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Keynote Session

Development of Preclinical Diagnostics of Neurodegenerative Diseases – Illusions or Reality?

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Institute of Developmental Biology RAS, Russia

Abstract

Numerous attempts to develop a preclinical diagnosis of Parkinson's disease (PD) by searching peripheral biomarkers as changes in biological fluids and premotor functions are not fully successful. A drawback of this methodology is the search for markers in PD patients at the clinical stage without guarantees that they are characteristic for preclinical stage. Indeed, all markers detected so far are nonspecific. We propose to upgrade this methodology, using only markers found both in patients and animals at modeling clinical (symptomatic) and preclinical (presymptomatic) stages of PD. Detection of the same marker in patients and symptomatic animals is believed to indicate adequate reproduction of pathogenesis along this metabolic pathway, and detection of this marker in presymptomatic animals proves its specificity for preclinical stage. We showed that 50% and 20% of the markers found in blood of patients were characteristic of MPTP-treated symptomatic and presymptomatic mice, respectively. Besides, we propose a different approach to early diagnosis of PD - a provocative test that has been successfully used for decades in internal medicine. We showed that the systemic administration of α-methyl-p-tyrosine, a reversible inhibitor of dopamine synthesis (provocative agent), to MPTP-treated mice at presymptomatic stage results in a reversible decrease in dopamine level in the striatum up to the threshold (30%) and short-term motor disorders. In controls, although the dopamine level decreases under α-methyl-p-tyrosine administration, it does not reach the threshold level and is not accompanied by motor disorders. Thus, we proposed a new complex methodology for the development of preclinical diagnosis of PD.

Biography

Michael Ugrumov PhD, MD, is the Head of Laboratory of Neural and Neuroendocrine Regulations at the Institute of Developmental Biology RAS and Professor at the National Research University "Higher School of Economics" (Moscow, Russia), vice-president of the Russian Society for Physiology and the president of the Russian Neurochemical Society. He was a visiting Professor in Japan (Tokushima University Medical School), US (SUNY Upstate Medical University, Syracuse, NY), France (University P. et M. Curie, Paris; University of Tours), Germany (University of Ulm). His main interests are addressed to Neurosciences with focus on Developmental Neurobiology, Neuroendocrinology, and Neurodegenerative Diseases.

Access Models for the Chemical Biology Infrastructure EU-OPENSCREEN ERIC

Wolfgang Fecke
EU-OPENSCREEN ERIC, Germany

Abstract

The European Research infrastructure EU-OPENSCREEN was founded in April 2018 with support of its member countries and the European Commission. Its distributed character offers complementary knowledge, expertise and instrumentation in the field of chemical biology from more than 20 European partner institutes while its open access working model ensures that both academia

and industry can readily access our collection of more than 100.000 chemical compounds, equipment and screening data. Biologists, chemists and data scientists in Europe and beyond can use our screening library, high-throughput and medicinal chemistry technologies for the identification and optimization of novel chemical tool compounds and therapeutic candidates.

Biography

Wolfgang Fecke obtained his PhD in 1994 at the Heinrich-Heine University in Düsseldorf on the function of protein complexes in mitochondria. After a postdoctoral fellowship at Columbia University, he worked as group leader in several pharmaceutical and biotech companies in the UK, Germany and Italy on drug discovery programs, always with a focus on assay development, screening and lead profiling. In 2015 he helped to establish the translational Discovery Sciences group at the VIB institute in Leuven, Belgium. Wolfgang returned to Germany in 2018, becoming director of the new European infrastructure EU-OPENSCREEN in Berlin.

Protoplast Fusion: A Novel Way to Create Antibiotics Against Multi-Drug Resistant Microbes

Omar Bagasra
Claflin University, USA

Abstract

The emergence of multiple drug resistant microorganisms (MDRs), especially methicillin resistant Staphylococcus aureus (MRSA) and oxacillin-resistant Staphylococcus aureus (OMSA) are two leading causes of hospital-acquired infections and have become a serious global problem. With the antibiotic pipeline drying up and lack of development of new antimicrobials, there is an urgent need to develop new ways to ensure antibiotics work for future generations. One reason for the lack of new antibiotic development is the paucity of new microbes and fungi that can generate them. Our laboratory has developed a novel patented technology to create new microorganisms in vitro that can create antimicrobials. This is done by fusing protoplasts from different Clostridia spp. to create genetically unique chimeras that produce unique chemicals with potent anti-bacterial effects. We have used this powerful new synthetic biology technology to create new antibiotics effective against MRSA and other multidrug resistant microorganisms. Our preliminary data has demonstrated successful generation of chimeras that exhibit antibacterial effects against MRSA, and MRD E. Coli and P. aeruginosa. Currently, we are fusing various species of Streptomyces to create more anti-bacterials. We believe that this innovation will bring: 1) a powerful new technology to create new antibiotics against MRSA and other MDRs, and 2) structural identification of these new antibiotics, which may result in new future antibiotics to combat these human pathogens. The rationale is that new antibiotics are critically needed to fight microorganisms, particularly MRSA and other MDRs.

Biography

Omar Bagasra received his Ph.D. from University of Louisville, KY in 1979 and completed his Medical education at UACJ and Temple University, Philadelphia, PA. He did his residency in Pathology at Hahnemann and Temple University and fellowship in Clinical Laboratory Immunology at St. Christopher's Hospital for Childrens. He has held full professorships at several large medical universities including Hahnemann University, Thomas Jefferson Medical College. Currently, he serves at Professor and Director of South Carolina Center for Biotechnology at Claflin University.

New Insights into the Congenital Clotting Disorders

Guglielmo Mariani
Westminster University, London, UK

Abstract

Disorders of the clotting system are a group of disorders that have in common the difficulty of yielding an effective blood clot; as a result, a mild, moderate or severe hemorrhagic tendency can occur. The bleeding syndrome may be the result of a congenital, inherited defect of may be acquired, i.e. be caused by intercurrent illnesses. Within the realm of the inherited bleeding syndromes, hemophilias were the first to be recognized, but the most frequent (is the disease described by von Willebrand, which comprises a number of variants displaying different phenotypes and subtypes. A number of other, rarer, bleeding syndromes share with vonWillbrand's Disease (vWD) the autosomal modality of inheritance. Only the Hemophilias (A & B) are characterized by an X-linked recessive

manner. Recently, large and collaborative studies have provided epidemiological data that demonstrate that bleeding disorders may, at least in some cases, be more frequent than previously thought. Other collaborative studies provided insights useful for the laboratory and clinical diagnosis, others again have opened the door to specific, more effective and less harmful therapies. We will review these collaborative studies that have enormously increased the interest in this area of research as testified by the very large and qualified scientific papers published.

Biography

Guglielmo Mariani, MD, has been Professor of Hematology at the Universities of L'Aquila, Palermo and Rome, Italy. Since 2015 GM has been teaching at the U. of Westminster in London, UK and at the LuDes University, Lugano, Switzerland. GM has been coordinator of or PI in numerous EU projects focused on the modernization of teaching in eastern European and Southern Caucasus universities and is the author of 380 publications mostly in high-ranked scientific journals. GM is also author of novels and books of scientific communication on Jogging (published) and Blood (unpublished).

Blood CD34 Cells Dynamics in Diabetic Patients with or without Cardiac Diseases: The Bridge Influence between Nuclear Factors STAT1/6 and PPAR ∞/α

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[2] *University Hospital Habib Bougatfa of Bizerte, Bizerte, Tunisia*

Abstract

Accumulating evidence considered the CD34 as a hematopoietic precursor biomarker for stem Leukocyte; CD34 could be used to follow and analyze the stem dynamic during the immunophysiology pathology stress like the Chronic Diabetic type 2 Diseases. In the same time two class of transducer nuclear factor are chosen, STAT and PPAR. They distinguish by the nature of their liguands, their signaling ways but could implicate together in the energetic stress phenotype of cells. The aim of this work is to study the dynamic of Blood PBMC –CD34 cells in two group of chronic diabetic human type 2 patients with or without cardiac disease; CD 34 mobilization in relation to the couple STAT1/6 and PPAR∞/α expression. For this PBMC from 20 patients of each group are harvested by Ficoll-paque gradient, the expression of CD34 , STAT1/6 and PPAR ∞/α are detected by the quantitative reverse transcription polymerase chain reaction (q-RTPCR) using a specific primer. Our work show a pronounced significantly expression of CD34+ in Diabetic group with Cardiac Vascular Disease (DCVD) than Diabetic case (D) with respectively (1,7 ±0,9) vs (1,17±0,06) p≤0,05. The DCVD group stress promote clearly the expression of STAT1with (o,81±0,08) vs (0,12±0,40) for (D) group. This profile is associated with an advantage expression of PPAR α (0,89±1,37) vs (0,76±1,62) to gammaPPAR. However in D group patients the nature of stress promote STAT6 expression with (1,04±0,32) vs(0,35±0,24) for DCVD . This situation favorite significantly more PPARgamma expression (2,7±0,8) against (0,99±1,06) for PPAR alphap≤ 0,003 .The distinguish stress phenotype marked by the bridge between PPAR ∞/α and STAT1/6 demonstrate that CD34 Stem cells value is sensitive and adapt to immunophysiology status linked to its endogen and exogen liguands. Finally, our model seems interesting to explore this type of stress situation.

