2252nd Conference

Bio America & CRISPR-2018

24TH BIOTECHNOLOGY CONGRESS: RESEARCH & INNOVATIONS

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Annual Congress on

CRISPR CAS9 TECHNOLOGY AND GENETIC ENGINEERING

October 24-25, 2018 | Boston, USA

Scientific Tracks & Abstracts

Day 1

Bevin Engelward, J Biotechnol Biomater 2018, Volume 8
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Novel cytotoxicity and broad-spectrum genotoxicity platforms

Bevin Engelward
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Toxicity and Genotoxicity testing are fundamental to drug safety and drug development. Here, we leverage cell microarray technology to create a robust and highly sensitive cytotoxicity platform and a broad-spectrum genotoxicity platform. Quantification of cell viability is one of the most fundamental and broadly used endpoints in the life sciences. The gold standard is the colony forming assay. While the assay has an impressive dynamic range (over several orders of magnitude), it is relatively low-throughput (10-21days), laborious and requires large dishes/high volumes of media, thus requiring large amounts of test compounds. Microtiter cytotoxicity assay has thus been developed, including the XTT and the CellTiter-Glo assays. The XTT assay suffers from low sensitivity, and the CellTiter-Glo assay is subject to artifacts due to its indirect measure of cell viability. To overcome these limitations, we developed the MicroColonyChip (uCC) assay, which directly measures the ability of cells to divide (like the gold-standard colony forming assay), but with the scale and speed of microtiter assays. Microcolonies grow in a microarray and toxicity is derived using a novel metric, namely the change in the distribution of microcolony sizes. The result is an exquisitely sensitive assay that is robust to artifacts. For genotoxicity testing, the comet assay is a commonly used approach. We recently developed a higher throughput version of the comet assay that exploits a cell microarray. The "CometChip" is more than 1000X faster, far more sensitive and includes automated data analysis. Further, we broadened the spectrum of detectable lesions to include bulky lesions, a class of DNA damaging agents that have the potential to cause cancer. Accurate cytotoxicity and genotoxicity testing hold a central role in drug development. Having reliable and sensitive assays enables identification of untoward deleterious effects of drug candidates, providing immense savings by narrowing the candidate pool. Further, cytotoxicity and genotoxicity assays are also pivotal for the development of novel DNA damaging chemotherapeutics, the mainstay of cancer treatment today.

Biography

Bevin Page Engelward graduated from Yale University in 1988 and from the Harvard School of Public Health in 1996. She continued at Harvard for a one year postdoc, after which she joined the faculty at the Massachusetts Institute of Technology. She is currently Professor of Biological Engineering, Deputy Director of the Center for Environmental Health Sciences, and Director of the MIT Superfund Research Program Center. The main interests of her research is DNA damage and repair, and development of novel technologies relevant to cancer etiology and drug development. In particular, she leads efforts to exploit photolithography to create cell microarrays. Most recently, a high throughput cytotoxicity assay has been developed wherein toxicity is measured by a change in the distribution of microcolony sizes. She has also helped to develop a higher throughput DNA damage assay that is based on the comet assay. The "CometChip" has been further developed to detect diverse classes of DNA damaging agents, including potentially carcinogenic bulky lesions. In addition to studies of cytotoxicity and genotoxicity, her laboratory was the first to develop a mouse model wherein mutations (caused by homologous recombination) can be detected *in situ* via fluorescence.

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Ligninolytic enzymes and their possible role in future sustainable waste management of non-recyclable paper

Gabriela Kalcikova and **Andreja Zgajnar Gotvajn**
University of Ljubljana, Slovenia

Statement of the Problem: With the improvement of paper quality by various coating and additives the waste management is facing many difficulties regarding recycling of this new type of waste. Particular problems cause laminated paper where polyethylene is used as a coating. Recycling and/or recovery of plastic-coated paper are considerably difficult and unprofitable and thus such waste is usually aimed to be disposed in a landfill or incinerated. The aim of our work was to develop an environmentally friendly method for delamination of plastic-coated paper by extracellular ligninolytic enzymes of white rot fungus *Dichomitus squalens*. Such enzymes as laccase (Lac) and manganese peroxidase (MnP) are involved in the degradation of lignin and their natural lignocellulosic substrates and are even capable to degrade various pollutants.

Methodology & Theoretical Orientation: Plastic-coated waste paper was obtained from a front and back page of an ordinary journal and was degraded by enzyme filtrate obtained by *Dichomitus squalens*. Various ratios between the amount of plastic-coated paper and enzyme filtrate were tested. The best results were applied to set up laboratory bioreactor.

Findings: Enzymes were very efficient for delamination of plastic-coated paper. The paper part was partially dissolved and partially stayed in bulk form while foils were free-floating. The efficient delamination proceeded already after one day of the experiment, the final delamination efficiencies after 15 days were from 88.6% to 91.5%. An activity of Lac and MnP enzymes reached up to 1000U/L and 300U/L, respectively and stayed active during the whole experiment. The treatment of plastic-coated paper in a laboratory bioreactor was also very efficient reaching about 90% of treatment efficiency. Results showed, that ligninolytic enzymes can be very effective for delamination of plastic coated paper and enzymatic treatment could become an environmentally friendly alternative to current disposal option.

