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Posters

Inhibition of Lysyl oxidase in breast cancer cells by small-molecule inhibitors

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Lysyl oxidase (LOX) is an extracellular matrix, copper-dependent, amine oxidase that catalyzes a key crosslinking step in collagen and elastin. This enzyme has also been shown to play a role in promoting metastasis. The correlation between high LOX activity and cancer metastasis is strong enough that upregulated LOX activity can be used as a diagnostic marker for the severity of cancer in patients. β-aminopropionitrile is a known potent inhibitor of lysyl oxidase; however, this inhibitor is not selective and, therefore, cannot be used as a therapeutic agent. β-aminopropionitrile has been derivatized using aromatic sidechains and has been used to selectively target lysyl oxidase in breast cancer cells. The inhibitor LP-1-2 has been shown to reduce breast cancer cell viability with a 100 μM dose and 72-hour incubation period. The effect on cell viability increased with increasing amounts of inhibitor. The selective targeting of lysyl oxidase was verified using western blot analysis and lysyl oxidase activity assays. The activity assays showed that addition of increasing amounts of inhibitor decreased the activity of lysyl oxidase. The highest level of inhibition detected was with lysyl oxidase isolated from cells treated with 5000 μM of LP-1-2 for 3 days, which decreased the activity three-fold as compared to lysyl oxidase isolated from untreated cells.

Recent Publications

1. Johnston, K. and Lopez, K. (2017) Minireview: Lysyl Oxidase in Cancer Inhibition and Metastasis, Cancer Letters, 417, 174 – 181.
2. Oldfield R, Johnston K, Limones J, Ghilarducci C and Lopez K (2017) Identification of histidine 303 as the catalytic base of lysyl oxidase via site - directed mutagenesis. The Protein Journal. Doi: 10.1007/s10930-017-9749-3.
3. Smith M A, Gonzalez J, Hussain A, Oldfield R N et al. (2016) Overexpression of soluble recombinant human lysyl oxidase by using solubility tags: effects on activity and solubility. Enzyme Research. 2016. Doi: 10.1155/2016/5098985.

Biography

Kathryn A Johnston is a fourth–year Senior Student at California State University, Bakersfield and has worked in Dr. Lopez's laboratory for the past two and a half years. During this period, she has published three papers. Her work deals primarily with the inhibition and reduction of viability of breast cancer cells using derivatized inhibitors of β-aminopropionitrile. She has presented her work as posters and invited talks at regional meetings, as well as national meetings of the American Chemical Society.

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Notes:

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Design, construction and extracellular expression of L-asparaginase from *Dickeya chrysanthemi* in yeast

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L-asparaginase (L-ASNase) is an important enzyme used as a biopharmaceutical to treat acute lymphoblastic leukemia (ALL). Currently there are two L-ASNase approved by FDA: native and of bacterial origin, both from *E. coli* and *D. chrysanthemi*. Due to L-ASNase's immunogenic effects, it is necessary to seek alternatives such as recombinant expression in yeast. This expression system can provide extracelullar secretion and glycosilation process, which can decrease immunogenicity and facilitate downstream process. We report the construction of three different expression vectors in order to obtain extracelullar L-ASNase from *D. chrysanthemi* using eukaryotic exrpression system. *asnB* gene from *D. chrysanthemi* was cloned in pJAG-s1 plasmid in fusion with endogenous signal sequence (SS), that addresses protein to bacterial periplasm, and with or without histidine tag (His). SuperMan$_5$ yeast strain was transformed with pJAG-s-*asnB* constructs in order to be able to express the recombinant protein. Aspartic acid β-hydroxamate method was applied for activity determination of L-ASNase recombinant in culture supernatants. When both SS and His-tag were removed (expression of mature protein), protein expression and secretion process were improved considerably compared to other constructions, indicating that for this gene, additional structures added to the recombinant protein may interfere with the expression, final enzyme activity and cell secretion. Purification processes are being executed.

