reached pandemic proportions in the industrialized countries. ASIT is performed with allergen extracts that can cause severe, even life-threatening reactions, but also new IgE sensitizations to other allergens present in the extract. Such obstacles can be circumvented by replacement of poor-defined allergen extracts with recombinant allergens. However, recombinant allergens have poor immunogenicity, and that disadvantage could be overcome by using suitable carriers. As gold nanoparticles are biocompatible and can be easily functionalized with antigens, they have been proposed as possible carriers for the delivery of antigens. The study of gold nanoparticles (GN) in vaccine research has been of interest in recent years and important advances have been made. In this study, gold nanoparticles (GP) modified with allergen Der p 2 and chimera composed of Der p 2 and hemagglutinin H1 from human influenza virus (N1H1) were tested as carriers for mice immunization.

Recombinant Der p 2 (D2) and construct Der p 2-hemagglutinin (H1D) were expressed in E. coli, refolded and purified by IMAC chromatography. After purification of the recombinant proteins, endotoxin was removed and than they were used for conjugation with gold nanoparticles, previously synthesized by the citrate method. Conjugated GN-D2 and GN-H1D were used for immunization of mice. Specific IgG and IgG2 was induced in sera of mice after immunization with antigens conjugated for gold nanoparticles.

Gold nanopartiocles seems to be a suitable delivery system for allergen-specific immunotherapy.

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BIOREMEDIATION OF WATERS POLLUTED WITH HEAVY METALS

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Due to intensive industrial work in mines from Banat region, a lot of soil and water is contaminated with heavy metals. Separation of these metals from soil or water is an important ecological task.

A lot of methods are studied, among them the use of different biochemical or biological materials. Biopolymers can bind heavy metals by different physical or physico-chemical mechanisms [1, 2]. Also a lot of plants or parts of plants such as sweet water algae can bind heavy metals [3].

In our work we describe a preliminary retention of copper by ionic exchange on alginate beads coupled with advanced retention on Spirogyra or Chladophora entrapped in carrageenan. This continuous retention in colons, in two steps, coupled also with in situ regeneration of alginate could be an attractive method for advanced bioremediation at large scale.

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