Biography

Gabriela Kalcikova has her expertise in waste management, landfill leachate treatment and using of white rot fungi in bioremediation. Her interest is to introduce new biotechnological approaches for treatment of so-called non-recyclable and non-biodegradable waste. She has also particular specialization in plastic waste - microplastics and their impact on the environment. She is an assistant professor and researcher at University of Ljubljana in Slovenia.

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Enabling a new era of stem cell medicine: A first technology for determining the dose of therapeutic tissue stem cells

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Adult tissue stem cells have essential roles in the renewal, repair, aging, and diseases (e.g., cancer) of mammalian tissues. These involvements make them the major principle for success in stem cell medicine. Ironically, given their importance, tissue stem cells pose many challenges to scientific investigation and medical use. Their cellular fractions in tissues are extremely low (typically less than 1 per thousand tissue cells), they are difficult to isolate, and it is difficult to expand their numbers by culturing. A particularly troublesome, long-standing barrier to their use has been the lack of a means to count them specifically. This difficulty has persisted for more than half a century because of the well-known lack of specific molecular biomarkers that identify tissue stem cells, but not their more numerous progeny, early committed progenitor cells. Asymmetrex recently solved the specific tissue stem cell counting problem with its AlphaSTEM Test™ technology developed in partnership with computer simulation leader AlphaSTAR Corporation. The new technology can be used to determine the stem cell-specific fraction of complex cell preparations. It can be applied to determine, for the first time, the stem cell-specific dose of stem cell transplantation treatments, including for example hematopoietic stem cells in bone marrow, mobilized peripheral blood, and umbilical cord blood or mesenchymal stem cells derived from bone marrow, adipose tissue, or amniotic fluid. In addition to better informing approved stem cell therapies, dose data will improve the design, optimization, and interpretation of ongoing stem cell transplantation clinical trials. The AlphaSTEM Test™ technology will enable a new era of quantitative measurement of therapeutic tissue stem cells that will greatly accelerate progress in stem cell medicine.

Biography

James L Sherley, MD, PhD is the founder and director of Massachusetts stem cell biotechnology company Asymmetrex, LLC. Asymmetrex develops and markets technologies for advancing stem cell medicine, including the first in kind technology for specific counting of adult tissue stem cells. This technology is also applied to design optimized procedures for more efficient manufacturing of therapeutic adult tissue stem cells at a greatly reduced cost. He is a graduate of Harvard College, with a BA degree in biology, and the Johns Hopkins University School of Medicine, earning joint MD and PhD degrees. Prior to founding Asymmetrex, he held academic research appointments at the Fox Chase Cancer Center, Massachusetts Institute of Technology, and Boston Biomedical Research Institute. His professional awards include Pew Biomedical Research Scholar, Ellison Medical Foundation Senior Scholar in Aging Research, and NIH Director's Pioneer Award.

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Notes:

Joel I Osorio, J Biotechnol Biomater 2018, Volume 8
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RegenerAge system: Therapeutic effects of combinatorial biologics (mRNA and allogenic MSCs) with a spinal cord stimulation system on a patient with spinal cord section

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As it has been previously demonstrated that electroporation of Xenopus laevis frog oocytes with normal cells and cancerous cell lines induces the expression of pluripotency markers and in experimental murine model studies that mRNA extract (Bioquantine®) purified from intra and extra-oocyte liquid phases of electroporated oocytes) showed potential as a treatment for a wide range of conditions, including Spinal Cord Injury (SCI) among others. The current study observed beneficial changes with Bioquantine® administration in a patient with a severe SCI. Pluripotent stem cells have therapeutic and regenerative potential in clinical situations CNS disorders even cancer. One method of reprogramming somatic cells into pluripotent stem cells is to expose them to extracts prepared from *Xenopus laevis* oocytes. The positive human findings for spinal cord injury with the results from previous animal studies with experimental models of traumatic brain injury and SCI respectively as our evidence and due to ethical reasons, legal restrictions and a limited number of patients, we were able to treat only a very small number of patients, deciding to include in our protocol the RestoreSensor SureScan to complete it. Based on the electrical stimulation for rehabilitation and regeneration after spinal cord injury published by Hamid and MacEwan, we designed an improved delivery method for the *in situ* application of MSCs and Bioquantine® in combination with the RestoreSensor® SureScan®. To the present day the patient who suffered a complete section of spinal cord at T12-L1 shows an improvement in sensitivity, strength in striated muscle and smooth muscle connection, 13 months after the first treatment and 6 months after the placement of RestoreSensor® at the level of the lesion, showing an evident improvement on his therapy of physical rehabilitation (legs movement) on crawling forward and backwards and standing on his feet for the first time and showing a progressively important functionality on both limbs.

Biography

CEO and Founder of Biotechnology and Regenerative Medicine at RegenerAge International™ (www.regenerage.clinic). Vice President of International Clinical Development for Bioquark, Inc. (www.bioquark.com) and Chief Clinical Officer at ReAnima™ Advanced Biosciences (www.reanima.tech). Advance Fellow by the American Board of Anti-Aging and Regenerative Medicine (A4M), Visiting Scholar at University of North Carolina at Chapel Hill (Dermatology). Fellow in Stem Cell Medicine by the American Academy of Anti-Aging Medicine and University of South Florida.