Biography

Brian Effer is a PhD candidate at the University of La Frontera and University of São Paulo in Cell and Molecular Biology and Biochemical and Pharmaceutical Technology areas, respectively. He has published seven papers in reputed journals and has been serving as a referee in several journals.

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Notes:

Short S et al., J Biotechnol Biomater 2018, Volume 8
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Study of the potential use of antifreeze proteins of *Deschampsia antarctica* in the cryopreservation of *Salmo salar* spermatozoa

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Cryopreservation allows to preserve genetic resources in aquatic species, such as Atlantic salmon (*Salmo salar*). However, freezing may cause cell damage affecting the sperm quality. New procedures including antifreeze proteins (AFPs) seem to improve sperm quality after cryopreservation. AFPs have the ability to bind to ice crystals inhibiting their growth, and ice recrystallization (IRI) *in vitro*. *Deschampsia antarctica* is a freezing tolerante vascular plant species (LT50 -27°C) exhibiting apoplastic antifreeze activity. We hypothesize that AFPs from *D. antarctica* favor the sperm quality of cryopreserved *S. salar* spermatozoa. The aim of this work is to evaluate cryoprotection of AFPs from *D. antarctica* in *S. salar* spermatozoa. Cryopreservation of S. salar spermatozoa has been made with a standard freezing medium (C^+) and different treatments with protein extracts (20 µg/ml) of *D. antarctica* supplemented with permeating, DMSO 1.3 M, glucose 0.3 M, and non-permeating, BSA 2% w/v cryoprotectants. Post-thawing plasma membrane integrity (PMI) by SYBR-14/PI and mitochondrial membrane potential (MMP) by JC-1 markers were assessed using flow cytometry. Thawed cells in the presence of protein extracts from *D. antarctica* without BSA maintained PMI as well as C^+ and showed significant differences respect to the other treatments. The percentage of cells thawed with protein extracts of *D. antarctica* and with cryoprotectants showed higher MMP than C^+. While, treatments without permeating and non-permeating cryoprotectants maintained a similar MMP to C^+. AFPs from *D. antarctica* showed a cryoprotective effect in *S. salar* spermatozoa and these would act as non-permeating cryoprotectant, replacing BSA in standard freezing medium.

Biography

Short S completed her Biotechnology Engineering at Universidad de La Frontera and started her Doctoral studies in Applied Cellular and Molecular Biology at the Universidad de La Frontera. She is currently TA of Enzymology, Protein Structure and Immunology at the same institution. She has published five articles in reputed journals.

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Farías J G et al., J Biotechnol Biomater 2018, Volume 8
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Characterization of antifreeze activity in apoplastic extract of *Deschampsia antarctica*

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Deschampsia antarctica Desv. is a vascular plant species that colonized maritime Antarctica exhibiting extreme freezing tolerance (-27°C). This has been associated with apoplastic antifreeze activity. Antifreeze proteins (AFPs) have the ability to bind to the growing surface of ice crystals inhibiting their growth; however, this activity has been poorly characterized in this species. Therefore, the aim of this work is to characterize the antifreeze activity of apoplastic extracts from *D. antarctica*. To understand how this plant can tolerate freezing temperatures year-around, and in order to evaluate the potential antifreeze activity of apoplastic proteins from *D. antarctica* as future applications, experiments have been developed in cold-acclimated and non-aclimated plants. To identify the best apoplastic proteins accumulation after plant cold-acclimation, apoplastic extracts were quantified every four days for 21 days of low temperature exposure. Antifreeze activity was determined by ice recrystallization inhibition (IRI), thermal hysteresis activity (TH) and ice crystal growth in dilution series of apoplastic extracts. The results indicate that the minimum IRI activity was evident in extracts with a concentration equal to 0.005 µg/µl at cold-acclimated condition, while in non-aclimated plants the IRI activity was lost at 0.05 µg/µl. At concentration equal to 2.5 µg/µl, ice crystals showed a bipyramid shape and a TH equal to 0.4°C. In conclusion, we observed that cold-acclimation increased apoplastic antifreeze activity, obtaining higher IRI but low TH in these apoplastic extracts. This high IRI is remarkable and further studies are needed to characterize the apoplastic extract to associate this activity to apoplastic antifreeze proteins which could be of interest for later studies as a cryoprotectant.