Biography

Oueslati Ridha began his professional work as short-term replacement assistant at Thameur Tunis Hospital in Hemato-Immunology laboratory. In 1990 he transfer to Military Principal Hospital at Tunis as contract assistant in Hemato-Immunology department until 1993.His first government position is as Professor associate (MCA) in Immunology at this department until 2000. In this period Dr Oueslati create the laboratory of cellular immunology and a cryo cell line Bank. In the same way he introduced the screening of the serological Tumor markers and cell immunologic profile for kidney and cardiac transplant patients. He transfers in the end 2000 to Sciences Faculty of Bizerte- Jarzouna as MCA in Immunology and from 2005 as Professor of Immunology and Microbiology. As a Teacher, he promotes the academic formation in Immunology and Microbiology. The gradual evolution of its academic activity permits him to introduce the immunologic comparative and Molecular microbiology at Master level linked to Inflammatory pathways. In parallel he create the immune Microbiology laboratory in this faculty ; Followed by the national Unit research in Immune Microbiology Environmental (IME) converted later in IMEC (Cancerogenesis).More than thirty master and Thesis are already out of this unit , work permit us to collaborate with prestigious international team linked to their thematic. Prostate, Mammary and colic pathways tumors are their targets. The immunological inflammatory pathways in target tumors cells are our reference. The comparative with some stem cells is an active form and markers. Our works are discussed and some from them are

published in the international journals. More than seventy international paper are numbered. The dynamic form integrative way of IMEC is open to its city, Town, country and the international groups.

Oueslati is actually an International Reviewer and Bored Editor in prestigious journals as Nature Reports Journal, Global Journal of of Immunology and Allergic Diseases, Applied Clinical Research Clinical Trials and Regulatory Affairs.

Erythroid Progenitor Targeted Gene Therapy using Bifunctional TFR1 Ligand-Peptides in Human Erythropoietic Protoporphyria

Laurent Gouya[1, 2, 3, 5*], Arienne Mirmiran[1], Caroline Schmitt [1, 2, 3] Thibaud Lefebvre[1, 2, 3] , Hana Manceau [1,4], Raed Daher[1], Vincent Oustric[1] , Jean-Jacques Lacapere[6] , Boualem Moulouel[3] , Herve Puy [1, 2, 3] Zoubida Karim[1, 2] Katell Peoc'h[4] Hugo Lenglet[1] Sylvie Simonin[3] Jean-Charles Deybach[1, 2, 3] and Gael Nicolas[1]

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[4]*Laboratoire de Biochimie, France*

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[6]*Universite Pierre Marie Curie, CNRS, France*

Abstract

Erythropoietic protoporphyria (EPP) is a hereditary disease characterized by a deficiency in ferrochelatase (FECH) activity that is responsible for the accumulation of protoporphyrin IX (PPIX). EPP manifests as severe photosensitivity without etiopathogenic treatment. Ninety-five percent of patients present a hypomorphic FECH allele trans to a loss of function FECH mutation, resulting in a reduction in FECH activity in erythroblasts below a critical threshold. The hypomorphic allele promotes the use of a cryptic acceptor splice site, generating an aberrant FECH mRNA, is responsible for the reduced level of the wild-type FECH mRNA and ultimately FECH activity. We have previously identified an antisense oligonucleotide, AON-V1 (V1) that redirects splicing to the physiological acceptor site and reduces the accumulation of PPIX. Here, we developed a new strategy to deliver V1 to the erythroid progenitors using transferrin receptor 1 (TRF1) as a Trojan horse. We designed a bifunctional peptide (P1-9R) including a TFR1-targeting peptide coupled to a nine-arginine cell-penetrating peptide that facilitates the release of the AON from TFR1 in endosomal vesicles. We demonstrated that P1-9R/V1 nanocomplexe promote the efficient and prolonged redirection of splicing towards the physiological splice site and subsequent normalization of the WT FECH mRNA and protein levels. Finally, P1-9R/V1 nanocomplexes increase WT FECH mRNA production and significantly decrease PPIX accumulation in primary cultures of differentiating erythroid progenitors of an overt EPP patient. P1-9R is the first method designed to target erythroid progenitors and represents a potentially powerful tool for the in vivo delivery of therapeutic DNAs in many erythroid disorders.

Biography

Laurent Gouya worked as a Full Professor in Genetics (Paris VII University) in the year of 2006. In 2015 he was the Head of the department of biochemistry and molecular genetics University Paris Diderot Hospital Louis Mourier after the he worked as a Head Centre de Reference National Maladies Rares Porphyries. In 2014 he worked as Co-Head of the research unit Inserm U1149 CRI and Executive committee DHU UNITY. His Hospital Activities at APHP, Louis Mourier Hospital, Colombes includes Biochemistry and Molecular Biology department, Genetic counseling in the reference center for Marfan disease and related syndromes, Head of clinical genetic department, Head of department of Biochemistry and Molecular Biology, Head of French Referral Center on Porphyria.

Inhibition of Calcium Carbonate Scaling by Precipitation using Secondary Nucleation Coupled to Degassing with Atmospheric Air

Hamza Elfil*, Khaled Touati, Hayet Cherif, Nesrine Kammoun and Montassar Jendoubi

Carthage University, Tunisia

Abstract

The hydrological system of southern Tunisia is mainly based on the waters of underground aquifers, which currently constitute the major exploitable resources. However, the use of these waters has caused scaling problems. The formation of undesirable deposition, especially calcium carbonate precipitation, is mainly influenced by the high temperature and CO_2 escapement in atmospheric air which is inevitable during cooling process. Scaling by calcium carbonate precipitation is an important challenge during exploitation of geothermal water or geothermal district heating systems. Water decarbonation is a promising solution to mitigate calcium carbonate precipitation and reduce the scaling risks. In the current work, the reduce of scaling power of geothermal water by coupling secondary nucleation to atmospheric air bubbling was investigated. For that, several operating conditions were studied and optimized such as air and water flows, hydrodynamics, residence time, and bubbling rate. The calcium carbonate secondary germination is induced by seeding with aragonite crystals. Synthetic and real solutions were tested. To reveal the efficiency of the proposed process, the addition of chemicals was avoided. Results showed that, using continuously operating reactor and under optimized operating conditions, the decarbonation rate of a calcium carbonate solution tested (4 mmol/L of $CaCO_3$, T = 50°C) can exceed 30% without using chemicals. It was also shown that he residence time in the reactor is relatively short (2.5 min) and the protection of the walls from precipitate adherence was guaranteed.

Biography

Hamza Elfil is a Research Professor in Chemical Process Engineering and the Director of Laboratory of Natural Water Treatment in Water Researches and Technologies Center (CERTE), Ecopark of BorjCedria, Tunisia. In 1999, he got his PhD about Process Engineering - Water Treatment from INSA Toulouse, France. His main research interests are related to Scaling phenomena, Water membrane desalination, Management of desalination waters, and water quality.