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Felice A de Jong, J Biotechnol Biomater 2018, Volume 8
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Towards a better understanding of the biochemical response to stressors such as disease or toxins: IROA® measurement system and workflow

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Statement of the Problem: Metabolites such as glucose respond to stressors that disrupt homeostasis, such as toxins. Humans produce 1000's of metabolites and the measurement of these molecules provides a treasure-trove of information that can be used to elucidate the health status of an organism. The measurement of metabolites (metabolomics) is widely achieved using liquid chromatography-mass spectrometry (LC-MS) whereby molecules are charged or ionized, fragmented and separated to generate peaks for 100s of metabolites from a single sample, i.e. a drop of blood. Advances in mass spectrometry, including accurate mass and high resolution, have enabled the measurement of many metabolites but still, over 60% of the data generated remains a mystery. One bottleneck is piecing together fragments with their parent molecules to achieve accurate identification of as many metabolites as possible. Confounding the issue is that most unknown peaks arise from artefacts including contaminants. The purpose of this study is to describe a QC IROA metabolomics workflow that removes artefactual peaks and increases accurate metabolite identification.

Methodology & Theoretical Orientation: Two sets of 100's of stable-labeled internal standards were generated; one enriched in one isotope and depleted in its other isotopic form, and the other its mirror image (95% ^{13}C6 and 5% ^{12}C6 and 5% ^{13}C6 and 95% ^{12}C6) so that all of the molecules in each standard exhibited unique mass spectral patterns, all mathematically calculable and easily characterized by Clusterfinder software algorithms. The paired internal standards (mixed 1:1; "Matrix") was analyzed by LC-MS and ClusterFinder to generate an accurate library of compound and peak identifiers, including mass and retention time. The heavier isotopic standard (95% ^{13}C6) was spiked into samples and analyzed by LC-MS. The Matrix was analyzed every ten samples to account for fluctuations in mass and retention time drift during the run. ClusterFinder located the labeled mass spectral peaks in the heavy standard and then located, identified and quantitated matching native (unlabeled) metabolite peaks in samples using the library. Peak correlation was performed to pair fragments and parent peaks. An example of this technology will be shown that illustrates the toxicological mechanism of action of flucytosine in yeast.

Conclusion & Significance: Metabolite identification and quantitation are achieved using a reproducible QC workflow enabling accurate results which can be used to interrogate living systems.

Biography

Felice de Jong has been an active leader and proponent in the field of metabolomics since its emergence, initially during her 6 years as Senior/Director of Business Development for Metabolon and more recently as CEO and co-founder of IROA Technologies. Here she works with collaborators to develop services and products that remove bottlenecks to streamline the measurement of biological response to stressors such as disease, adverse environmental conditions, drugs, and toxins.

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Ipsita Roy, J Biotechnol Biomater 2018, Volume 8
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Natural polymers of bacterial origin and their medical applications

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Polyhydroxyalkanoates (PHAs) are natural polymers produced by bacteria under nutrient limiting conditions. These polymers are biodegradable and biocompatible in nature and hence can be used in a variety of medical applications such as tissue engineering, wound healing, medical device production, and drug delivery. PHA synthases are the main enzymes involved in the biosynthesis of PHAs. There are two main types of PHAs, short chain length PHAs, scl-PHAs, with monomer chain length C3-C5, these are generally hard and brittle; mcl-PHAs, with monomer chain length C_6-C_{16}, these are soft and elastomeric in nature. Due to their varied mechanical properties and degradation rates, PHAs can be used to replace a range of tissue types including bone nerve cartilage pancreas cardiac and skin. In addition they can be used for short-term and long-term controlled drug delivery. PHAs are known to be particularly cardio-regenerative in nature. Myocardial infarction results in the generation of scar tissue with limited or no regeneration. The concept of a cardiac patch is tailored to meet the unmet medical need of cardiac regeneration where a biomaterial-based patch with/without cells would be used to induce efficient cardiac regeneration. Mcl-PHAs have been shown to be excellent substrates for the growth and function of neonatal cardiomyocytes. We have carried out an in-depth study of the potential of MCL-PHAs for the development of functional cardiac patches.PHAs are also known to be highly neuro-regenerative in nature. Peripheral nerve injuries caused due to accident or disease are highly debilitating in nature. Gaps longer than 5mm do not regenerate naturally and lead to loss of function. We have developed Nerve guidance conduits using PHA based blends. These have been tested using animal models and were found to result in functional regeneration comparable to autografts, an excellent outcome.In conclusion, PHAs are a highly promising family of medical polymers with huge potential in the future.

Biography

Ipsita Roy is an expert in Microbial Biotechnology, Biomaterials and Tissue Engineering. She is currently a Professor at the Faculty of Science and Technology, University of Westminster, London. She was awarded the Inlaks Scholarship to study for her PhD at the University of Cambridge. Her postdoctoral work was at the University of Minnesota, USA. Subsequently, she has been at the University of Westminster since 2000 and leads the Applied Biotechnology Research Group. She has published over 100 papers in high Impact Factor journals such as Biomaterials, Biomacromolecules, Journal of Royal Society Interface. Her group is currently focussed on the production of novel polyhydroxyalkanoates (PHAs), a group of FDA-approved natural polymers and their characterisation. Her work has been funded by the EPSRC, EU, DuPont and WESTFOCUS, London. Professor Roy's current projects involve the use of PHAs for the production of drug eluting biodegradable stents, nerve guidance conduits, antibacterial polymers and wound healing.