Biography

Farías J G is the Associate Professor and Director of the Chemical Engineering Department, Universidad de La Frontera. His research interest is in Pharmaceutical Biotechnology, focusing on Molecular Therapeutics and Drugs Production. He has published 51 articles in reputed journals.

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Notes:

Shahid Ullah, J Biotechnol Biomater 2018, Volume 8
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Computer aided screening of Mangrove ecosystem derived compound against Acetyl-cholinesterase

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Alzheimer's disease (AD) is considered as the most common type of dementia among older people. Almost 9 million people are suffering from AD in china and increasing with the course of time. Currently many different herbs are used for the treatment of AD including six Flavors Rehmannia Pills , Gastrodia and Uncaria Drink. It is been suggested that some Acetyl-cholinesterase inhibitors induced molecular and cellular change that directly influence AD pathogeneses. In our study literature search was perform to find Mangrove eco-system phytochemical structures by using Builder software implemented in Molecular operating environment (MOE 2009). Acetyl-cholinesterase (PDB ID 1EVE) structure with bound ligand was retrieved from protein data bank. Molecular docking was performed by triangular matcher placement method and rescore by London dG parameter. The crystal structure has bound ligand which was active against acetyl-cholinesterase. It can be concluded by docking analysis of different compounds that mangrove ecosystem compound may serve as good inhibitors against Acetyl-cholinesterase

Biography

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Efficacy of neutral and negatively charged liposome-loaded gentamicin on planktonic bacteria and biofilm communities

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We investigated the efficacy of liposomal gentamicin formulations of different surface charges against *Pseudomonas aeruginosa* and *Klebsiella oxytoca*. The liposomal gentamicin formulations were prepared by the dehydration-rehydration method, and their sizes and zeta potential were measured. Gentamicin encapsulation efficiency inside the liposomal formulations was determined by microbiologic assay, and stability of the formulations in biologic fluid was evaluated for a period of 48 h. The minimum inhibitory concentration and the minimum bactericidal concentration were determined, and the *in vitro* time kill studies of the free form of gentamicin and liposomal gentamicin formulations were performed. The activities of liposomal gentamicin in preventing and reducing biofilm-forming *P. aeruginosa* and *K. oxytoca* were compared to those of free antibiotic. The sizes of the liposomal formulations ranged from 625 to 806.6 nm in diameter, with the zeta potential ranging from 0.22 to -31.7 mV. Gentamicin encapsulation efficiency inside the liposomal formulation ranged from 1.8% to 43.6%. The liposomes retained >60% of their gentamicin content during the 48 h time period. The minimum inhibitory concentration of neutral formulation was lower than that of free gentamicin (0.25 versus 1 mg/L for *P. aeruginosa* and 0.5 versus 1 mg/L for *K. oxytoca*). The negatively charged formulation exhibited the same bacteriostatic concentration as that of free gentamicin. The minimum bactericidal concentration of neutral liposomes on planktonic bacterial culture was twofold lower than that of free gentamicin, whereas the negatively charged formulations were comparable to free gentamicin. The killing time curve values for the neutral negatively charged formulation against planktonic *P. aeruginosa* and *K. oxytoca* were better than those of free gentamicin. Furthermore, liposomal formulations prevent the biofilm-formation ability of these strains better than free gentamicin. In summary, liposomal formulations could be an effective lipid nanoparticle to combat acute infections where planktonic bacteria are predominant.