Lecithin Extracted from a Marine Source in the form of Nanoliposomes as New Generation of Active Vector

Elmira Arab-Tehrany[1]*, Shahrzad Latifi[2], Cyril Kahn[1], Miche[l] and Linder[1]

[1] *Universite de Lorraine, France*

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Abstract

Phospholipids, the main constituents of liposomes, in brain membranes, contain different polyunsaturated fatty acids (PUFA) that are critical for nervous system function and structure. The main PUFA in the brain are docosahexaenoic acid (DHA), derived from the omega-3 fatty acid, alpha-linolenic acid, arachidonic acid (AA) and docosatetraenoic acid, the last two of which derived from the omega-6 fatty acid, linoleic acid. Several studies have demonstrated the positive relevance of purified omega-3 and omega-6 PUFAs in brain structure and function. In this work, we developed highly specific natural nanoliposomes, extracted by low temperature enzymatic process without any organic solvent. Administration of these nanoliposomes to primary cortical neurons results in a significant increase of metabolic activity. Furthermore, the nanoliposomes from salmon lecithin promote neurite outgrowth and dendritic arborization. Our data suggest that the variety of fatty acids composition, especially mono and polyunsaturated fatty acids and different lipid classes, can significantly improve the formation and activity of neuronal networks. Nanoliposomes derived from natural sources such as fish can be used as carrier systems to transport MUFAs and PUFAs. These systems will not only be prepared from lecithins in an innovative green enzymatic extraction process that does not involve any solvents, but promise to be very efficient drug carriers.

Biography

Elmira Arab-Tehrany is a Full Professor at Lorraine University, and team leader in nanovectorisation in her laboratory. She realized two international mobilities: Italian Institute of Technology (Gevova-Italy) and Harvard-MIT (USA). She provides her expertise as member of the committee at the French Agency for food safety since 2009, and as expert of scientific projects for Romanian and Poland Governments since 2009. She organized the international conference of Lipids and Brain (Lipid and Alzheimer disease) in 2017. She has coauthored more than 80 scientific publications in international journals (2933 citation, h-index:27), 7 book chapters, 16 invited speakers, 50 oral presentations

Development of a Platform for Generation of Small Bispecific Affinity Domains

Sarah Lindbo*, Emma von Witting and Sophia Hober
KTH Royal Institute of Technology, Sweden

Abstract

Engineered non-immunoglobulin proteins have proven to be well suited as alternatives to antibodies formany diverse clinical and biotechnological applications as these molecules can be engineered in vitro to affinities and potencies comparable to monoclonal antibodies. Furthermore, properties includingsmall size, high solubility and lack of disulfide bonds give these molecules advantageous characteristicsthat even challenge and outperform antibodies in certain applications. The small size improves thetissue penetrations but also allows for rapid systemic elimination via the kidneys. For diagnosticapplication this is an ideal scenario while for most therapeutics long serum life is desirable. Numerousstrategies have been developed to increase the in vivo half-life of small therapeutic proteins andassociation with albumin is one promising approach. Binding to serum albumin will increase thehydrodynamic radius of the protein as well as take advantage of the FcRn-mediated recycling of albumin.

We have engineered the albumin binding domain (ABD) into bi functional ABD-derived affinity proteins (ADAPTs) with the capability to bind albumin and simultaneously interact with a target. We believe thatthese ADAPTs will possess properties enabling both good tissue distribution and improved serum half-life. Careful selection and design of the randomized positions were crucial in order to engineer bi-specificity into the 5 kDa small ABD scaffold. Phage display has been applied to select ADAPTs thattarget clinically relevant biomarkers and simultaneously bind albumin. I will discuss our approach fordevelopment of bifunctional ADAPTs and give some highlights from our latest results.

Biography

Sarah Lindbo is a Postdoctoral researcher in Sophia Hober's research lab at the division of Protein Technology at Royal Institute of Technology, KTH, Stockholm, Sweden. She acquired a master of Science in Engineering in Biotechnology in 2013 and a PhD degree for research on small affinity domains for clinical applications in 2018 from KTH.

Factor VII Deficiency: Clinical Phenotype, Genotype and Therapy

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Abstract

Factor VII deficiency is the most common among the rare inherited autosomal recessivebleeding disorders: in the latest Italian Bleeding Disorders Registry reports, a frequency similar to that of Hemophilia B has consistently been observed. FVII deficiency is a chameleon disease due to the correlation between plasma levels of coagulation Factor VII and bleeding manifestations, less consistent than in hemophilias. Clinical phenotypes range from asymptomatic conditions——to severe life-threatening bleeds (central nervous system & gastrointestinal bleeding and hemarthrosis) occurring, namely, perinatally. In between, a bleeding phenotype similar to that of a mild platelet disorder is observed, which is, not rarely, undiagnosed or diagnosed late. A typical example is that of menorrhagia, a frequent symptom whose underlying cause may be found in adulthood leading to FVII deficiency diagnosis. Prediction of bleeding risk is based on multiple parameters that challenge disease management. Spontaneous or surgical bleedings require accurate treatment schedules, and patients at high risk of severe hemorrhages may need prophylaxis from childhood onwards. The aim of the current report is to depict an updated analysis of genotype and clinical & laboratory phenotypes in parallel, carried out in 347 subjects with an inherited Factor VII deficiency. Treatment options related to the clinical variants will be dealt with by the analysis of 175 treatments performed in 129 symptomatic individuals.

Biography

Guglielmo Mariani, MD, has been Professor of Hematology at the Universities of L'Aquila, Palermo and Rome, Italy. Since 2015 GM has been teaching at the U. of Westminster in London, UK and at the LuDes University, Lugano, Switzerland. GM has been coordinator of or PI in numerous EU projects focused on the modernization of teaching in eastern European and Southern Caucasus universities and is the author of 380 publications mostly in high-ranked scientific journals. GM is also author of novels and books of scientific communication on Jogging (published) and Blood (unpublished).

Chances and Challenges of Clinical High-Throughput Sequencing

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[2]*University of Zurich, Switzerland*

Abstract

High-throughput sequencing (HTS) is widely used for clinical applications such as the molecular diagnosis of Mendelian disorders. As the applied technology/workflow substantially affects the diagnostic yield, knowledge about the pitfalls and advantages of HTS technologies and analysis pipelines is crucial for the successful application of hitherto unprecedented large-scale genetic testing (PMID: 25820422). We address the chances and challenges of HTS in the molecular diagnosis of Mendelian disorders as well as assess the sensitivity/recall, precision, computation time, and disk footprint of four corresponding HTS analysis pipelines (PMID: 28916731). We exemplify the limitations of targeted (gene panel) and whole-exome sequencing (WES) as well as emphasize the potential of whole-genome sequencing (WGS) in the detection of single nucleotide variants (SNVs) and copy number variations (CNVs). In addition, we elucidate limitations of short-read HTS including the influence of homologous/repetitive regions (mappability 60×) instead of WES or panels and the inclusion of CNV analysis can contribute to increased diagnostic yield in molecular diagnosis with lifetime value. As long-read HTS may overcome limitations of short-read HTS, it is envisioned as the future of (clinical) sequencing (PMID: 29206278).

Application of HighResolution Melting for Spa Methicillin Resistance Staphylococcus Aureus and Shigella Sonnei Rapid Genotyping Method for Epidemiological Purposes

Waleed AbdulmotalibMazi[1,2*], Jun Yu[3], Amir Saeed[2,4], Vartul Sangal[2], Francois-Xavier Weill[5], Elizabeth Dickson[6] and Gunnar Sandstrom[2]
[1]*King Faisal Medical Complex-Taif, Saudi Arabia*
[2]*Karolinska Institutet, Stockholm, Sweden*
[3]*University of Strathclyde, Glasgow, UK*
[4]*Hail University, Saudi Arabia*
[5]*Institute of Pastur, Francez*
[6]*Scotttish MRSA Reference Laboratory, UK*

Abstract

High resolution melting (HRM) analysis has been used in laboratory medicine as acurate, rapid and cost effective scheme method. Methicillin resistant Staphylococcus aureus (MRSA) infections impose huge risk to public health in healthcare and community settings worldwide. Shigella sonnei has been predominantly responsible for dysentary worldwide. The organism has only one serotype and is genetically homogeneous. We evaluated MRSA spa typing and introduced new tools for Shigella sonneil genotyping using HRM analysis for epidemiological purposes. Fifty clinical MRSA isolates were selected randomly from Scotland, Brazil, Sudan and Saudi Arabia. Methicillin-resistant phenotype was determined in accordance with BSAC standards using the Vitek 2system. Ten Shigella sonnei DNA samples were provided by Institut Pasteur, France. Primers for the polymorphic X region of the spa gene and the six single nucleotide polymorphisms (SNPs) within kduD, deoA, emrA, fdX and menFwere amplified by colony PCR using the SensiMix HRM kit, and the melting temperature (Tm) and melting curves of the amplicons were analyzed in close tubes using a Rotor-Gene 6000 instrument.

