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Danielle da Silveira dos Santos Martins et al., J Biotechnol Biomater 2017, 7:1(Suppl)
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Lactic acid production from hemicellulosic fraction of sorgum bagasse by *Lactobacillus pentosus*

Danielle da Silveira dos Santos Martins, Ludmila de A N Viana, Elcio R Borges, Edmar da M Penha and **Nei Pereira Jr**
Federal University of Rio de Janeiro, Brazil

Among the various renewable feedstocks available for bioproducts synthesis, the sweet sorghum stands out as one of the most promising due to its wide adaptability to different types of climate and soil. Furthermore, it is the only crop that provides stalks and grains which can be used in the food industry, and the exceedance biomass can be used such as second generation organic acids. Most organic acids on the market are produced via chemical synthesis with high levels of pollution. The production of organic acids of second generation is inserted in the biorefinery context, advancing towards emerging future technologies. In this context, lactic acid is considered a commodity with multiple industry applications, as well the polylactic acid (PLA) syntesis. The aim of this work was the preliminary study of the lactic acid production from hemicellulosic fraction of sorgum bagasse by *Lactobacillus pentosus*. Initially, the bagasse was submitted to a pretreatment with diluted acid to fractionate and extract the hemicellulose component from the solid residue named cellulignin. Batch fermentation experiments were performed under the principles of the statistical methodology of response surfaces to define the optimum process conditions- inoculum, xylose and KH_2PO_4 concentrations- under the 37°C, 120 rpm. The experiments were performed until a statistical model to study the effect of several variables and to seek optimum conditions for a multivariable system. The *Lactobacillus pentosus* strains exhibited increased ability to uptake and ferment xylose, reaching until 20 g/L lactic acid production.

Biography

Danielle da Silveira dos Santos Martins has completed her PhD in Sciences from the Post-graduation Program on Technology in Biochemical and Chemical Process from Federal University of Rio de Janeiro, Brazil. She is a Professor at Federal University of Rio de Janeiro, in Biotechnology with emphasis on Industrial Microbiology and Fermentation. She participated in theoretical-experimental projects development, scientific research and development in bioprocesses. Her subjects of research includes "The process development aimed at biofuels production, enzymes, biosurfactants, organic acids, waste treatment and industrial effluents and molecular biology techniques development".

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Notes:

Biorefinery safety: The role of INAIL in Italy

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The Italian Workers Compensation Authority (INAIL) research in the biotech field is focused on occupational safety in biotech plants and the promotion of industrial applications in the perspective of environmental remediation and sustainable development according to the framework of the European legislation. Existing lessons on safety public concern of biotech plants suggest that the development of effective, responsive and responsible safety standard can improve the trust of the public opinion and affected industries in biotech and in the new generation plants (biorefinery). The first step should be replacing the current retrospective risk-based paradigm for governing biotechnology with a proactive safety paradigm applied early in the design process. Although the environmental and health risks posed by bioprocesses for the valorization of biomass are usually expected to be lower than the traditional chemical and petrochemical processes, there is still a lack of information about safety aspects of biorefinery plants, especially for novel bioprocesses under development. In 2014 INAIL carried out a research project on biorefinery safety through the investigation of some industrial plants in Italy. Furthermore, INAIL has a partner in the Circular Economy European project RES URBIS mostly focusing to convert urban bio-waste into valuable biobased products like polyhydroxyalkanoate (PHA) based bioplastics and bio-based solvents and fibers in an integrated single biowaste biorefinery and by using one main technology chain. As part of the RES URBIS project, the role of INAIL is to study the occupational risk for the workers who are involved in the production process of PHA from bio-waste.

Biography

Pietrangeli B was a Biologist at the Sapienza University of Rome in the year 1983 and Specialist in Hygiene and Public Health (1986). From 1988 to 1995, she was a Researcher at Enitecnologie (Italy) for the development of biotech processes for the disposal and valorization of industrial wastes. Since 1995, she is a Researcher at the Italian Institute for Occupational Health and Safety (currently INAIL) and is responsible for research projects in remediation of contaminated sites and biorefineries in relation to the safe application of biotech processes. Since 2008, she is an Adjunct Professor at the Sapienza University of Rome. She has published about 100 papers in national and international journals.

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Notes:

Solmaz Agha Amiri et al., J Biotechnol Biomater 2017, 7:1(Suppl)
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Construction, expression and characterization of a cancer-specific fusion protein targeting CD22 in B-cell malignancies