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A novel enzyme extracted from *Aloe vera* plant used in hide unhairng leather process

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Bagavathi Bio Tech Chemical Company, India

Leather has undergone chemical processing since time immemorial. We are to this day awed by beauty sophistication of leather products sometimes found in archeological excavations. After fabricating the mansions of fashion and comfort, leather products developments are now moving towards high–tech area of performance. This has brought up diversification and the sea change in the chemical processing of leather which was basically unchanged while years. Application of biotechnology in chemical processing of leather is one of the revolutionary ways to enhance chemical processing pinnate right from tanning to final finishing. The plant-based enzymatic process is a groundbreaking technology for the beam house stage of the leather production process. In this research work, an enzyme extracted from the aloe vera plant has developed a completely new, enzyme-based unhairing solution that overcomes current beam house challenges. A paradigm shift from chemical- to enzyme-based processes ensured that these noncollagenous materials were removed using enzymatic digestion rather than brutal osmotic forces employing chemicals like lime and sulfide. In the present study, protease enzyme extracted from aloe vera plant was used as experimental, and a lime and sulfide process was used as the control sample. Unhairing chemical composition was used 50% enzyme/50% lime and sulfide, 100% enzyme and 25% lime/sulfide and 75% enzyme in this trial work. Unhairing of sheepskin has been carried out with Protease enzyme alone at various concentration (5%, 10%, and 20%). The properties of enzymatic unhaired samples are compared with those of the conventional one. Encouraging results in term of fiber opening, excellent hair removal is obtained in case of 25% Lime/sulfide and 75% enzyme.Further, it reduces the volume of effluent as well as BOD, COD, TDS and nitrogen content. Using the enzyme permits a reduction in the chemical dosage and/or process time. The test results have strongly revealed that it is possible to produce commercially acceptable upholstery leather by using the enzyme in hair-save unhairing. The total consumption of lime and sodium supplied was reduced to 1.5% and 1.0%, respectively. Scanning electron microscopy (SEM) images of 25% lime/sulfide and 75% enzyme treated leathers showed the better degree of fiber opening, and an energy dispersive X-ray spectrum (EDS) shows the elements present on the skin matrix. This study provides a newer insight for a cleaner, economical, and sustainable method of leather processing.

Biography

Durairaj Jothi, M. Tech (Textile), PhD (Currently doing), working as a professor in the Department of Textile Chemistry, SSM College of Engineering, Anna University, Tamil Nadu, India. Earlier, he taught at the Bahirdar University, Ethiopia for several years before joining SSM college of Eng in 2012. He has more than 34 years of experience in teaching and conducting various researches in the organic product development for textile, leather and paper manufacturing. He has published more than 24 papers in National and International Journals, and authored two books.

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Effect of glycosylation and physico-chemical properties on biological activity of anti-HER2 molecule

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Background: The biological activity of an anti-HER2 molecule is profoundly influenced by the microheterogeneity of the N-linked glycan profile. A minor change in glycan profile of anti-HER2 molecule during post-translational modification showed an impact on its biological activity, Pharmacokinetics (PK) as well as the stability of the molecule. The complexities in glycan pattern of anti-HER2 molecule further encourage the need of new analytical challenges in evaluating the comparability with innovator molecule in recent scenario.

Aim/Objective: A comparison of glycan profiling and binding kinetics including charge variant heterogeneity analysis of anti-HER2 molecule with innovator molecule was aimed for the current investigation.

Methodology: In the current investigation, The PNGase F digestion of anti-HER2 molecule followed by UPLC analysis under Normal phase liquid chromatographic conditions using a platform approach to establish the chromatographic profile of various Glycan residues such as G0, G0F, GIFa and GIFb, G2F. Further, binding with the HER2 receptor under *in vitro* conditions through Biocore T200 analysis was evaluated to demonstrate the binding kinetics. Additionally, charge variant heterogeneity was subjected as a part of extensive characterization to evaluate the anti-HER2 molecule in comparison with innovator molecule.

Results and Discussion: Anti-HER2 molecule with a mass of 148kDa was purified and a comparison against innovator molecule in glycan profiling was identified by predominant residues, which represent 90% of the glycosylation profile of the anti-HER2 molecule. The terminal galactosylation and fucosylation of glycan residues influence the CDC and ADCC activity, respectively. The variability in the glycan profile can thus affect the biological activity. Further, the high mannose content in anti-HER2 molecule showed the early clearance and provides a strong impact on its PK. Additionally, binding with a HER2 receptor under *in vitro* conditions through Biocore T200 analysis was evaluated to demonstrate the binding kinetics. The dynamic binding nature of anti-HER2 molecule ($6.21x10^{-10}$M) and innovator molecule ($6.95x10^{-10}$ M) showed the closeness of KD value. Moreover, the weak binding of acidic variants with HER2 receptor also provides an impact on ADCC activity and hence, charge variant analysis was shown to be equally important to demonstrate the efficacy of the anti-HER2 molecule. Acidic variant (K0, deamidated Asn) and basic variants (K1, K2) were determined by cation exchange chromatography (CEX) and percentage of acidic variant (<35%) were evaluated based on separation on cation exchange column in UHPLC system. The comparisons were carried out based on the response of reference standard peaks under the identical condition and determine the percentage of acidic variants.

Conclusion: Overall, the efficacy and comparability aspects of the in-house anti-HER2 molecule were evaluated against the innovator's molecule and attested its candidature as a "Biosimilar" in the pharmaceutical therapeutic domain.