Fifteen spa types detected each had a distinct melting temperature (Tm) that unambiguously assigned 44 isolates. Both t008 and

t2770, as well as t311 and t021 spa types, shared the same Tm .

The first set run separated lineages I, II and III with distinctive melting curves and the Tm of each allele was at least a half degree away from that of other alleles. The second set run distinguished the sublineages IIIa, IIIb and IIIc with distinctive melting curves.

Analysis of Mutation Rate of 17 Y-Chromosome Short Tandem Repeats Loci using Tanzanian Father-Son Paired Samples

Fidelis Charles Bugoye[1*], Elias Mulima[1] and Gerald Misinzo[2]
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[2]*Sokoine University of Agriculture, Tanzania*

Abstract

The interpretation of DNA evidence in forensic analysis and paternity testing is based on the similarities or differences at a genetic loci used. Since the spontaneous mutations in the germline of the putative father at any genetic marker locus used in the analysis can lead to an erroneous exclusion. Therefore the aim of the present study was to determine the mutation rate of Tanzanian population using 17YSTRs loci commonly used in forensics. In our study, hundred unrelated father-son buccal swab sample pairs collected from consented Tanzanian population were examined to establish mutation rates using 17 Y-STRs loci of the AmpFlSTRYfiler kit commonly used in forensics.

Father-son pair biological relationships were confirmed using 15 autosomal STRs markers and found to be paternally related. Using 17 YSTRs loci, a total of four single repeat mutational events were observed between father and sons. Two mutations resulted in the gain of a repeat and the other two resulted in a loss of a repeat in the son. All observed mutations occurred at tetra nucleotide loci DYS389II, DYS385a, and DYS385b.

The locus specific mutation rate varied between 0 and 1.176 x10−3and the average mutation rate of 17Y-STRs loci in the present study was 2.353x10−3 (6.41x10−4-6.013x10−3) at 95% CI.

Based on the findings of the observed mutation rates in this study, the precise and reliable understanding of mutation rate at Y-chromosome STR loci is necessary for a correct evaluation and interpretation of DNA typing results in forensics and paternity testing involving males.

Regulation of Gene Expression for Oil Accumulation System in Oleaginous Yeast Lipomyces starkeyi

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[2]*Niigata University of Pharmacy and Applied Life Sciences, Japan*

Abstract

In Biotechnology field, production of oil by microorganisms is attracting an alternative to petroleum, and oleaginous yeasts are considered as one of the effective measures because of their high oil accumulation abilities. Among the oleaginous yeasts, Lipomycesstarkeyi is known to have great industrial potential for oil accumulation, and its products are expected to use for industrial field as functional food and biodiesel ingredients. Recently, the artificial control of oil accumulation system in L. starkeyi have been investigated, however, the amount of oil produced by L. starkeyi is insufficient for industrial use yet.

In this study, we applied our developed computational approaches to seek the target genes for the regulation of oil accumulation in L. starkeyi. The whole genome of L. starkeyi have been uncovered at 2016 and their triacylglycerol biosynthesis pathways have been estimated. To infer the target genes which regulates the oil accumulation system in L. starkeyi, we applied our developed network analysis methods to systematic measured expression profiles. First, we complied dozens of time-series data measured in several types of mutant strains in L. starkeyi with high oil accumulation abilities. After that, we developed some statistical methods combined with theoretical operation and signal processing to estimate the related genes with oil accumulation. Finally, we applied network inference methods based on Structural Equation modelling. From our analysis, we selected 14 genes as regulatory factors for oil accumulation

in L. starkeyi, and only 6 genes among them were considered as candidates to affect the volume of oil accumulation.

Biography

Sachiyo Aburatani is a vice director of Com. Bio Big Data Open Innovation Lab. in National institute of Advanced Industrial Science and Technology (AIST). Her field is systems biology and computational biology.

Molecular Mechanisms of Tolerance to Toxic Metal Ions in Thermus thermophilus for Biosensor Development

Gabriella Fiorentino[1*] Giovanni Gallo[1], Immacolata Antonucci[1], Danila Limauro[1], Patrizia Contursi[1], Jose Berenguer[2] and Simonetta Bartolucci[1]

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[2]*Universidad Autonoma de Madrid, Spain*

Abstract

The exploration of life in extreme environments has led to the isolation of a large number of hyperthermophiles able to cope with toxic metals and even to use them for their metabolism (1). A detailed understanding of the molecular mechanisms responsible for resistance to toxic metals is of growing interest also because it is crucial to develop biosensors for the detection of these chemicals in the environment or to enhance bioremediation strategies. However, such knowledge is still at an age of infancy.

In the last years, we started an investigation on the mechanisms of arsenic resistance in the thermophilic bacterium Thermus thermophilus HB27 originally isolated from a Japanese hot spring. This microorganism exhibits tolerance to both arsenate (V) and arsenite (III) and owns, interspersed in its genome, genes putatively involved in the arsenic resistance. Here we describe in vivo and in vitro studies performed to discover the main players involved in the mechanism and regulation of arsenic resistance in T. thermophilus; they are: an arsenate reductase (TtArsC), an ArsR/SmtB transcriptional repressor and a Zn^{2+}/Cd^{2+} dependent membrane ATPase (2, 3, 4). How such knowledge can be translated towards the development of robust enzyme-based and cell-based biosensors for the detection of arsenic in the environment will be discussed.

Session II: Agricultural Biotechnology

Soil Salinization: Approaches to Ameliorate Salt Stress Adaptation in Plants

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[2]*University of Natural Resources and Life Sciences, Italy*

[3]*Instituto di Ricerca sugli Ecosistemi Terrestri, Italy*

Abstract

The increasing of soil salinity, due to inappropriate agricultural management, decreases the cultivable areas, by turning fertile fields to marginal lands. Any modification of salt concentrations in soil may impose severe constrains on plant growth, which cause loss of crop yield. In these soils, plant growth is highly reduced because of plant water deficit and nutrient imbalance; at cellular level the osmotic stress, the production of reactive oxygen species (ROS), and decreasing of chlorophylls amount affect metabolic activities. In plant, salt stress can be counteracted by efficient physiological and morphological adjustments, elicited by stress signaling mechanisms. Different approaches can be used to ameliorate salt stress tolerance in sensitive species. The way to tackle this issue will be discussed by considering the responses obtained in cultivars of the crop species Brassica napus L., exposed to elevate saline conditions.

Biography

Cinzia Forni is an Associate Professor of Botany, and Group Leader of the Laboratory of Botany and Phytotechnologies of the Department of Biology at the University of Rome "Tor Vergata". She is the Author of 92 papers and 136 Contributions to National and International Congresses also as invited speaker. Co-author and Co-Editor of University Textbooks. She is referee of international journals, member of the Editorial Board of Journal of Plant Science and Phytopathology, and reviewer of Italian and International research projects. In October 2012 she was visiting scientist at The Hebrew University of Jerusalem, R. H. Smith Institute of Plant Sciences and Genetics in Agriculture, Rehovot, Israel. In October 2015 she was visiting professor at the Dept. of Biology, University of Waterloo, Waterloo, Ontario, Canada. She has over 30 years of experience in plant biology and physiology. Her main current research projects are dealing with abiotic stress response in plants, synthesis of plant metabolites with antitumor activity and phytoremediation of wastewaters.