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Dual-function proteins are a new class of therapeutics that composed of an antibody or antibody fragment linked to a cytotoxic molecule to facilitate the targeted delivery and destruction of malignant cells. CD22 is a highly internalizing B-cell specific surface antigen which overexpressed in 60%-80% of different types of B-cell malignancies. Therefore, anti-CD22 antibodies are ideal candidates for targeted intracellular delivery of antitumor agents. Apoptin is a small 13KDa protein which can induce apoptosis in tumor and transformed cells but not in normal cells. Hence, the apoptin protein can be used as a toxic moiety in development of cancer -specific fusion proteins. In this study, we generated a novel dual function protein by fusing apoptin to the C-terminus of a humanized anti CD-22 scFv; the anti-CD22 scFv portion of the protein targets the whole molecule to the tumors, while apoptin executes specific killing functions. Using the routine molecular methods, the recombinant anti-CD22 scFv-apoptin protein was expressed in *E. coli* and then purified. The *in-vitro* binding analyses by immunofluorescence and flow cytometry demonstrated that the anti-CD22 scFv specifically bind to Raji CD22 positive cells and almost not to Jurkat CD22 negative cells. Evaluation of apoptotic property of anti-CD22 scFv-apoptin using flow cytometry showed that following specific binding of anti-CD22 scFv-apoptin, the protein induced apoptosis significantly in Raji cells ($p<0.05$). In conclusion, we have successfully produced functional anti-CD22 scFv-apoptin in *E.coli*. This recombinant protein may offer a new opportunity for the treatment of CD22+ B-cell malignancies.

Biography

Solmaz Agha Amiri is currently pursuing her PhD in the field of Pharmaceutical Biotechnology at Shahid Beheshti University of Iran. She has published five papers in reputed journals.

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Notes:

Tuba Sevgi et al., J Biotechnol Biomater 2017, 7:1(Suppl)
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Antimicrobial activities of seed extracts of *Prunus persica, Prunus cerasus, Prunus avium* and *Prunus armeniaca*

Tuba Sevgi and **Elif Demirkan**
Uludag University, Turkey

Fruits contain phenolic additives which may show more or less antimicrobial effects. Depending on their antioxidant properties phenolic substances, which have effect mostly on color, flavor and durability of fruits and vegetables, are closely related with human health in terms of antimicrobial, anti-carcinogenic and anti-mutagenic activities. In the long term, bacterial resistance against antimicrobial agents may cause problems in fighting against several diseases. Therefore investigation of novel antimicrobial agents derived from new and natural sources have become important. In this study, seeds of some fruits (*Prunus persica, Prunus cerasus, Prunus avium and Prunus armeniaca*) were investigated against some clinically substantial pathogenic bacteria (*Salmonella typhimurium ATCC 14028, Klebsiella pneumoniae ATCC 700603, E. coli ATCC 25922, E. coli ATCC 35218, Staphylococcus aureus ATCC 25923, Yersinia enterocolitica ATCC 9610 ve Enterococcus faecalis ATCC 29212*) by using well diffusion method. For this purpose, seeds from fruits were crushed into small pieces and, were extracted by Soxhlet using methanol as solvent. After incubation, the highest zone diameter (15 mm) was obtained against *E. faecalis* in the sample of P. *cerasus* seed, and also against *S. aureus* in the sample of *P. armeniaca* seed (14 mm). Hence, this shows that the products can be potential new antimicrobial agents pharmacologically.

Biography

Tuba Sevgi has completed her MSc from Technical University of Kaiserslautern in Molecular Biotechnology and Systems Biology, Germany. Currently, she is doing her PhD in the Department of Biology, Faculty of Arts and Sciences, Uludag University. She is a Research Assistant in the Department of Biology, Faculty of Arts and Sciences, Uludag University.

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Optimization of catalase activity by *Rhodotorula glutinis* using experimental design

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Rhodotorula glutinis is a pigmented, salt tolerant yeast and also gains attention due to its oleogenic property. It has high capacity to produce antioxidant molecules such as carotenoids. However, limited research has been conducted on the synthesis of other antioxidant molecules such as catalase (CAT) enzyme. CAT is a heme protein that is present in animal cells, bacteria and plants and it decomposes hydrogen peroxide to water and oxygen (Eqn. (1)). It is widely used in various industrial areas such as textile, food and cosmetics.

$2H_2O2 \rightarrow O_2 + 2H_2O$ (1)

The aim of this study is to investigate the parameters that provided the optimum conditions for high CAT activity by Rhodotorula glutinis, and to search for the potential utilization of glycerol as a carbon source for high CAT activity, which is a by-product of biodiesel plants. For this aim, central composite design (Design Expert 7.0.0) including 20 runs with 6 central points was performed and temperature (T^oC) (10.6-32.4^oC), initial medium pH (pH) (3.99-6.0) and glycerol concentration (Gly, gL^{-1}) (9.77-60.23) were selected as factors to be optimized for the response, CAT activity, according to the previous findings of the research group. The following second order model (Eqn. (2)) was proposed:

$$CAT\ (U) = -4.36106 + 1.34381(pH) + 0.051575(T) + 0.028907(Gly) - 0.13551(pH)^2 - 3.78071 \times 10^{-4}(Gly)^2 \quad (2)$$

The model was found to be statistically significant (R2=0.94, R2adj=0.92, model F value 40.95, lack of fit value 2.21). The most effective factor on CAT activity was found as temperature ($p<0.0001$). The response surface graphics are presented at Fig. 1. Fig. 1a was obtained when Gly was 37.03 mg mL^{-1}. According to the figure, the highest CAT activity was obtained at high T and low pH values. Similarly, Fig. 1b showed that activity increased with increasing T however, medium values of Gly provided higher activity values, maximum at 37.5 mg mL^{-1}. According to Fig. 1c, the highest CAT activity values were obtained at the medium values of both pH and Gly. As a result of the experiments, it was found that determination of the maximum predicted response required a shift of the experimental region to higher temperature values.