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Functional characterization of *Chlamydomonas reinhardtii* MTP1-4 by yeast heterologous expression for enhanced metal tolerance and uptake

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Metal pollution has been a great challenge in most industrialized countries as a result of waste generated from industrial activities being introduced into the environment. Algae have been considered a potential tool for bioremediation of metal pollutants due to its metal sequestration properties. In this study, a family metal transport protein named MTP1-4 from *Chlamydomonas reinhardtii* were screened by yeast heterologous expression for metal transport activity. MTP1 was able to strongly rescue the Zn and Co sensitivity of *zrc1 cot1* strain, MTP3 could weakly mediate Zn and Co growth, but MTP2 and MTP4 appeared to have no Zn or Co tolerance activity. MTP2, MTP3 and MTP4 but not MTP1 could strongly rescue the Mn sensitivity of *pmr1* strain. Metal transport proteins are potential tools that can be manipulated for enhance metal tolerance and uptake.

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From organic waste to biohydrogen: Approaches to enhance H_2 production by *Escherichia coli*

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Bioconversion of organic waste into biohydrogen (H_2) is a promising strategy both for inexpensive energy generation and for simultaneous waste treatment. Different waste materials, such as biodiesel production waste glycerol, lignocellulosic brewery spent grains (BSG), were used in the study. Lignocellulose, the most abundant renewable biomass with a huge amount of worldwide annual production, is an ideal candidate for biomass and H_2 production. Glucose and xylose are the two most abundant sugars derived from the breakdown of lignocellulosic biomass. Besides, Escherichia coli utilize many natural sugars to form biomass and to produce H_2. The later can be produced from formate decomposition via formate hydrogenlyase (FHL) during *E. coli* glucose, xylose or glycerol fermentation. FHL consists of formate dehydrogenase H (FDH) and membrane-associated four [Ni-Fe]-hydrogenase (Hyd) enzymes. The dilute acid pre-treatment method was used to hydrolyze the lignocellulose structure and the BSG hydrolysate (BSGH) optimal conditions for bacterial growth and H_2 production were designed. *E. coli* BW25113 parental strain and hydrogenase (Hyd) mutants with deletions of genes for key subunits of Hyd 1-4 *(ΔhyaB, ΔhybC,ΔhycE, ΔhyfG)*, respectively, as well as *ΔhyaBΔhybC* double mutant biomass formation, redox potential kinetics and H_2 production were investigated upon BSGH and glycerol utilization. Responsible Hyds for H_2 production upon both glycerol and BSGH utilization were revealed. Approaches, such as mutations in Hyd genes, heavy metals supplementation, pH, redox potential were used to enhance H_2 production upon both glycerol and BSGH utilization. The results obtained will contribute the efficient and economical biomass and H_2 production.

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Lipolytic enzymes of *Mycobacterium tuberculosis* as drug target

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Tuberculosis is one of the top ten causes of death worldwide and India alone accounts for one quarter of cases. MDR (Multi drug resistant) and XDR (Extensively drug resistant) strains of the causative pathogen *Mycobacterium tuberculosis* are the major hurdles in combating the disease. There is dire need to search for new drug targets/drugs that are effective against the drug resistant strains. A major chunk of the mycobacterial genome encodes for genes involved in lipid metabolism. Several lipases were reported to be essential for virulence, survival and pathogenesis of MTB. The antisense nucleotides against lipU, lipS and lipK genes inhibited the *in vitro* growth and survival of bacteria under stress conditions. Rv0774c, an iron stress inducible, extracellular esterase is involved in immune-suppression associated with altered cytokine and TLR2 expression. All these genes coded for esterases. LipS demonstrated epoxy hydrolase activity also. 3D model structure of these proteins was developed and stabilized through molecular dynamics. Natural compounds and FDA approved drugs were screened against these proteins. We identified some inhibitors by virtual screening against these enzymes and validated using bioinformatics tools. The results suggested high probability of these drugs to inhibit the enzyme activity of these enzymes and could be tested under *ex-vivo* and *in vivo* conditions.