Genetic Diversity in Red Clover (Trifolium pretense L.) Cultivars of Russian Breeding Revealed by SSR Analysis

Shamustakimova Anastasia* and Klimenko Irina

Federal Williams Research Center of Forage Production & Agroecology, Russia

Abstract

This study set out to: (1) analyze the genetic variability of red clover cultivars in a collection of forage crops from Federal Williams Research Center of Forage Production & Agroecology (FWRC) using SSR primers, (2) select molecular markers that may be useful for identification and certification of selected cultivars. Seven varieties of red clover, developed by breeders of Federal Williams Research Center, were analyzed. Genomic DNA was extracted from bulk-sample of 30 seedlings from each of 7 varieties, preliminary cultivated in Petri dishes during 7-days. PCR was carried out using a "touchdown PCR" program with the set of 7 microsatellite markers:

RCS0131, RCS3186, RCS1307, RCS3711, RCS3095, RCS4280, RCS2138 (Sato et al., 2005). Gene Analyzer and Excel Microsatellite Toolkit were used for the allele size estimation and for statistical treatment of the results, respectively. Amplification of the 7 SSR primers in the 7 red clover cultivars resulted in a total of 38 alleles, ranging in size from 111 to 257 base pairs. The number of alleles per locus ranged from 4 (RCS1307) to 9 (RCS3095), with an average of 1.62 alleles per locus. Markers detecting five alleles were most frequent. The expected heterozygosity level ranged from 0.73 to 0.84. The value of average polymorphic information content (PIC) for these markers was estimated to be 0.7. Despite the observed moderate differentiation between cultivars from FWRC, the unique bands in some tested varieties were identified. The selected markers may be useful for the identification and certification of red clover cultivars.

Biography

Shamustakimova Anastasia has completed her Master's thesis from Russian State Agrarian University, Russian Federation. Currently works at the laboratory of genome Analysis in Gene Pool Department. She is an author and co-author of the number of papers in academic journals.

Functional Characterization of the First Bioactive Peptide from the Archaeal Kingdom Showing Antimicrobial, Antifungal and Antitumor Activities

Angela Arciello[1], Martina Aulitto[1], Simonetta Bartolucci[1], Annarita Del Gatto[2], Eliana Dell'Olmo[1], Annarita Falanga[2], Bianca Farina[2], Roberto Fattorusso[3], Salvatore Fusco[1], Rosa Gaglione[1], Stefania Galdiero[2], Luciano Pirone[2], Emanuela Roscetto[3], Adriana Vollaro[3], Giovanni Smaldone[4], Laura Zaccaro[2], Anna Zanfardino[1], Maria Rosaria Catania[3], Mario Varcamonti[1], Eugenio Notomista[1] and Emilia Pedone[1] and Patrizia Contursi[1]*

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[2]Italian Research National Council, Italy
[3]University of Campania-Luigi Vanvitelli, Italy
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Abstract

Pathogenic bacteria and fungi easily develop resistance towards conventional antibiotics. Therefore new antimicrobials that do not lead to the selection of resistant strains, are urgently needed. Cationic antimicrobial peptides (CAMPs) are small positively charged peptides with an amphipathic structure, active against Gram-positive and Gram-negative bacteria and fungi. A novel (CAMP)-like peptide, namely VLL-28, was identified in the primary structure of the archaeal transcription factor Stf76. VLL-28 shows a broad-spectrum antibacterial activity and acquires a defined structure in the presence of membrane mimetics. This peptide localizes on the cell membrane as well as in the cytoplasm of Escherichia coli suggesting that it could exert its antimicrobial activity also on intracellular targets i.e. DNA and/or RNA thus interfering with fundamental metabolic processes. Interestingly, besides its antimicrobial activity, VLL-28 was found to exert antifungal and antibiofilm activities towards different species of Candida.

Finally, we have also found that VLL-28 selectively displays cytotoxic activity towards murine and human tumor cells and confocal microscopy data show that VLL-28 is internalized only into tumor cells. The VLL28 -induced cytotoxicity to cancer cells is exerted through a time-dependent activation pathway of apoptosis.

Calnexin and Calreticulin as Important Chaperones to Enhance Environmental Stress Tolerance in Plants

Maryam Sarwat[1*] and Altaf Ahmad[2]

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Abstract

Our country India is an agriculturally important country where about 600 million people are directly dependent on agriculture. Therefore, we must have a sustainable agriculture system in order to protect not only the livelihood of our countrymen but also the growing demand of food, fodder and fuel. Unfortunately, this is like a dream as we are suffering from the volatilities and vulnerabilities

of climate. Thus, production of resilient genotypes is the need of the hour. New genes are incorporated into various crop plants to their adaptability to adverse climatic conditions.

Our study on rice calnexin is one such attempt. We have elucidated new role of the Calnexin (CNX) protein in abiotic stress tolerance. This protein has a well known function in Endoplasmic Reticulum Quality Control Machinery. It is an integral membrane protein of endoplasmic reticulum (ER) and together with its luminal counterpart Calreticulin (CRT) constitute CNX/CRT cycle which is imperative for proper folding of nascent proteins. Its deficiency have reported to cause severe complications in organisms because of improper folding of proteins. CNX functions by maintaining calcium homeostasis within the cell. We have done heterologous expression of OsCNX (OsCNX-HE) in tobacco and the OsCNX-HE lines were observed to exhibit better germination under drought stress conditions. They are also showing better survival under dehydration stress conditions.

Keywords: Climate Change, ER Stress, Calnexin, Crops, Stress tolerance

Plant Growth Promoting Bacteria: A Strategy for Enhancing Growth and Protection to Tea Plants

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[2]*Cotton University, India*

Abstract

Tea (Camellia sinensis) is an important beverage crop extensively cultivated in Northeast India for its leaf. For plant growth promotion (PGP) and disease control, chemicals are commonly used in tea fields, which cause environmental pollution. Therefore, microbial control has drawn attention and has been considered as a potential alternative to chemicals inputs. Endophytic bacteria (EB) form a symbiotic relationship within the host plant and produces compounds useful for plants for their growth and protection. Various microbial inoculants have been developed and applied in different agricultural crops as biocontrol and biofertilizer agents. However, research on EB prevalent in Tea is still unexplored. This study is mainly focused in selection of potent endophytic bacteria prevalent in tea clones for PGP and antifungal traits.146 culturable EB were isolated using selective media and screened in-vitro for the production of PGP traits and antifungal metabolites against test Tea fungal pathogens.Qualitative and quantitative assays showed promising results for the PGP traits (IAA production, Phosphate solubilization, Ammonia production, ACC deaminase, and Nitrogen fixation). They exhibited antagonistic activity against phytopathogens. They produced chitinase, cellulase, amylase and protease enzymes that can degrade cell wall components of fungi.They may result in substantial decrease in the use of chemical fertilizer for the tea growing areas.This study can be utilized to explore the natural associations among endophytes and their hosts, and can be employed in future for sustainable agricultural crop production.

Biography

ShabihaNudrat Hazarika presently working as a Research Scholar at IASST, India. She received her M.Sc. in Molecular Biology and Biotechnology from Tezpur University. After my M.Sc. she worked as a Project fellow for 2 years. Thereafter,she worked as Assistant Professor at USTM in Department of Applied Biology for 2 years. she published 4 research papers in reputed journals. She also published 1 conference proceedings as corresponding author. One book chapter "Actinobacteria" has also been accepted for publication by Elsevier. She supervised 14 students in dissertation work during her teaching tenure. She is native of India, residing in Guwahati. Besides research she enjoys gardening, swimming, and cycling and keep interest in athletics.

The Use of a Recombinant Canarypox-based Equine Influenza Vaccine during the 2007 Australian Outbreak: A Systematic Review and Summary

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Abstract

Equine influenza (EI) is a major respiratory disease of the horse caused by the highly contagious

equine influenza virus (EIV). Together with Iceland and New Zealand, Australia had remained freefrom EI until August 2007 when the first cases of EI were diagnosed in New South Wales (NSW).This outbreak originated from the importation of one or more sub-clinically infected horsesfrom Japan, which was experiencing an EI outbreak at the time. These horses had respondedpoorly to recent vaccination or were infected during the immunity gap, a period of low protectiveimmunity that may occur in some vaccinated horses between the primary vaccination course and thefirst boost immunization. Vaccination had not been permitted in Australia, which had remainedfree of EI, hence the horse population was naïve to EIV. The breach of quarantine at the Eastern CreekAnimal Quarantine Station and the subsequent virus escape led to a significant outbreak, with over76,000 horses infected with EIV detected on more than 10,600 properties.The EIV strain A/equine/Sydney/2888-8/07 (H3N8), representative of the Australian EI outbreak,was classified as a member of the Florida sublineage clade 1 (FC1), typical of viruses isolated in NorthAmerica at the time and closely related to A/equine/Ibaraki/07, the representative strain from theJapanese 2007 EI outbreak. The Australian EI outbreak was first detected on 24th August, peakedon 1st October and lasted 5 months, with the last known case reported in December 2007. Around8% of the susceptible Australian horse population was infected with EIV.