Biography

Ayse Ezgi Unlu has expertise in enzymes, enzymatic reactions, fermentation, protein synthesis, proteomics, enzymatic biopolymers and green solvents. The synthesis of Naproxen, a member of NSAIDs, was the subject of her Master's thesis by using commercial lipase subjected to various pre-treatment strategies that enhanced the activity. Investigation of different parameters on the production of lipase by *Candida rugosa* and also proteomic analysis of the isoenzymes was another subject of her interest. She has done her Post-doctoral research on the synthesis of flavonoids using green solvents.

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Notes:

The role of liver CYP1A1 and CYP2E1 enzyme activities and lipid peroxidation level in diabetic rats

Gökçe Kuzgun, Rahman Başaran, Ebru Arioglu Inan and **Benay Can Eke**
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Diabetes mellitus is one of the most common metabolic disease in which pancreas no longer produce enough insulin or the body cannot use it efficiently. Many studies have implicated that the increased oxidative stress is associated with the progress of diabetes and diabetic complications. Cytochrome P450 monooxygenases are one of the sources of reactive oxygen species in diabetes and lipid peroxidation may occur as a result of oxidative damage. The increased lipid peroxidation may cause cellular retardation, abnormality of blood coagulation, hypertension and cardiovascular disease in diabetic patients. The expression of CYP450 enzymes may be affected by various pathophysiological conditions such as diabetes, hypertension and cancer. It has been reported that the expressions and activities of CYP1A1, CYP2E1 and other drug metabolizing enzymes alter in diabetes. In this study, we used streptozotocin-induced diabetic rats, insulin treated streptozotocin-induced diabetic rats and control group to investigate how diabetes affects liver CYP1A1 and CYP2E1 enzyme activities and lipid peroxidation level. We observed that insulin regulates liver CYP1A1 and CYP2E1 activities and lipid peroxidation level in rats.

Biography

Gökçe Kuzgun has done her graduation from Hacettepe University, Faculty of Pharmacy in 2012. She also works as a Junior Patent Examiner at the Turkish Patent Institute. Currently, she is doing her Master's degree in the Department of Pharmaceutical Toxicology at Ankara University.

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Notes:

Novel human indoleamine 2,3-dioxygenase inhibitors form a long-lived complex with the enzyme

Julie Alexandre, Michael Swan, Mike Latchem, Dean Boyall, John Pollard, Stuart Hughes and **James Westcott**
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Human indoleamine 2,3-dioxygenase 1 (IDO) catalyzes the conversion of L-tryptophan (L-Trp) to N-formylkynurenine through a heme and O_2-dependent oxidation process. IDO is recognized as a central regulator of immune responses in a broad variety of physiological and pathological settings and is thus considered an attractive therapeutic target. In search of novel IDO inhibitors, we identified 4-amino-1,2,3-triazoles. Using crystallographic, biochemical and spectroscopic techniques we have fully characterized a representative molecule of this molecular series (VIDOi1) and shown that: VIDOi1 is non-competitive for D-Trp; VIDOi1 interacts with the IDO heme iron VIDOi1 binds to both the ferric and the ferrous form of the enzyme; VIDOi1establishes a slow complex with the ferrous form of IDO; and the VIDOi1-IDO complex is long-lived. The generation of this tight binding complex between IDO and the 4-amino -1,2,3-triazoles leads to exceptional potencies of this molecule series in a cellular context.

Biography

Julie Alexandre is specialized in Kinetics at the Vertex Pharmaceuticals Europe Ltd., (Abingdon, UK). She holds a PhD in Biochemistry from the University of Edinburgh (Scotland, UK) and undertook Post-doctoral Research in Enzymology at the Pierre-and-Marie-Curie University (Paris, France). She has been working at Vertex since 9 years and has contributed in many internal drug discovery efforts in oncology, targeting kinases, proteases and redox enzymes, through characterization of enzymes substrates and inhibitors kinetics and mode of action.

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Notes:

Mohammed F. EL-Shiekha, J Biotechnol Biomater 2017, 7:1(Suppl)
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Molecular study on the potential therapeutic activity of novel nanocomposite on cancerous tumor bearing mice

Mohammed F. EL-Shiekha
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Nanoparticles are making significant contributions in the development of new approaches of drug delivery in cancer and can provide a platform for combined therapeutics with subsequent monitoring of response. Basic curcumin and zinc oxide (ZnO) nanocomposites modified with vitamin C and CTAB have been exerting chemo-preventative activity against cancer in mice animal model. The present results observed that nanocomposite have distinct effects on liver cell viability via killing cancer cells, while posing no effect on normal cells (hepatocytes). The marked difference in cytotoxicity between cancer cells and normal cells suggests an exciting potential for nanocomposite as novel alternatives to cancer therapy. Our molecular data showed that both mRNA and protein levels of tumor suppressor gene *p53 were* upregulated and induce activity of DNA fragmentation in liver cells.