Biography

Sourav Majumdar serves as a Deputy Manager in Serum Institute of India Pvt. Ltd, Pune. He has developed his core expertise in the area of Bioanalytical method development in proteomics platform. He is leading the group of biosimilar especially dealing with recombinant monoclonal antibody. After completion of his PhD degree he joined EPR Centre for cancer research, Hyderabad as ADL lead. He developed several analytical methods for routine and extensive characterization of mAb molecule. Formerly, he worked in Intas Bio Pharmaceutical R&D stability division as Senior Research Associate. He then joined for USV and was assigned to work on various recombinants Biosimilar Therapeutics with several analytical challenges especially with anticancer molecule. He joined as a Junior Research fellow in MBBT department for PhD His research was on a "Novel Fibrinolytic Enzyme and a comparison with commercially available Cardiovascular Drugs under *in vitro* and *in vivo* conditions". He has his majors in Bio-Chemistry from University of Pune, 2004. He is a recipient of "National Level fellowship" and "Ratan Tata fellowship". He was awarded the best speaker from Garware Chemical Association. He published a series of Research Article in reputed International Journals.

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Enhanced bioH2 and poly-hydroxyalkanoates production by a co-culture of *Syntrophomonas wolfei* and a photoheterotrophic mixed consortium using a dark-fermentation effluent as substrate

Z Vanegas-Zuniga, O Cortes De Luna, C Nino Navarro and **I Garcia Pena I Chairez**
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Nowadays, the pollution from oil-based derivatives such as gasoline, polyethylene, etc. is getting problematic. One possible way to overcome this issue is by developing alternative green technologies. The bio-plastics production seems to be a promising method to reduce the plastics production. Polyhydroxyalkanoates as the copolymers of PHB and PHV have similar characteristics of the polyethylenes, therefore many applications *Synthrophomonas wolfei (S. wolfei)* and some photoheterotrophic bacteria are able to produce this polymer from the effluents of organic residues treatment. Moreover, these microorganisms may produce bioH2 depending on the culture conditions. However, the production of biopolymers based on the bacteria metabolism is nowadays still more expensive than synthetic production. This condition motivates the research to optimize the biological process to make it competitive compared to the regular oil-based method. The purpose of this study was to develop the syntrophic consortium composed by *S. wolfei* and a photoheterotrophic mixed consortium named C4. This strategy would allow improving the simultaneous production of bioH2 and PHA. The dark-fermentation effluent was used as a substrate during the photoheterotrophic process. This effluent consists of a complex mixture of volatile fatty acids including acetic, butyric, lactic, propionic and some others. The data demonstrated the syntrophic activity between *S. wolfei* and C4 based on the comparison of PHA and H_2 productions from the individual and co-culture fermentations. The individual cultures showed that consortium C4 and *S. wolfei* can use the effluent as a carbon-nitrogen source. *S. wolfei* produced a higher concentration of bioH2 but lower PHA production compared with C4. The co-culture produced this bioproduct simultaneously, with 25%PHA and 90mmolv/vH2 at 100 and 75 hours respectively.The profile of volatile fatty acids consumption explained the interaction between C4 and *S. wolfei* suggesting a mutualism.

Biography

Zaira Jovana Vanegas Zuniga is a Biotechnology Engineer recently graduated from UPIBI at the National Polytechnic Institute. She is working on a research project named “Enhanced the bio-hydrogen and poly-hydroxyalkanoates production by a co-culture of *Syntrophomonas wolfei* and the photoheterotrophic mixed consortium using the dark-fermentation effluent as substrate”. Her current research interests are the production of biofuels (Hydrogen and biopolymers) using residues from agro-industries as carbon-nitrogen source.

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Notes:

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Detection of adulteration/substitution in herbal market samples

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Medicinal plants are the basic raw materials for herbal market samples which are available either in the form of raw or powdered forms or sometimes either in processed forms such as capsules, tablets, tinctures etc. Of approximately 17,000-18,000 species of flowering plants existing in India, 6,000-7,000 are used in alternative or traditional systems of medicine. Only 960 of these are used in trade, of which 178 are actively traded. Due to the unrestricted collection of these medicinal plants, most of their populations are declining and instances of substitution/adulteration by look-alike substitutes have increased. Thus, it becomes essential to authenticate herbal market samples, which is difficult to validate using the contemporary taxonomic methods. DNA barcoding, a novel technology, that uses simple molecular tools can identify such materials even if they are fragmented or available in small quantity. In the present investigation, Internal Transcribed Spacer 2 (ITS2), because of its ease of amplification from degraded samples and sufficient informative sites despite its short length, was tested as a barcode for authentication of herbal market samples. The availability of barcode quality ITS2 sequences was checked on NCBI. Of the 960 lists of medicinal plants of India, ITS2 sequences of 172 were available. A reference barcode library of these species was prepared. 160 herbal market samples belonging to 50 of these species were procured from various markets. The amplification and sequencing success rates from these samples were 80.6% and 68.1%, respectively. In the ML tree based on 109 ITS2 herbal sample sequences, 46 samples belonging to 32 species could be authenticated unambiguously. For the remaining samples, a BLAST search was performed on NCBI GenBank, which assigned seven more samples belonging to four species correctly. The identity of the remaining samples could not be deciphered unambiguously.

Biography

Akanksha Priya planned and executed the experiment. She has expertise in the field of molecular biology. Alka Sinha helped in the collection of herbal samples and writing of the manuscript. The work was performed under the guidance of Professor Girish Kumar Sinha.