Nanoscale Metal-Microbial Interface of the Metal Immobilizing Thermoacidophilic Archaeon Metallosphaera sedula

Tetyana Milojevic
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Abstract

A great variety of evolutionally diversified metallophilic microorganisms are equipped with unique capabilities and fascinating metabolic routes for manipulating minerals and dissolving them to access useful metals. Biomining is an increasingly applied biotechnological procedure for processing of ores in the mining industry (biohydrometallurgy), which relies on metal solubilization mediated by microorganisms. Metallo sphaerasedula is an extreme metallophilic and thermoacidophilic archaeon, which flourishes in hot acid and respires by metal oxidation. The metal-microbial interactions and microbial bioprocessing of terrestrial and extraterrestrial materials are the focus of the current study. The biotransformation of metal-bearing terrestrial and extraterrestrial materials performed by the extreme thermoacidophile Metallo sphaerasedula leads to the breakage of mineral structure and subsequent metal solubilization. Total soluble metals are significantly higher in cultures containing M. sedula grown on studied terrestrial and extraterrestrial materials than the abiotic controls, indicating active bioleaching. The nature of the mineral–microbe interface, where electron and mass transfer processes arise, is a key element in the understanding of the transition of geochemistry to biochemistry. Here the nanoscale interface between metal-bearing terrestrial and extraterrestrial materials and M. sedula is presented. Our results highlight unique metallophilic life in hostile environments extending the knowledge of microbial biogeochemistry. Based on these findings biohydrometallurgical processing of mineral ores can be further explored.

Biography

Tetyana Milojevic has her expertise in the area of metal-microbial-mineral interactions. Since 2014 she is a deputy head of the Department of Biophysical Chemistry at the Faculty of Chemistry, University of Vienna and a leader of Biochemistry/Space Microbiology group investigating biotransformation of terrestrial and extraterrestrial minerals and microbial survivability in outer

space environment. She has been leading an "excellence" Elise-Richter FWF research project to decipher metal-oxidizing machinery of the extreme thermoacidophile Metallospaerasedula.

Evaluating Bioreactor Performance of a Surface-Aerated Novel Horizontal Tubular Bioreactor with Spiral Impeller for Mammalian Cells

Rajesh Sharma, Sylva L. Schwager, Edward D. Sturrock, Susan T. L. Harrison and Siew L. Tai*
University of Cape Town, South Africa

Abstract

Mammalian cell lines, such as Chinese hamster ovary (CHO) cells, are often the preferred choice for expressing recombinant proteins because of their superior cellular machinery for post-translational modifications; however, they are very shear-sensitive. Liquid shear forces from stirring, bubble formation and direct sparging have drastic effects on the overall cell growth and productivity of the fermentation batch. Therefore, we designed and fabricated a horizontal tubular bioreactor (HTB) with a spiral impeller for the propagation of mammalian cells. The HTB was evaluated by growing CHO-K1 cells expressing angiotensin-converting enzyme (ACE) to assess its suitability for the growth of mammalian cells. . It was evident from the cell density achieved (4.2 x 106 cell/mL) and the morphology of the cells that the bioreactor conditions were conducive to cell growth and exerted low shear conditions. The maximum ACE activity measured was 465 mU/mL. The mobility of the proteins on SDS PAGE, using a polyclonal antibody to identify ACE, corresponded to that of purified somatic ACE. HTB with a spiral impeller provides a combination of surface aeration and impeller mixing with enhanced surface renewal rates which resulted in cell density of more than 4 x 106 cells/mL with extended longevity. It is predicted that in fed-batch mode, target cell densities of more than 10 x 106 cells/mL are achievable based on the obtained (kLa) value of 16 hr-1 for this reactor.

Biography

Siew L. Tai holds a B.Eng. Hons in Chemical Engineering, University of Manchester, an M.Sc. (cum laude) in Biochemical Engineering, and a PhD in Industrial Microbiology from Delft University of Technology. He carried out his post-doctoral fellowship at the University of Stellenbosch, South Africa. Following that, he joined the Biovac Institute in Cape Town where he was part of the team in building a vaccine manufacturing plant. His research encompasses the intricate networking between biotechnology and bioprocess engineering. His current research at UCT concentrates on the fields of bioprocess design, techno-economics, and bioproduct formation.

Xyloglucan Utilization by The Cellulolytic Bacterium Ruminiclostridium cellulolyticum

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[2]*Waters SAS, France*

Abstract

Ruminiclostridium cellulolyticum is a strict anaerobic and cellulolytic bacterium that secretes large multi-enzymes complexes called cellulosomes, which depolymerise the plant cell wall cellulose, and related polysaccharides. It was shown that this bacterium is also able to grow on xyloglucan. The latter is a major polysaccharide of the hemicellulose and is composed on a cellulose main chain, highly decorated with alpha-xylosyls and beta-galactosyls. Our studies have shown that at least four cellulosomal enzymes whose encoding genes are split on the chromosome, are involved in the extracellular depolymerisation of xyloglucan, but their end products are 4-glucosyl backbone xyloglucandextrins. These dextrins, which can be made of up to 9 monosaccharides, are subsequently imported in the cytosol via an ABC transporter whose solute binding protein displays an exceptionally affinity (KD in the nM range) for the 4-glucosyl backbone xyloglucandextrins. The genes encoding the ABC transporter form an operon located downstream of a 5-gene divergent operon, encoding a two-component system, and cytoplasmic beta-glucosidase, alpha-xylosidase and beta-galactosidase. Genetic studies have shown that indeed, the ABC transporter, as well as the two-component system are essential for growth on xyloglucan. We have also shown that these three enzymes can very rapidly depolymerise the imported dextrins into galactose, xylose, glucose and surprisingly the disaccharide cellobiose. We are currently investigating how the bacterium handles the simultaneous release in the cytosol of these four sugars by investigating the kinetic properties of the first enzymes involved in each corresponding

pathway (glycolysis, Leloir, Pentose phosphate, and cellobiose pathway).

Detection of CTX-M-15 Harboring Escherichia Coli Isolated from Wild Birds in Tunisia

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Abstract

The increased incidence of antibiotic-resistant E.coli is a public health problem world wide[1]. This investigation aims to analyse the potential role of wild birds, given their capacity of migrating over long distances, in the spreading of extended-spectrum β-lactamase (ESBL). One hundred and eleven faecal samples were collected from free-living wild birds in northern Tunisia. Samples were inoculated on MacConkey agar plates supplemented with cefotaxime. One colony per sample was selected. ESBL-producing E. coli were detected in 12 of 111 samples (10.81%). All cefotaxime resistant E. coli exhibited an ESBL-phenotype and expressed the CTX-M-15 enzyme. Four isolates co-expressed CTX-M-15 and TEM-1 enzymes. The twelve ESBL-producing E. coli were multi-resistant. After molecular characterization, by PCR and sequencing, our results showed that these strains harbored the tet(A), qnrB1 and aac(6')-1b genes, responsible for bacterial resistance to tetracycline and ciprofloxacin. The molecular typing of the ESBL producing E. coli highlighted different STs profiles including ST297, ST410 and ST349. These results appear to be common types in isolates from clinical, animal and environmental origin. The Same STs were detected in different birds which were sampled at a distance of 160 km from each other. In conclusion, this current investigation highlighted the potential role of wild bird's migration phenomenon and its implication in the worldwide spreading of multi-resistant bacteria, especially those identified as ESBL-producing strains. These findings could contribute to the development of a more integrated bio-surveillance system.