Biography

Mohammed F El-Shiekha has completed his PhD from the Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt. He is a Faculty Member in the Department of Biochemistry and pharmacy in October 6 University, Egypt. He has published 6 papers in reputed journals.

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Ameliorative effect of some natural products on hepatic and renal functions in female mice bearing cancerous tumor

Mohammed F. EL-Shiekha
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Curcumin and tannic acid which are naturally occurring dietary polyphenols, have exerted and found to be chemo-preventative against cancer in various animal models. This study was carried out on 220 (12-14 weeks old, 25-30 g each) female mice. Mice were classified into two main large experiments. Experiment 1: Non-tumor bearing mice (NTB) included 100 animals and divided into four groups, each one comprised 25 mice. Group 1: NTB- control saline treated. Group 2: NTB-treated with curcumin orally (350 mg/kg/day) for 6 weeks. Group 3: NTB-treated with tannic acid orally (160 mg/kg/day) for 6 weeks. Group 4: NTB-treated with curcumin and tannic acid orally at ratio (50%:50%) for 6 weeks. Experiment 2: Tumor bearing (TB) mice. The total 120 animals were divided into four groups, each one comprised of 30 mice. Group 1: TBM-control saline treated. Group 2: TBM-treated with curcumin orally (350 mg/kg/day) for 6 weeks. Group 3: TBM-treated with tannic acid orally (160 mg/kg/day) for 6 weeks. Group 4: TBM-treated with curcumin and tannic acid orally at ratio (50%:50%) for 6 weeks. Blood samples were collected from all animal groups after 2, 4 and 6 weeks from treatment. Serum were separated and processed directly for glucose, insulin, total cholesterol, triacylglycerol, total protein determination. The obtained results revealed that, a highly significant decrease in serum glucose, total cholesterol, total protein concentration, meanwhile, a highly significant increase in serum triacylglycerol concentration was also observed. But a non-significant decrease in serum insulin levels were observed in tumor bearing mice when compared with control. The results of this study indicated that curcumin, tannic acid and their combination treatment have potential benefits in cancer treatment.

Biography

Mohammed F El-Shiekha has completed his PhD from the Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt. He is a Faculty Member in the Department of Biochemistry and pharmacy in October 6 University, Egypt. He has published 6 papers in reputed journals.

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Notes:

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Catabolic route for 3-guanidinopropionic acid utilization by *Aspergillus niger*: Involvement of 4-guanidinobutyrase

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Aspergillus niger is a metabolically versatile filamentous fungus that utilizes various guanidinium compounds as nitrogen source. The fungus utilizes 4-guanidinobutyric acid (GB), whereas its lower structural homologue 3-guanidinopropionic acid (GP) is very poorly metabolized. The enzyme 4-guanidinobutyrase (GBase) facilitates GB catabolism in this fungus. There is no specific 3-guanidinopropionase (GPase) in *A. niger* but the purified GBase itself exhibits low GPase activity. Based on these observations we hypothesized that the inability of the fungus to mobilize GP as a nitrogen source is because GP is a poor GBase substrate. Two strategies were employed to test this; one was to increase the mycelial GBase levels and tailoring the GBase specificity towards GP was the second approach. A constitutive expression of GBase in *A. niger* resulted in normal growth on GP indicating that intracellular GBase levels essentially limit GP utilization in this fungus. There was a direct correlation between growth on GP and cellular GBase levels. In the second approach, altering GBase substrate specificity was attempted. *A. niger* spores were exposed to ethyl methane sulfonate (EMS) and the mutants were selected through differential growth on GP versus GB. One mutant that better utilized GP than the parent strain was selected and analyzed. Neither an increased GBase activity nor a specific GPase activity was observed in this mutant. Furthermore, no mycelial GPase activity was detected when the mutant was grown on GP. The presence of urea in the spent media when the mutant was grown on GP however implicates a GPase. The possibility of an alternate route for GP catabolism, not involving a GBase needs further study.

Biography

Tejaswani Saragadam is an Integrated MSc-PhD student working under Professor N S Punekar at IIT Bombay. She is working on the aspects of enzymology and metabolism in *Aspergillus niger*, an industrially well-known fungus for citric acid production and various enzymes. Understanding the nitrogen metabolism in this fungus and studying new pathways and enzymes involved in nitrogen metabolism forms her major work. Further characterizing these enzymes and understanding their role in the novel metabolic pathways forms the basis of her study.