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Structure/function of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) synthase (PAPSS)

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3'-Phosphoadenosine 5'-phosphosulfate synthase (PAPSS) catalyzes the formation of PAPS from inorganic sulfate and ATP. In the first step inorganic sulfate combines with ATP to form adenosine 5'-phosphosulfate (APS) and pyrophosphate (PPi) catalyzed by the ATP sulfurylase domain. In the second step APS kinase catalyzes the phosphorylation of APS at the 3' hydroxyl of APS to form PAPS and ADP. PAPSS utilizes 2 moles of ATP and cleaves it differently during PAPS formation, a feature that is unique only to PAPSS. ATP sulfurylase domain cleaves the ATP at the alpha-beta position whereas the APS kinase domain splits the ATP between beta-gamma position. The alpha-beta cleavage of ATP among all ATP sulfurylases share the common motif RNPxHxxH and henceforth it is called Venk-Ettrich motif. Site directed mutagenesis and computational modeling confirms the role of HNGH residues on alpha-beta splitting of ATP. The beta-gamma splitting of ATP contains a typical Walker A motif. Further studies are underway to look at the details of the reaction mechanism. Our overall aim is to look at 1. How 3D structure of PAPSS determines the enzyme function? 2. Studying the roles of specific amino acid residues and the dynamics of it in aqueous solution. 3. Making explicit structural and thermodynamic predictions of ligand binding. 4. Understanding the structural/functional consequences of the proteins due to DNA mutations among various human populations.

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Between two paradigms of mushroom biotechnology: solid-state cultivation and submerged growing

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Any microbial biotechnology involves the use of biocatalysts, as whole microorganisms or their enzymes to synthesize useful metabolites or convert the raw materials into new products. Basically, there are two paradigms of mushroom biotechnology, as solid-state cultivation of mushroom species and the new procedure regarding their submerged growing in safety conditions. The specific status of all native or indigenous mushrooms is to grow and develop in natural habitats by colonizing only solid substrates. None of the known mushroom species has the ability of growing and developing in natural aquatic habitats, and much more than that, none of them is adapted to form fruiting bodies inside a liquid medium. This is a restrictive condition for all native mushrooms by which they are compelled to live inside terrestrial ecosystems but through the application of submerged cultivation, many of these species can be grown in safety conditions. In this respect, it should be taken into consideration that the physical and chemical factors interact and affect the efficacy of the bioprocess regarding the mycelia growth inside the liquid medium. The submerged cultivation of mushrooms (SCM) has an exclusive and specific character concerning the mycelial growth in very different conditions by comparison with solid-state cultivation. SCM refers to a biotechnological process of mushrooms growing inside an artificial environment represented by the volume of a liquid medium where they are provided with all the needed physical and chemical factors for optimal development of mycelium without any risk of chemical or biological contamination, as shown in this study.

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Imaging of vitamin B_{12} dynamics by genetically encoded fluorescent nanosensor in living cells

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Vitamin B_{12} (cobalamin) is a co-factor of various enzymes and involved in the metabolism of every cell of human body. Deficiency of vitamin B_{12} causes various neurological abnormalities and pernicious anemia. Various methods like isotopic labeling MS and NMR have been used for measuring the level of cellular metabolites or signaling molecules, but these methods require the disruption and fractionation of tissues which suffer from contamination. Genetically encoded FRET-based sensors have been constructed to determine the metabolite concentration in live cells. Here, we report the designing of fluorescence resonance energy transfer-based nanosensors for direct visualization of changes in cobalamin concentration in intact living cells. Initially, a construct was designed by using the vitamin B_{12} binding protein (BtuF), cyan (CFP) and yellow (YFP) variants of green fluorescent protein. This construct was then shuttled in different expression vectors. This FRET sensor was named as SenVitAL (sensor for vitamin anemia linked) which is found to be very specific for vitamin B_{12}. This sensor is stable to pH changes, and measures the vitamin B_{12} in a concentration-dependent manner with an apparent affinity of (K_d) ~157 µM. In case of *E. coli*, increase in the emission intensity ratio was specifically observed after exposure to vitamin B_{12}. Both *in vitro* and *in vivo* measurements, FRET ratio rises after with the addition of vitamin B_{12}. Moreover, the results show that the SenVitAL can evaluate the vitamin B_{12} concentration in the cytosol of yeast and mammalian cells, proving its potential in eukaryotic system. Consequently, the sensor can serve as novel indicator to investigate the vitamin B_{12} flux and, would help to elucidate their complex roles in metabolism.