Soluble Recombinant Protein Production in Escherichia Coli: Effect of Temperature, Agitation Rate and Media Composition on Recombinant Lipase Solubility

Angela Liliana Meza*, Ingrid Yamile Pulido and Rosa Erlide Prieto
Universidad de La Sabana, Colombia

Abstract

Recombinant lipases derived from Pseudomonas have been overexpressed in E. coli several years ago due to their importance in the industrial area. However, most of recombinant enzymes form aggregates of insoluble protein that affects solubility and enzymatic activity. The strain E. coli BL21(DE3) used for this study contains the LipA gen from P. aeruginosa, however more than 30% of the recombinant lipase is produced in inclusion bodies. Therefore, the present study evaluated the effect of reducing temperature (5°C), the agitation rate(30rpm) and the addition of chemical chaperones (CH) to the medium on the total protein content, enzyme activity and solubility of the recombinant lipase in soluble (SF) and insoluble fractions (IF). Both fractions were evaluated using Bradford protein and p-NPPassays and analyzed with SDS-PAGE, respectively. Results were compared with a control group (18°C-110 rpm). The total protein concentration in the SF when glycerol was added to the medium increased by 4-fold (From 35,67 ± 0,17 to 159,9 ± 6,1 % ug/mL). Lipase specific activity in the SF was increased by 2,6 and 3-fold when proline and glycerol were added to the medium (From 21,37± 3,0 % to 57,15 ± 3,65% and to 65,15 ± 6,91% nmol/min/mL, respectively). The densitometric analysis showed that only proline increased by 1,25-fold the relative density of the recombinant lipase produced in the SF. Results are possibly explained due to a decrease in hydrophobic interactions at low temperatures and the stabilization of the recombinant lipase during the folding process when CH are added to medium.

Biography

Angela Liliana Mezais a Biologist with a huge interest in proteins obtained from natural products. During his career he worked with tumoral cell lines and the cytotoxic effect of compounds derived from natural products, He got very motivated after obtaining a merit in my undergraduate project. Two years of work experience in consulting of liquid chromatography (LC) and attendance to a LC course at Milford, USA. He is doing a Master's degree in Design and Process Management (Bioprocess specialization) thanks to a full scholarship. Very interested in sharing my knowledge, learning from other researchers and looking for PhD possibilities abroad.

Metabolic Fluxes-Oriented Control of Bioreactors: A Novel Approach to Tune Micro-Aeration and Substrate Feeding in Fermentations

Teresa Zangirolami[1*], Leandro Benedini[1,2], Viviane Gonçalves[3], Joaquin Cabrera-Crespo[3] and Felipe Furlan[1]
[1]*Federal University of Sao Carlos (UFSCar), Brazil*
[2]*Federal Institute of Sao Paulo, Brazil*
[3]*Butantan Institute, Brazil*

Abstract

Besides ethanol, several industrially relevant products such as vaccines, chemical derivates, and other biofuels are produced under limited oxygen conditions. These conditions are difficult to monitor and control using in situ dissolved oxygen measurements and conventional control strategies. In this work, a novel control strategy based on metabolic fluxes was set up and evaluated in micro-aerated ethanol fermentations. All the experiments were carried out with one replication each in fed-batch mode, using fresh commercial Saccharomyces cerevisiae as well as defined medium and glucose as carbon source. Simulations of a genome-scale metabolic model for Saccharomyces cerevisiae (iND750) were used to identify the range of oxygen and substrate fluxes that increased the ethanol fluxes. Oxygen supply and feed flow rate were manipulated to control oxygen and substrate fluxes as well as the respiratory quotient (RQ) at the identified levels. The results were further compared to the performance of other two fermentation strategies: a conventional "Brazilian fuel-ethanol plant" fermentation and a strictly anaerobic fermentation (ultra-pure nitrogen as inlet gas). The concentrations of ethanol, glycerol, and glucose were measured off-line by HPLC with refractive index detection. The proposed cultivation control strategy showed the best average volumetric ethanol productivity (7.0 g.L-1.h-1), with a final ethanol concentration of 87 g.L-1 and yield of 0.46 gethanol.gsubstrate-1. The other fermentation strategies showed lower yield (below 0.40 gethanol.gsubstrate-1) and ethanol productivity around 4.0 g.L-1.h-1. The control based on fluxes was successfully implemented and could also be adapted and improved for other bioprocesses that require restrict aeration.

Biography

Teresa Zangirolami joined the Chemical Engineering Dept. of Federal University of Sao Carlos as a lecturer in 1989. She got her PhD in Chemical Engineering from the Technical University of Denmark in 1998. Back to Brazil, she engaged on research projects in the Biochemical Engineering field. Her scientific production comprises 45 papers, 2 patents, 1 registered software, 12 PhD theses and 16 MSc dissertations. She is the leader of the Cell Factories Lab (CeFaL), which has as main target to apply System Biology to find more efficient production routes for biological products and novel control strategies for bioprocesses.

Sewage Sludge Anaerobic Co-Digestion with Agricultural Straw and its Components: Experimental and Kinetic Study

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[2]*Henan Agricultural University, PR China*

Abstract

Co-digestion of sludge with straw is a promising technology to improve the performance of anaerobic digestion. Performance of straw (wheat straw, corn stalk) andthe individual component of straw (cellulose, hemicellulose and lignin) during anaerobic co-digestion withsludge was investigated to improve the understanding of co-digestion process and enhance the methane production. In the first group experiment, the objective was to explore the synergistic effects obtained from the combination of sludge and straw. The total methane released from sludge+wheat straw and sludge+corn stalk reached to 187.01, 157.33 mL/g VSadded, and the measured methane yield of sludge+wheat straw and sludge+corn stalk increased by 9.46% and 1.48% respectively when compared to the estimated methane yield. In the second group experiment, microcrystalline cellulose, xylan and klason lignin were employed for anaerobic co-digestion with sludge. The results showed that anaerobic digestion of hemicellulose was hydrolysed and acidified more quickly than that of cellulose. With the help of pH adjustment, the methane yield from hemicellulose (292.56 mL/g VSadded) performed much better than that of cellulose (259.13 mL/g VSadded). The cumulative methane yield of co-digestion of cellulose with sludge reached to 230.52 mL/g VSadded, which was 15.61% higher than that of hemicellulose co-digestion with sludge. The start-up and peak for methane formation were all shortened for the co-digestion compared to the individual digestion of components. It needs

to be noted that lignin exhibited as the most stable substrate among the three components regardless of individual digestion or co-digestion. The results would help develop the bio-chemical technology on utilization of agricultural wastes for methane production.

Key words: Co-digestion; Sludge; Straw; Cellulose; Hemicellulose; Lignin; Methane

Natural and "Green" Nanoemulsions from Chia Seeds (Salvia hispanica L.) as Potential Active Vectors

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Abstract

Polyunsaturated acids (PUFA) have been of particular interest in reducing risk of disorders that develop during aging. The content of PUFA in chia seeds (Salvia hispanica L.) and in particular, the ω-3 fatty acid, α-linolenic acid (ALA), represents about 60% of the total lipid content, one of the highest levels in known plant sources. Compared to traditional extraction methods, the use of enzymes simplifies the extraction process and reduces both energy and manufacturing expenses. The objective of this study was to develop an enzymatic water-based green method for the extraction of oil from chia seeds. Chia seeds of the ORURO variety were used that are adapted for growth and cultivation in France (Agrofün).Solvent- (Soxhlet and Folch) or enzymatic-based extraction methods were compared for their efficacy in the production of chia seed oil lipids. After extraction, chia seeds oil-derived nanoemulsions were prepared using sonication and homogenization techniques. Various analytical techniques were used to characterize the size and charge of the particles (dynamic light scattering), the encapsulation efficiency (HPLC), and fatty acid composition (gas chromatography). In this case, chia seeds oil-derived nanoemulsions could be used as active particles with high bioavailability for their functions, both for their inherent biological properties (ALA and PUFA-rich), and as vectors for molecules of interest.

Biography

Daria Kuznetcova was born in Vologda, Russia. Daria has an engineering diploma in "Technology of milk and dairy products" (Vologda State Dairy Farming Academy named after N.V. Vereshchagin, Russia) and Master degree in "World Economy" (Saint-Petersburg State University, Russia). Daria is currently working on her thesis project since 2016 in ITMO University, Saint-Petersburg, Russia with joint co-direction program with University of Lorraine, Nancy, France. Daria is a 2018 laureate of the Eiffel excellence scholarship program.

Session II: Nano Biotechnology | Biotechnology Trends | Biomaterials & Tissue Scaffolds

Zca – A Calcium-Dependent Protein a Domain that Allows for Purification of Antibodies with Elution at Neutral PH

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Abstract

ZCa is an engineered variant of the B domain of Protein A that contains a calcium-binding motif and has a calcium-dependent binding to IgG. In a purification setup this allows for elution of IgG at a milder pH, by using EDTA, than what is traditionally used to break the antibody-Protein A interaction.