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Notes:

Molecular characterization of glutathione transferase M1-1 from the *Camelus dromedarius*

Fereniki Perperopoulou[1], **Farid Ataya**[2] and **Nikolaos E Labrou**[1]
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Glutathione transferases (GSTs, EC. 2.5.1.18) are a large family of multifunctional enzymes, best known for their involvement in the metabolism and inactivation of a broad range of xenobiotic compounds. GSTs catalyze the nucleophilic attack of the reduced form of glutathione (γ-L-Glu-L-Cys-Gly, GSH) on the electrophilic center of a variety of compounds such as pesticides, herbicides, etc. The result of the conjugation of GSH to such molecules is the increase of their solubility and the reduction of their toxicity. GSTs could be useful tools with a variety of biotechnological applications in many fields. Many studies have been carried out exploiting the natural ability of the GSTs to interact with xenobiotic compounds in order to develop simple and selective biotechnological applications. In the present work, we report the cloning, kinetic and structural characterization of the GSTM1-1 from camel (*Camelus dromedarius*). The Cd-GSTM enzyme was expressed in *E. coli* and purified by affinity chromatography. The ligand in function of the enzyme was evaluated by measuring the ability of 47 xenobiotic compounds to bind and inhibit the enzyme activity. The inhibition potency was measured with the CDNB/GSH assay system. The IC50 value and the kinetic analysis of the compound that showed the highest inhibition were determined. The results demonstrated that the enzyme exhibits high selectivity towards the fungicide Zoxium/ zoxamide. Hence, this method can be used as an optical biosensor for the determination of Zoxium/zoxamide in environmental samples.

Biography

Fereniki Perperopoulou has studied Agricultural Biotechnology from the Agricultural University of Athens. She has done her Master's degree in Bioactive Protein Products and Technology at the Agricultural Biotechnology department of Agricultural University of Athens. Currently, she is a PhD candidate in the Department of Biotechnology at the Agricultural University of Athens, working on the protein engineering and molecular study of transferase glutathione.

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Notes:

Engineering of Tau class GSTs for the development of biosensor

Foteini M Pouliou and **Nikolaos E Labrou**
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Glutathione transferases (GSTs, EC 2.5.1.18) constitute one of the most important families of detoxifying enzymes in nature with multiple biotechnological applications. GSTs are involved in the detoxification mechanism of endogenous and xenobiotic electrophile compounds by catalyzing the nucleophilic attack of reduced glutathione (GSH) on the electrophilic center of xenobiotic compounds including pesticides. This catalytic activity is the basis for the development of enzyme biosensor for herbicide determination in environmental samples. A library of Tau class GSTs was constructed by DNA shuffling using the DNA encoding the *Glycine max* GSTs GmGSTU2-2, GmGSTU4-4 and GmGSTU10-10. The DNA library contained chimeric structures of alternated segments of the parental sequences and point mutations. Chimeric GST sequences were expressed in *Escherichia coli*, purified by affinity chromatography and their enzymatic activities towards CDNB (1-chloro-2,4-dinitrobenzene) were determined. A selected chimeric enzyme which exhibited high catalytic activity and stability was used for the development of enzyme biosensor. The inhibition potency of 47 different pesticides towards the chimeric enzyme was evaluated using activity assays. Five compounds, one insecticide and four fungicides, showed high inhibition potency (IC50) towards the chimeric GST. Kinetic inhibition studies revealed that pesticides appeared to bind at the substrate-binding region in a competitive manner with respect to the substrate. The chimeric enzyme will be immobilized and will be explored for the construction of an optical biosensor. This biosensor will be portable, easy to use, allowing the direct determination of pesticides in environmental samples.

Biography

Foteini M Pouliou is a PhD candidate at the Agricultural University of Athens since 2014. She majored in Biotechnology from the Agricultural University of Athens in 2012. She has done her Master of Science studies in 2013 focusing on the Bioactive Products and Protein Technology. Her research interests include protein engineering, enzyme and environmental biotechnology.

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Angiotensin converting enzyme inhibitory activity in the mealworm *Tenebrio molitor* (Coleoptera, *Tenebrionidae*) protein hydrolysates

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Hypertension is well known as one of the major risk factors for cardiovascular disease. The angiotensin converting enzyme (ACE) plays a key role in blood pressure regulation process. Hypertension treatment by synthetic ACE inhibitors (e.g. captopril, lisinopril, enalapril) is effective but their use can cause serious side effects, such as hypotension, cough, reduced renal function and angioedema. Therefore, research was focused on natural ACE inhibitory peptides sources such as foodstuffs and recently, also insects, promoted by the Food and Agricultural Organization of the United Nations (FAO) as a more environmentally sustainable, nutritious and functional alternative food to conventional livestock for human consumption. The purpose of this study is to investigate the ACE inhibitory activity in protein hydrolysates derived from the larval and pupal stages of the edible insect *Tenebrio molitor* (Coleoptera: *Tenebrionidae*). Each insect protein extract was hydrolyzed by the gastrointestinal enzymes (pepsin, trypsin and chymotrypsin) to simulate digestive process and compared to the crude extract. ACE inhibitory activity was measured by an indirect assay method based on the quantity of hippuric acid released by ACE from hippuryl-L-histidyl-leucine and determined by reverse-phase high performance liquid chromatography. Captopril was used as positive control and ACE inhibition degree expressed as the concentration of protein extract that inhibits 50% of ACE activity (IC50), assuming that the activity of the blank is equal to 100%. The IC50 value of captopril was 2.6x10-6 mg/mL. A significantly lower IC50 was detected after gastrointestinal hydrolysis of the protein extracts obtained from larvae (0.720 vs. 0.097 mg/mL after gastrointestinal hydrolysis) and pupae (0.484 vs. 0.132 mg/mL after gastrointestinal hydrolysis). Based on experimental data, *T. molitor* larvae represent the most promising development stage for the purification and identification of bioactive ACE inhibitory peptides, confirming the potential benefits of this coleopteran for human health.