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Trehalose mediated oxidative stress tolerance in maize seedlings under salinity and low P stress

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Statement of the Problem: Over production of reactive oxygen species (ROS) under salinity and low P stress limits crop growth and production. Therefore, they must be removed for cellular survival. Trehalose (Tre), a disaccharide, is reported to have environmental stress tolerance role. Therefore, exogenous Tre might have an important role in the regulation of antioxidants (enzymatic and non-enzymatic) responsible for lessening oxidative damage. The objective of the study was to investigate the regulatory role of Tre on enzymatic antioxidants in maize seedlings under salinity and low P stress.

Methodology & Theoretical Orientation: Seven days old seedlings of two maize genotypes, BARI Hybrid Maize-7 (BHM-7) and BARI Hybrid Maize-9 (BHM-9), were imposed to 12 dSm^{-1} salinity and low P (10 ppm) in the presence and absence of Tre (10 mM) for seven days. Growth parameters as well as reactive oxygen species (ROS), lipid peroxidation (as melondialdehyde, MDA), lipoxygenase (LOX) and enzymatic antioxidant activities were investigated in fully expanded leaves.

Findings: Tre increased the length and volume of roots as well as delayed the loss of chlorophyll. Both salinity and low P increased ROS, MDA and LOX activity enormously, and Tre lessened all of the contents. Although Tre failed to increase the activity of superoxide dismutase (SOD) under salinity stress, it maintained higher activity under low P and salinity +low P stress. Increased activities of catalase (CAT) and ascorbate peroxidase (APX) under both stresses indicated their importance in scavenging H2O2. In case of double stress, Tre restored the stress inhibited activities in both genotypes. Increased glutathione reductase (GR) and dehydroascorbate reductase (DHAR) activities by Tre under salinity and P stress signified their role in maintaining glutathione (GSH) and ascorbate (ASA) in maize.

Conclusion & Significance: Exogenous Tre maintained growth parameters and reduced oxidative damage. It is recommendation that Tre be applied to reduce oxidative stress in maize.

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Mutated and wild type Gossypium universal stress *protein-2* (GUSP-2) gene confers resistance to stresses in *Escherichia coli, Pichia pastoris* and cotton plant

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Gossypium arboreum is considered to be a rich source of stress responsive genes and EST data base revealed that most of its genes are uncharacterized. The full length *Gossypium universal stress protein-2* (GUSP-2) gene (510bp) was cloned in E. coli, Pichia pastoris and *Gossypium hirsutum*, characterized and point mutated at three positions separately at 352-354, Lysine-60 to proline (*M1-usp-2*) and 214-216, aspartic acid-26 to serine (*M2-usp-2*) and 145-147, Lysine-3 to proline (*M3-usp-2*) to study its role in abiotic stress tolerance. It was found that heterologous expression of one mutant (*M1-usp-2*) provided enhanced tolerance against salt and osmotic stresses, recombinant cells have higher growth up to 10-5dilution in spot assay as compared to *Wusp- 2* (wild type *GUSP-2*), *M2-usp-2* and *M3-usp-2* genes. *M1-usp-2* in Pichia pastoris transcript profiling exhibited significant expression (7.1-fold) to salt and (9.7) and osmotic stresses. *M1-usp-2* gene was also found to enhance drought tolerance and significant expression (8.7) in CIM-496-*Gossypium hirsutum* transgenic plants. However, little tolerance against heat and cold stresses both in recombinant yeast and bacterial cells was observed. The results from our study concluded that activity of *GUSP-2* was enhanced in *M1-usp-2* but wipe out in *M2-usp-2* and *M3-usp-2* response remained almost parallel to *W-usp-2*. Further, it was predicted through in silico analysis that *M1-usp-2*, *W-usp-2* and *M3-usp-2* may be directly involved in stress tolerance or function as signaling molecule to activate the stress adaptive mechanism.