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Biodegradation of woods used for roof construction as one of the causes of roof failure in Nigeria: An overview

Kalada Itelima and **Janet Itelima**
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Statement of Problem: This overview focuses on the biological degradation of woods utilized for the purpose of roof construction as one of the causes of the rising incidences of roof failure in Nigeria. Understanding the degradation of wood by biological agents, the factors that influence the rate of degradation and the consequences of such effects is important for developing protection strategies to curtail these occurrences.

Methodology & Theoretical orientation: The information used for this write up was obtained from published articles and the internet. Wood is defined as the hard fibrous material that forms the main substances of the trunk or branches of a tree. It occupies a unique position as the world's most important raw material for construction purposes. The roof is one of the most important components of a building. It is that part of the building that offers protection against sun and rain; hence without it, the content of the building would be damaged. The rising incidences of roof failures in recent times in many parts of Nigeria have become a matter of concern. A roof is said to have failed if is no longer capable of performing any of the desired functions. The consequences of roof failures include damage to personal belongings and the exposure of inhabitants and livestock to bad weather conditions. Wood can be degraded or decomposed by a variety of biological agents such as fungi, bacteria, and insects. Fungi are can colonize wood and degrade the cell wall component to form brown, soft or white rot. Bacterial species can directly attack wood to cause erosion cavities and tunneling patterns of deterioration. Damage caused by insects such as termites and carpenter ants is one of the most serious types of damage to wood in service. In general, the activities of these biological agents would weaken the structural integrity of wood and make it easier for enzymes to access cellulose chains.

Conclusion & Significance: To avoid biodegradation of wood, possible failure and collapse of roofs, it is necessary to subject woods utilized for building construction to some prophylactic treatments, which may be in form of seasoning and application of chemical preservatives.

Biography

Kalada Itelima qualified as an Architect. He is presently the Director of Kalite Associate Jos, Nigeria. He is also involved in designing, building construction and consultancy services. He has very keen interest in attending conferences. He is into community services in Jos, Plateau State, Nigeria, where he is residing and to other parts of the country

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Day 2

Hikmet Budak, J Biotechnol Biomater 2018, Volume 8
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Genome editing in wheat, polyploid and complex genome

Hikmet Budak
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Genome editing technology is relatively easy to use and more cost-effective than others. By combining versatile tools to study and modify plants at different molecular levels, the CRISPR/Cas9 system is paving the way toward a new horizon for basic research and crop development. Wheat (*Triticum aestivum L.*) is a stable crop providing more than 20% of daily calories intake for humans and has a complex genome formed with the combination of three different genomes: A, B, and D. The hexaploid wheat genome makes this plant an important model for studying and optimizing the genome editing system. In our study, we applied the CRISPR/Cas9 genome editing system for abiotic stress response genes and small RNA genes. The CRISPR/Cas9 genome editing system in wheat was effectively established using different proteins as well.

Biography

Hikmet Budak currently works at the Department of Plant Sciences and Plant Pathology, Montana State University. Hikmet does research in Molecular Biology, Genetics and Agricultural Plant Science.

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Analytical next-generation sequencing approaches for CRISPR-Cas9-mediated cell engineering

Haythem Latif
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CRISPR-Cas9-mediated cell lines hold great promise to transform therapeutics in various disease areas. Next-generation sequencing (NGS) is a powerful analytical tool with applications designed throughout the CRISPR-Cas9 workflow from discovery, through screening, and to validation of CRISPR-Cas9 effects. Here we will review contemporary workflows for CRISPR-Cas9 knockout libraries and briefly highlight how NGS and advanced informatics facilitates drug target identification. We will also detail various routes for optimizing and screening CRISPR-Cas9 pools and isolates for on- and off-target activity. Moving beyond screening and lead identification, we will review novel unguided NGS approaches to demonstrate on-target specificity while confirming low/no off-target activity prior to clinical trials.

Biography

Haythem Latif is a life science professional with a passion for next-generation sequencing technologies and applications. His interests in NGS began during his time as a graduate student pursing a PhD in Bioengineering from the University of California, San Diego. There, he generated, analyzed, and integrated multi-omic datasets to deeply characterize the genome organization of various microbial organisms in search of novel biological insights. Upon graduation, he worked for ThermoFisher in the Ion Torrent applications team focusing on AmpliSeq panel development. He is currently managing NGS Business & Strategic Partnerships at GENEWIZ.

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CRISPRseek and GUIDE-seq for design of target-specific guide RNAs in CRISPR-Cas9 genome-editing systems

Lihua Julie Zhu
University of Massachusetts Medical School, USA

The most recently developed genome editing system, CRISPR-Cas9 has greater inherent flexibility than prior programmable nuclease platforms. Because of its simplicity and efficacy, this technology is revolutionizing biological studies and holds tremendous promise for therapeutic applications. However, imperfect cleavage specificity of CRISPR-Cas9 nuclease within the genome is a cause for concern for its therapeutic application. To facilitate the adoption and improvement of this technology, we have developed CRISPRseek for designing target-specific gRNAs, and GUIDE-seq for identifying genome-wide off-target sites from GUIDE-seq and CIRCLE-seq experiments to assess the precision of engineered CRISPR-Cas9 nucleases. In this talk, I will give an introduction to the CRISPR genome editing, GUIDE-seq and CIRCLE-seq technologies, followed by an overview of the functionalities of CRISPRseek and GUIDE-seq. By the end of the talk, the participants should be able to design target-specific gRNAs for various cas9 nucleases and genomes using CRISPRseek and analyze GUIDE-seq and CIRCLE-seq data using GUIDE-seq.