Biography

Annarita Cito has completed her PhD in Biochemistry and Enzymology from the University of Siena (Italy) in 2010. Her dissertation investigated the role of homocysteine and some oxidative stress markers in neurodegenerative disorders (Alzheimer disease) and in autoimmune digestive disorders (such as celiac disease). She has expertise in cardiovascular disease mechanism and prevention. Currently, she is conducting research, as a Post-doctoral Researcher at CREA-Research Centre for Agrobiology and Pedology in Florence (Italy), on the evaluation of the potential use of the edible insect species *Tenebrio molitor* and *Galleria mellonella* as human diet supplement of polyunsaturated fatty acids and ACE inhibitory bioactive peptides for cardiovascular disease prevention.

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Notes:

The state of oxidative stress in the body of women living in the Sub-Aral area

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The Aral crisis is recognized as one of the global environmental problems of our time. Existing environmental trouble in the region is reflected on the health of the population in almost all areas of the Aral Sea region marked increase in the number of diseases of the endocrine, nervous, digestive and urinary systems. Numerous studies conducted by scientists of Kazakhstan shows that the health of population in recent decade' s sub-Aral area continues to deteriorate. In the period of 2014-2016 years, the research team of Karaganda State Medical University (KSMU) carried out the study of health status of population in Sub-Aral area in the medical and biological direction under the state program. The study was conducted to determine the integrated approaches in solving problems of the region, to carry out systematic monitoring of the health status of the population of Sub-Aral area and development of complex of therapeutic and preventive measures based on the results obtained. This approach provides multidirectional nature of health research not only in the zone of ecological adversity of Kyzylorda region, but also regions adjacent to Sub-Aral area, namely: Aktobe region and South Kazakhstan regions. As a result of the research, we have established higher values of indicators of oxidative stress on the markers of lipid peroxidation and DNA damage in the blood of women living in zone of ecological disaster in the Aralsk-city and Aiteke-Bi-village (Kzylorda region) and women living in the area environmental pre-crisis state Kyrgyz-village (Aktobe region) and Ulytau-village (Karaganda region), in the age group 30-39 years. The presence of elevated levels in blood markers of lipid peroxidation and DNA damage indicates the development of a general oxidative stress in the body of women surveyed and indicates the presence of most acute diseases, aggravation of chronic processes, intoxication and other pathological changes.

Biography

Kultanov B Zh has done his PhD from Kazakh Academy of Nutrition in Almaty in the year 2006. He is Head of the Department of Molecular Biology and Medical Genetics of the Karaganda State Medical University. The main focus of his research is the study of the biochemical, morphological and molecular indicators of reproductive status under the influence of physical and chemical factors. He is the author of various domestic and foreign editions of the textbooks of Biology developed in the state language and Russian languages.

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Notes:

A new approach to obtain the catalytic sites region of human sACE with correct fold and activity

Regina Affonso[1], Suelen de Barros Sampaio[1], Fagner Sant'Ana Januario[1], Larissa Miranda Pereira[2], Danielle S Aragão[2], Dulce E Casarini[2] and **Caroline Cristina Elias[1]**

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Angiotensin-converting enzyme I (ACE) is a membrane-bound that catalyzes the conversion of angiotensin I to the potent vasopressor angiotensin II. ACE is a key part of the renin-angiotensin system, which regulates blood pressure and is widely distributed throughout the body. There are two isoforms of human ACE, including the somatic ACE (sACE) present in somatic tissue and the testicular ACE (tACE) present in male germinal cells. The sACE possesses two domains, N- C- domains, with catalytic sites which exhibit 60% sequence identity. These domains differ in terms of chloride-ion activation profiles, rates of peptide hydrolysis of angiotensin I, bradykinin, Goralatide, Luliberin, substance P, angiotensina, beta-amyloid peptide and sensitivities to various inhibitors. A more detailed analysis shows that these regions are composed of HEMGH and EAIGD sequences that bind zinc ions to facilitate catalytic activity (Fig. 1). Our question is: If the synthesis of catalytic sites with corrects structure and activity could be a good model *per si* to study new drugs. The objective was to obtain the Ala^{361} a Gli^{468} and Ala^{959} to Ser^{1066} catalytic regions sACE in a structural conformation that resembles its native form. The catalytic regions were obtained from bacterial system; the expression of this protein in soluble form enables completion of the solubilization/purification steps without the need for refolding. The characterization of Ala^{959} to Ser^{1066} region shows that this has an α-helix and β-strand structure, Fig. 1b, which zinc ion (essential for its activity) binds to, and with enzymatic activity. Our conclusion is that the strategy used to obtain the Ala^{959} to Ser^{1066} region in the correct structural conformation and with activity was successful.