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Nigerian plant resources, an incredible generosity with an incredible responsibility

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Nigeria is a physically and climatically diverse country that has been endowed with substantial plant resources. The natural vegetation varies from rain forest to savannas with nine distinct ecological zones which permit the growth of a wide variety of crops. The country is generously blessed with a lot of plant resources to the extent that, there will be no reason whatsoever to live in hunger or suffering. This magnanimity is an incredible generosity of Mother Nature which carries with it an equally incredible responsibility. This paper attempts to highlight the value and incalculable magnanimity of Mother Nature in Nigerian plant genetic resources. It also discusses the need to utilize the God-given plant genetic resources with responsibility and wise exploitation. It is concluded that, there is need for the scientific research community in every country to wake up to this reality and be engaged in not just knowing their heritage in plant genetic resources but also to do what is necessary to ensure food security in their country.

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Enzymatic production of bioactive peptides from raps flour

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The biologically active peptides may exert systemic effects, such as antihypertensive, antioxidant, antiproliferative, among others. The defatted flour of raps, a byproduct of rape oil, has between 30-40% of proteins, being an interesting source for the production of bioactive peptides. Given this, our goal is to obtain peptides with different degrees of hydrolysis, in order to study their ability to inhibit the proliferation of MCF-7 cells (breast cancer) as part of their biological properties. Protein from rape flour was recovered by alkaline solubilization and isoelectric precipitation. Afterwards, the peptides were obtained by enzymatic hydrolysis with Alcalase® 2.4 L (50 °C), analyzing two processes, one without pH adjustment (starting at pH 10) and another controlling the pH at 8 (optimum pH of the enzyme), by means of a pH-stat, at enzyme/substrate ratios of 3 and 5%, for a time of 24 h. The degree of hydrolysis was measured by the method of orthophthaldehyde (OPA) and antiproliferative activity by the resazurin method. The hydrolysis kinetics obtained, showed that by controlling the pH at 8 throughout the reaction, the enzymatic rate is higher and thus the degree of hydrolysis, reaches up to 22% at 20 hours when using 3% of enzyme. With regards to antiproliferative activity, it is possible to determine an inhibition of MCF-7 cell proliferation of up to 80% when a 10 mg/L peptic extract is used, demonstrating the effectiveness of the enzymatic treatment with Alcalase® 2.4 L to obtain peptides with potential anticancer activity.

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Studies on optimization of improved amylases developed by protoplast fusion of *Aspergillus* species

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Improved amylase producers were developed by the fusion of protoplasts from two indigenous amylase-producing *Apergillus* species. Twenty randomly selected, regenerated fusants were screened for improved amylase activities. Selected fusnats were used for further comparative studies with the parent isolates. Effect of incubation period on amylase activities of parent and fusant isolates were studied by assay of crude enzymes produced by solid state fermentation on rice bran and used for cassava starch hydrolysis. The interaction between temperature, pH and enzyme types (parental and selected fusants) was studied and optimized using the central composite design (CCD) of the response surface methodology (RSM). Fusant designates, T5 T13 and T14 were selected for improved activities over parents and were used in further comparative studies. Assay of amylase activities of randomly selected regenerated fusants at room temperature and at 80°C showed designates, T5 (920.21 U/ml, 966.67 U/ml), T13 (430 U/ml, 1011.11 U/ml) and T14 (500.63 U/ml, 1012.00 U/ml) as preferred fusants. Amylases produced by the fusants were observed to be active over the range of pH studied. Analysis of the sequences generated suggests the occurrence of genetic recombination in selected fusants, fusants T13 and T14 had optimum amylase production at 72 hours, while parents TA, TC and fusant T5 produced amylase maximally after 96 hours of incubation. Optimum amylase activity was observed from amylase from fusant T5 at pH 4 and temperature of 40°C. These results show the efficiency of protoplast fusion technique in strain development for improved amylase activity.