Biography

Lihua Julie Zhu is a research professor and the head of Bioinformatics Core in the Department of Molecular, Cell and Cancer Biology at University of Massachusetts Medical School. Prior to joining UMASS, she served as the Director of Bioinformatics Consulting Core and the Director of Clinical Informatics Group of the Robert H. Lurie Comprehensive Cancer Center (RHLCCC) at Northwestern University. Her work is devoted to the understanding of gene regulation and cancer biology, biomarker discovery, and development and application of genome editing technology. Her expertise is algorithms and computational tool development. Her group is an active contributor to the open-source open-development Bioconductor project. They have developed a dozen packages with various utilities, ranging from gRNA design, machine learning, peak calling, annotation, data integration to visualization. Among them, two packages are for designing and evaluating gRNAs for the genome editing technology using engineered CRISPR-Cas9 nucleases: CRISPRseek is for designing target-specific and efficient gRNAs, and GUIDE-seq is for evaluating the specificity of gRNAs using GUIDE-seq and CIRCLE-seq technologies. Among her 76 publications

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Notes:

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RegenerAge system: Therapeutic effects of combinatorial biologics (mRNA and allogenic MSCs) with a spinal cord stimulation system on a patient with spinal cord section

Joel I Osorio
RegenerAge, USA

Bioquantine® a mRNA extract from *Xenopus laevis* frog oocytes (purified from intra- and extra-oocyte liquid phases of electroporated oocytes), showed potential as a treatment for a wide range of conditions in animal models, including Spinal Cord Injury (SCI) and Traumatic Brain Injuries (TBI) among others. The current study observed beneficial changes with Bioquantine® administration in a patient with a severe SCI. Pluripotent stem cells have therapeutic and regenerative potential in clinical situations CNS disorders. One method of reprogramming somatic cells into pluripotent stem cells is to expose them to extracts prepared from *Xenopus laevis* oocytes. Due to ethical reasons and legal restrictions we selected a No Option patient, deciding to include in our protocol the RestoreSensor® SureScan® to complete it. Based on the electrical stimulation for rehabilitation and regeneration after spinal cord injury published by Hamid and MacEwan, we designed an improved delivery method for the *in situ* application of MSCs and Bioquantine® in combination with the RestoreSensor® SureScan®. To the present day the patient who suffered a complete section of spinal cord at T12-L1 shows an improvement in sensitivity, strength in striated muscle and smooth muscle connection, 14 months after the first Bioquantine® and MSCs treatment and 9 months after the placement of RestoreSensor® at the level of the lesion, showing an evident improvement on his therapy of physical rehabilitation (legs movement) on crawling forward and backwards and standing on his feet for the first time and showing a progressively important functionality on both limbs.

Biography

Joel I Osorio is an innovative businessman with a distinct entrepreneurial mindset concentrated adding value on areas of Biotechnology (mRNA), Reprogramming & Regenerative Medicine for translational use in humans and a variety of clinical applications aimed for both the private and the public health sectors. He is the Founder, President, and CEO of RegenerAge Clinic and RegenerAge Beauty initiatives for transnational implementations. Vice President and International Clinical Developer for Bioquark, Inc. Executive Vice President: Chairman of the WAMS Americas Division, member of the WAMS Executive Council (WAMS Executive Board), a member of the WAMS Education and Training Board (ETB), a member of the WAMS Editorial Board, an Honorary Member of the Academy Faculty FWAMS, an Honorary Fellow of the Academy and is also a Senior Partner at WAMS, The World Academy of Medical Sciences.

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Implications of cGMP for CRISPR-Cas9 cellular therapy

Kimberley Buytaert-Hoefen
Parexel International, USA

Many current therapeutic treatments are not able to address the underlying cause of a disease, alter its course, or reverse the damage that has already occurred. Cellular therapies offer the power of the human body to heal and regenerate itself. Regulatory precedents for cellular therapy products continue to evolve for a widening array of the product of types. An exciting new discovery of clustered regularly interspaced short palindromic repeat (CRISPR) technology when incorporated with cellular therapy may lead to the cure of many diseases. CRISPR-Cas9 offers the hope of a cure for various maladies including genetic diseases and cancers. The CRISPR-Cas9 medical cellular therapies involve removing cells from the body, modifying their DNA, and administering them to the patient. These modified cells are able to either replace or attack diseased cells. Medical cellular therapies are required to demonstrate quality, safety, and efficacy standards to obtain a marketing authorization. Medicinal cellular therapy products are regulated as drugs, devices, and biological products, which adds the regulatory requirement of manufacturing under cGMP conditions. With the high value of CRISPR-Cas9 source cell material, having ample amount for process-development and validation of the manufacturing processes is an industry challenge. Furthermore, limited shelf life and quantity of cells can complicate quality control testing and stability determinations. Defining critical quality attribute's (CQA's) for these products and developing assays for their potency are essential to the commercialization of these cellular therapy products. CRISPR-Cas9 source cells are characterized based on the presence of surface markers, size, and combinations of attributes associated with cell source and mode of action. Due to their ability to alter DNA, CRISPR-Cas9 cellular therapies offer the possibility to move beyond conventional disease treatment by addressing the underlying cause of disease, altering its course, or reversing the damage that has already occurred. The transitions from discovery, to research and development, to commercially manufactured products, brings the challenge of the regulatory requirements for incorporating cGMPs into the collection, production, and delivery of these products. These developments will allow for CRISPR-Cas9 cellular therapy to become increasingly available to patients and will offer new treatments and the hope to cure many diseases.