Biography

Regina Affonso has experience in the field of Biochemistry, with emphasis on protein in the area of molecular biology, working on the following topics: RNA extraction, RT-PCR, PCR, cloning, expression and purification of recombinant human proteins in bacterial system and cell culture. In the structural area, she is interested in: circular dichroism, fluorescence, crystallography, and bioinformatics.

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Notes:

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Accepted Abstracts

Plant tissue culture applied biotechnology provides safe, effective and sustainable active ingredients for skin care applications

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The use of plants as a source of active ingredients for health care was a common practice in all the passed civilizations, and still plant derived products have been extensively used for many types of therapeutic applications. It has been evaluated that in the last 20 years around 40% of the newly developed compounds or extracts for human health care have been natural products or derived from products of plant origin. Skin care research is constantly looking for new plant ingredients, which can be guaranteed for quality and safety to final consumers, as these need to be utilized in unregulated quantities. Unfortunately, several plant derived products can be used limitedly because it contains potential allergenic compounds, and may be subjected to environmental contaminations, such as pollution, pesticides or agrochemical residues. To bypass these limitations, plant tissue culture techniques can provide alternative solutions for producing valuable metabolites with skin care applications. These systems allow to cultivate plant cells or tissues in sterile conditions, totally independent from geographical and climatic factors, and with no risk of biological nor chemical contamination. Starting from different plant species and adopting new biotechnological approaches, we developed different types of tissue cultures, including cell, hairy root and somatic embryo suspension cultures, and used them as sources of active ingredients for skin care applications. The obtained ingredients, tested on skin cell cultures *in vitro* and on human volunteers *in vivo*, showed a wide range of cosmetic and dermatological activities, ranging from UV protection, anti-inflammation, hydration to anti-ageing.

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Isolation and characterization of a new *Bacillus thuringiensis* strain with a promising toxicity against lepidopteran pests

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Bacillus thuringiensis is an aerobic, spore-forming, gram-positive soil bacterium. This microorganism produces at the sporulation stage of its growth an intracellular crystal composed of one or more δ-endotoxins. BLB459 is a new *B. thuringiensis* isolated in the laboratory of biopesticides from a Tunisian soil sample. In the present study, we focused on the screening of *B. thuringiensis* collection of 200 strains isolated from different variety of Tunisian soils and the characterization, using RFLP and molecular hybridization, of *B. thuringiensis* strain BLB459 having a distinctive plasmid profile. *Sma*I-PFGE typing confirmed the uniqueness of the DNA pattern of this strain, compared with BUPM95 and HD1 reference strains. PCR and sequencing assays revealed that BLB459 harbored three *cry* genes (*cry30, cry40* and *cry54*) corresponding to the obtained molecular sizes in the protein pattern. Interestingly, PCR-RFLP assay demonstrated the originality of the BLB459 cry30-type gene compared to the other published cry30 genes. Insecticidal bioassays showed that BLB459 spore-crystal suspension was highly toxic to lepidopteran comparing with that of the commercial strain HD1 used as reference. Important histopathological effects of δ-endotoxins on the tested larvae midgut were detected, traduced by the vacuolization of the apical cells, the damage of microvilli, and the disruption of epithelial cells. Such results indicated the interest of the new selected *B. thuringiensis* strain BLB459 and their cry toxins in the biological control of different lepidopteran insects such as lepidopteran and the possibility of its use for the formulation of new bioinsecticides.

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Quantitative RP-UPLC analysis of quercetin in three *Grewia tenax* phenotypes

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Grewia tenax (Forssk.) Fiori. (*Malvaceae*) is commonly found in Africa, Asia and Australia. It has been used traditionally to treat various diseases. The extracts from various plants, which are expected to be safe, exhibited various biological effects, e.g., anti-oxidant, antibacterial, hepatoprotective, anti-inflammatory, anti-emetic, anti-malarial, analgesic, and anti-pyretic activities. Such effects might be attributed to the flavonoidal content of the species, e.g., quercetin. A total of 25 accessions of *G. tenax* were selected for this study from trees grown within the same geographical area. Seven morphological traits were measured for each accession. Three phenotypes were identified according to their distinct variations in leaf and stem morphology. Air dried leaves and stem were extracted separately using 80% methanol. The methanolic extracts were fractionated sequentially using petroleum ether, dichloromethane and ethyl acetate. Phytochemical analysis was carried out to detect variations in quercetin content in leaves and stems within the phenotypes. A reversed-phase ultra-performance liquid chromatography, using an ultraviolet diode array detector (RP-UPLC-UV/DAD) assay was standardized for quercetin detection and quantification in the ethyl acetate fractions. The results showed variation in quercetin contents between different phenotypes, and between leaves and stem. The highest quercetin content (14.09 mg/L) was present in stem of *G. tenax* phenotype SUST1. These results reinforce the strong phenotypic effect on the secondary chemical profile. The variability in quercetin content in *G. tenax* might be related to genotypic or parent of origin effects. The clear morphological characters variation measured in studied plants provided a good indicator to distinguish between them in quercetin contents.