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Osteocyte-specific Cas knockout mice exhibit decreased bone mass through increased osteoclastic bone resorption

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The skeleton is a metabolically active organ that undergoes continuous remodeling throughout life. Osteoporosis, which is fostered by advancing age, is the most common clinical disorder affecting bones. Although it has been postulated that osteocytes play an important role in sensing mechanical load in bone tissues, detailed molecular mechanisms of how osteocytes regulate bone metabolism remain largely unclear. The adaptor molecule p130Cas (Crk-associated substrate, hereafter referred to as Cas), which is phosphorylated at focal adhesions upon extracellular matrix engagement, is involved in various cellular processes including migration, survival, transformation, and invasion. In addition, we reported that Cas binds to the cytoskeletons in a stretch-dependent manner. This suggests that Cas can function as an initiator of intracellular signaling cascades through force-dependent changes in the cytoskeleton network. To investigate the role of Cas in bone metabolism, we generated osteocyte-specific Cas conditional knockout (cKO) mice by mating $Cas^{flox/flox}$ mice with Dentin matrix protein 1 (Dmp1)-Cre transgenic mice, in which the Cre recombinase gene was specifically expressed in osteocytes. The resulting Dmp1-Cre+/−; $Cas^{flox/flox}$ mice (referred to herein as Cas cKO mice) exhibited a significant decrease in bone volume, as determined by μCT analysis. Histomorphometric analysis of Cas cKO mice revealed a significant increase in the eroded surface/bone surface ratio, osteoclast surface, and osteoclast number. Furthermore, the expression levels of RANKL genes were significantly increased in the osteocyte fractions derived from Cas cKO mice. Collectively, these findings suggest that the bone loss in Cas cKO mice was caused by increased osteoclatstic bone resorption.

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Solid-phase extraction of protein by using magnetic chitosan and graphene oxide-functional guanidinium ionic liquid composite

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A series of novel cationic functional hexaalkylguanidinium ionic liquids and anionic functional tetraalkylguanidinium ionic liquids have been synthesized. Magnetic chitosan-graphene oxide (MCGO) composite has been prepared and coated with the functional guanidinium ionic liquids for protein extraction. Vibrating sample magnetometer, x-ray diffraction spectrometer, field emission scanning electron microscopy and Fourier transform infrared spectrometer were used to characterize the MCGO-functional guanidinium ionic liquid composite. After extraction, concentrations of protein were determined by an ultra violet visible spectrophotometer. Lysozyme, trypsin, BSA and ovalbumin could be well extracted by the proposed MCGO-functional guanidinium ionic liquid magnetic solid-phase extraction system. Compared to magnetic chitosan, graphene oxide, MCGO and MCGO-ordinary imidazolium ionic liquid, the MCGO-functional guanidinium ionic liquid has higher extraction capacity. Single factor experiments proved that the extraction efficiency of protein was affected by temperature, pH value, extraction time, amount of MCGO-functional guanidinium ionic liquid composite and concentration of protein. Moreover, the MCGO-functional guanidinium ionic liquid can be easily regenerated; the extraction capacity after being used three times was 94% of the first time.

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The transient kinetic mechanism of protein arginine methylation

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Protein arginine methyltransferases (PRMTs) catalyze the transfer of the methyl group from S-adenosyl-L-methionine (AdoMet) to the guanidino group of arginine residues in protein substrates, resulting in mono and di-methylarginine residues. Protein arginine methylation is an important posttranslational modification mark regulating epigenetics and many other cellular pathways. We sought to resolve significant kinetic steps of PRMT catalysis by combining steady-state and transient kinetics techniques. We have constructed a novel turnover model which reveals critical information about the ternary complex formation and methyl transfer process. Methyl transfer was found to be the rate limiting step. Significantly, the catalysis is found to follow a unique mechanism in which PRMT1 is able to randomly bind AdoMet or peptide substrate to form binary complex but follows a kinetically preferred (ordered) pathway to form the ternary complex. The delineation of PRMT1 transient kinetic mechanism provides new insights to understand biological function of arginine methylation and to design potent PRMT inhibitors.

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