Biography

Buytaert-Hoefen obtained a Bachelor's degree in Psychology at the State University of New York at Binghamton and then went on to complete her Master's and Doctorate degrees in Neuroscience at the University of Colorado at Boulder. She completed two post-doctoral fellowships at the University of Colorado Health Sciences Center where she specialized in embryonic and adult stem cell research. She then entered the industry with a position as a Lead Scientist at Navigant Biotechnologies. After which, she accepted a position as a Consumer Safety Officer at the FDA, where she specialized in pharmaceutical inspections with an emphasis on biotechnology and sterile processing. Currently, as a Consultant at Parexel, she works closely with clients to develop and implement effective compliance solutions in accordance with client needs. She performs GXP audits, conducts laboratory data review including chemistry, microbiology, and data integrity assessments.

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Generation of genetically modified ducks by CRISPR/Cas9-mediated gene insertion into the duck genome

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Statement of the Problem: The bird egg is a potential bioreactor for heterologous production of protein, especially for the production of recombinant therapeutic proteins in the biopharmaceutical industry. The vast majority for the last 30 years of studies on the transgenic poultry focus on chickens (Gallus gallus) and quails (Coturnix japonica). Since duck (Anas platyrhynchos) eggs are larger than chicken and quail eggs, they have an advantage of being used as bioreactors over chickens and quails. The limiting factor of widespread distribution of genetic modification technologies in poultry, especially in waterfowl, is often the high cost of generating transgenic birds primarily due to the relatively low efficiency of transgenesis.

Methodology & Theoretical Orientation: We used CRISPR/Cas9-mediated homology-directed repair to edit the duck genome. Three different approaches were used to deliver the transgene into the host genome: lipofectamine transfection of sperms followed by artificial fertilization; microinjection of transfected blastoderm cells into duck blastoderm-stage embryos; direct injection of the transgene into the cavity under the germinal disc of duck embryos.

Findings: In the approach of sperm-mediated gene transfer, 20.6% of the founder ducks were positive for the transgene. In the other two approaches, 65% and 77.8% chimeric founders transmitted the transgene to the next generation. Transgene transmission to the next generation was observed in three different approaches, suggesting an appropriate genome editing. Therefore, we have successfully generated transgenic ducks using all the three approaches. Genome engineering of ducks is significant because it can be used as a model of waterfowl.

This study was supported by the Earmarked Fund for National Waterfowl-industry Technology Research System (CARS-42-06) and the Zhejiang Major Scientific and Technological Project of Agricultural (livestock's) Breeding (grant number 2016C02054-12).

Biography

Professor of the Department of Genetics, Breeding and Biotechnology of Animals of the National University of Life and Environmental Science of Ukraine, Doctor of Biological Sciences (in genetics) theoretically substantiated and experimentally demonstrated the species-specific destabilization of karyotype of different species of animals under the influence of chronic low-dose irradiation (Kostenko SA, 2001). Scientific interest is currently associated with increasing the efficiency of introducing transgenic structures into the genome of waterfowl and creating new lines of ducks based on the polymorphism of the genes of quantitative traits. International expert of the company Zhejiang Generation Biological Science and Technology Co., Ltd.

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Efficient animal model generation using zinc-finger nucleases and CRISPR/Cas systems

Guojun Zhao
Horizon Discovery, USA

Genetically modified animal models have been powerful tools for studying gene functions and human diseases. Over the past two decades, the predominant gene-modified animal models have been mouse models largely because the mouse embryonic stem cell-based genome editing technology was the primary method to create precise gene modifications in animals. The development of engineered nucleases has not only accelerated animal model production but also expanded animal models to numerous species or organisms other than the mouse. Engineered nucleases create double-strand breaks (DSBs) at targeted genomic loci, which can be repaired either by non-homologous end joining (NHEJ) or by homology-directed repair (HDR). Since 2009, our Lab (Horizon Discovery, formerly SAGE Labs) has been using zinc-finger nucleases (ZFNs) to create hundreds of genome engineered mouse, rat and rabbit models by direct pronuclear microinjection. While ZFN-based gene editing was efficient and specific, the time-consuming ZFN engineering has hampered the further extensive application of this technology. In recent 5 years, we have primarily used CRISPR to create genetically engineered animal models. We found that while both ZFN and CRISPR are equally efficient in creating different types of rat models, CRISPR is more efficient in HDR-based mouse model generation. After continuing optimizations, we have established methods to achieve highly efficient genome editing with relatively low off-target effect. We will present some case studies to show ZFN and CRISPR/Cas system-mediated point mutations, targeted integrations, megabase deletions as well as some challenges in an animal model generation.

Biography

Guojun Zhao has been working in in vivo genome editing since 2011. He joined Sigma-Aldrich (SAGE Labs) in 2011 and since then he has been using ZFNs and CRISPR/Cas systems to create hundreds of genetically engineered mouse, rat and rabbit models for customers worldwide. Those animal models help scientists in big pharma companies and universities study gene functions and find methods to control human diseases.

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