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Biotransformation of inexpensive natural platform chemicals to higher value flavor compounds

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The project focuses on biotranformations of relative inexpensive natural platform chemicals derived from distillation of essential oils and non-volatile compounds to higher value flavour compounds through biocatalysis. Experimental processes using a range of enzymes (cytochrome P450s, aldo-keto reductases/alcohol dehydrogenases and carotenoid cleavage oxygenases) from various sources have been previously described and a number of high value flavour components produced from inexpensive starting materials. In this project similar processes will be used to transform platform molecules using an array of enzymes focussed around those previously described.

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Different structure-oriented design of selective butyrylcholinesterase probe and its application in drug discovery

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The two major human cholinesterases are acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8), and are very important enzymes in multiple areas such as pharmacology, neurobiology and toxicology due to their significant roles in human body and health. Although the biological function is uncertain, the BChE levels have been implicated in lipid metabolism and various human diseases such as liver damage, cirrhosis, Alzheimer's disease (AD) and liver metastasis. BChE is also responsible for detoxifying xenobiotics like organophosphates and cocaine and is a well-known biomarker for clinical diagnosis. Thus, the quantification of BChE activity and its inhibition is not only important in diseases diagnosis, but also indispensable for drug discovery. We report on the different structure-oriented design and application of a selective fluorogenic molecular probe (BChE-FP) for human butyrylcholinesterase (BChE). This probe, rationally designed by mimicking the native substrate and manipulating the steric feature of the recognition group of designed probes targeting the structural difference of the active sites for BChE and acetylcholinesterase (AChE), exhibits near-zero background fluorescence but produce remarkable fluorescence enhancement upon the catalysis by BChE in a fast biochemical reaction. To the best of our knowledge, BChE-FP is the first probe that can discriminate BChE from AChE, which is successfully applied for BChE inhibitor screening and characterization under physiological conditions, and BChE detection in human serum. These results demonstrate that this molecular probe can function as a useful molecular tool for high-throughput drug discovery against BChE-related diseases, as well as the biosensing for neuromuscular blocking agents.

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A new tRNA-assisted mechanism of post-transfer editing by aminoacyl-tRNA synthetases

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Statement of the Problem: Aminoacyl-tRNA synthetases (aaRSs) maintain fidelity during protein synthesis by attaching amino acids to their cognate tRNAs. For many aaRSs, the required level of amino-acid specificity is achieved either by specific hydrolysis of misactivated aminoacyl-adenylate intermediate (pre-transfer editing) or by hydrolysis of the mischarged aminoacyl-tRNA (post-transfer editing). Both reactions are depend on a tRNA cofactor and required translocation to the editing site located in the separate domain. In this work we have studied molecular mechanisms of editing by synthetases from two different classes: Thermus thermophilus leucyl-tRNA synthetase (LeuRSTT) from class I and Enterococcus faecalis prolyl-tRNA synthetase (ProRSEF) from class II.

Methodology & Theoretical Orientation: To investigate the mechanism of post-transfer editing of norvaline by LeuRSTT and alanine by ProRSEF, we used molecular modeling, molecular dynamic (MD) simulations, quantum mechanical (QM) calculations, site-directed mutagenesis of the enzymes and tRNA modification. The transition states of the reactions were identified.

Findings: The results support a new tRNA-assisted mechanism of hydrolysis of misacylated tRNA which directly involves two water molecules. The most important functional element of this catalytic mechanism is the 2' or 3'-OH group of the terminal adenosine 76 of aminoacyl-tRNA, which forms an intra-molecular hydrogen bond with the carbonyl group of the misacylated residue. Bonding increases the electrophilic character of the carbon atom and strongly facilitates the subsequent nucleophilic attack by water molecule.

Conclusion & Significance: Class I LeuRS and class II ProRS with a different architecture of editing site have both tRNA-assisted mechanism of post-transfer editing in which free 2' or 3'-OH group of the substrate plays a key role in hydrolysis by forming an intra-molecular hydrogen bond with the substrate amino-acid carbonyl group. Proposed editing mechanism is significantly different from those described in the literature for class-I and class-II aaRSs.

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Advances in recent enzymology

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Canonical enzymology has been carried out under the pre-requisite conditions of [S] >> [E]. However, advances in analytical instrumentation allow us to investigate enzymes systems with minute quantities of both enzymes and substrates, of very high-affinity reactions, of membrane-bound enzyme-substrate interactions, and hydrophobic environments.

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