Antibodies are of great importance in the fields of biotechnology, biology and medicine, where approved therapies based on monoclonal antibodies are ever increasing. Therefore, the need for robust and convenient purification processes is vast, and Protein A has since long filled this demand by being a high affinity, straight forward method yielding pure and concentrated product in a single purification step. However, the low pH required to break the interaction between Protein A and the antibody can be detrimental to

acid sensitive antibodies or Fc-fusion proteins which makes them unsuitable for this type of purification and in the end may hinder their development. Presented here is ZCa, an engineered variant of the B domain of Protein A, that displays a calcium-dependent binding to IgG antibodies which allows for elution of monoclonal antibodies using EDTA at neutral pH for IgG2 and IgG4 and at pH 5.5 for IgG1. Elution at these pH present significantly milder conditions then the low pH used in traditional Protein A purification. Thus, ZCa is a novel and exciting improvement for antibody purification, opening up for the purification of molecules previously too acid labile to take advantage of the specificity and convenience of Protein A chromatography.

Biography

Sara Kanje holds a Ph.D. in Biotechnology from KTH (Royal Institute of Technology) in Stockholm where she currently works as a researcher in Professor Sophia Hober's lab. Her work revolves around protein engineering, affinity proteins, antibody labelling and protein purification.

New Generation of PUFA-Rich Nanoliposomes: In vitro Neurotrophic Effect

Elodie Passeri*, Aurelia Poerio, Catherine Armand, Elmira Arab-Tehrany and Frances Yen Potin
University of Lorraine, France

Abstract

Polyunsaturated fatty acids (PUFAs) are required for the correct development and functioning of the brain. All fatty acids have a role in cells where they can act as regulators of gene expression, be used as precursor of lipid mediators or play a structural role. Fatty acids are the main constituent of cell membranes where they are present in the form of phospholipids. Quantitative and qualitative variations in membrane lipid composition play a crucial role in regulation of membrane function. In neurons, optimal organization and function of membranes is essential not only during synaptogenesis and neuronal development to form new connections between neurons, but also in adult brain to preserve synapses. Synaptogenesis is a series of events that transform the synaptic terminals into specialized membranes with precise functions. Different cell activities can influence the formation of new connections including axonal growth and dendrite formation, time-dependent pre- and post- synaptic proteins expression, and receptor formation and/or distribution. These activities influence synaptic plasticity, which depends on the lipid membrane composition and organization. Our hypothesis is that enrichment of neuronal membranes in PUFAs, in particular omega-3 PUFAs, can play a role in synaptogenesis and neuronal development by favoring membrane fluidity and synaptic plasticity. To enrich membranes in fatty acids we used "pure" nanoliposomes derived from fish or rapeseed lecithin that are naturally rich in fatty acids with a variable composition depending from their origin. Primary cultures of cortical neurons isolated from rat embryos were used to study the expression and localization over time of different proteins involved in synaptic function, neurite outgrowth, neuronal differentiation and cell survival. In parallel, the effect of PUFA-rich nanoliposomes was tested on the same markers of these functions. Our results show that these nanoliposomes are able to accelerate synapse formation and neuronal development and to promote neuronal survival.

Nanofunctionalized Hybrid Hydrogels for Wound Healing Applications

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Abstract

The massive progress in the tissue engineering field was further boosted by innovations in the field of hydrogels. Hybrid hydrogels, such as Interpenetrating Polymer Networks (IPNs) have emerged as advanced scaffolds for tissue engineering. Here, we present the fabrication of a natural IPN scaffold based on alginate and gelatin methacryloyl (GelMA) hydrogels nanofunctionalized with rapeseed nanoliposomes.

GelMA was produced from porcine skin gelatin by the substitution of the free amino groups with methacrylic anhydride. Lecithins, extracted with an enzymatic hydrolysis process, were probe sonicated to produce nanoliposomes. Dynamic light scattering technique was used to measure the size distribution and electrophoretic mobility of nanoliposomes. Stable IPN discs were formed by the initial crosslinking of alginate using CaCl2. This was followed by UV-crosslinking of GelMA. Nanomechanical properties of the

nanofunctionalized IPN system were characterized using Atomic Force Microscopy (AFM). Finally, standard scratch assay was used to investigate the IPN hydrogels interaction with human keratinocytes.

The nanofunctionalization with nanoliposomes (110 nm, -3.41mV) improved the mechanical properties of IPN hydrogels at the nanoscale level. Nanofunctionalized IPN hydrogels induced human keratinocytes growth and significantly increased the coverage percentage.

Overall, a natural nanofunctionalized scaffold was produced form alginate/GelMA IPN hydrogels and rapeseed nanoliposomes. The nanofunctionalization of the scaffold improved its nanomechanical and biological properties, which suggests that it can be used as a topical drug delivery system for wound healing applications.

Biography

Kamil ELKHOURY is pursuing a PhD in Biotechnology and Food Engineering at the University of Lorraine. His research is based on nanofunctionalizing natural based hydrogels with soft nanoparticles to control the cellular microenvironment and biofabricate complex tissue constructs. He received his Masters from the University of Birmingham, UK, and his Bachelors from the University of Balamand, Lebanon, both in Chemical Engineering.

Poster Pesentations

Tissue Engineering: Development of Scaffolds Composed for Gel of Aloe Vera and Fibroin, through a Device Recently Elaborate on behalf of Optimization of Dermo-Epidermal Equivalents

Pablo P. de Almeida[1*], Gerlane de S. P. da Costa[2], Thalia Henke[3], Mayra C. Corteletti[4] and Vytor H. Mendes[5]
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Abstract

For centuries humanity always searched to understand the constitution of the human body, as a result of these efforts there have been numerous advances, highlighting the ability to perform organ and tissue transplants, saving the lives of millions of people. But, considering the high incidence of cases of burns and cutaneous ulcers, it is evident that the availability of homologous skin currently is insufficient, making tissue engineering essential in the development of dermo-epidermal substitutes.

With the advances of biotechnology providing new treatments, in parallel with the development of technologies with higher processing rates and lower costs. It becomes the search for new techniques and materials with better physicochemical properties of great interest to the sector [1].

With the selection of materials and related equipment, this research constituted in the elaboration of a specific prototype for the development of scaffolds using a new combination of biomaterials, consisting essentially of gel of Aloe Barbadensis Miller and fibroin extracted from cocoons of Bombyx mori, preserving its molecular properties.

Acting on all stages of processing, the prototype is based on the removal of sericin and residues from cocoons, getting a fibroin structure, conjugated with certain amount of main solution, determined by a sequence of calculations, maximizing the similarities with native tissue.

Being its effectiveness evaluated by synthesis tests, it has been found that it has achieved the objectives, presenting great potential in biomedical applications, due to fast processing and better cost-effective, making the final product more accessible for those who they crave.

Biophysical Characterization of aFeruloil-CoA Synthetase and AnEnoyl-CoA Hydratase/Aldolase, Isolated from a Lignin-Degrading Consortium, Involved in the Conversion of Ferulic Acid to Vanillin

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Abstract

Ferulic acid (FA) is one of the potential vanillin precursors in the biotransformation process. FA can be converted to vanillin by the enzymes feruloyl-CoA synthetase (FerA3) and enoyl-CoA hydratase/aldolase (FerB2) in various microorganisms. In this study, we report the expression and purification of a FerA3 and an FerB2 isolated from a lignin-degrading consortium. The purified prokaryotic enzymes, FerA3 and FerB2, were characterized biophysically and biochemically. FerA3 forms a homodimer in solution and showed analyses notable secondary structure stability at alkaline pH values and high temperatures. FerA3 optimum pH and temperature were 9 and 37°C, respectively. Furthermore, the crystal structure of FerB2 was solved at 2.1 Å resolution. The results revealed that FerB2 is a hexamer composed of a dimer of trimers. These studies are important in the field of lignin valorization via conversion of FA to valuable molecules.

Keywords: feruloyl-CoA synthetase; enoyl-CoA aldolase; crystallization. Financial support: FAPESP- project 2017/16976-8.

Expression, Purification and Characterization of a Eukaryotic Lytic Polysaccharides Monooxygenase Belonging to the Family AA15 with Chitinase Activity

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Abstract

The lytic polysaccharides monooxygenases (LPMOs) constitute a new class of metalloenzymes copper-dependent involved in the biodegradation of recalcitrant polysaccharides, such as cellulose, via oxidative mechanisms, acting synergistically with cellulases and other hydrolases. These enzymes have attracted the interest of researchers due to its great potential for biotechnological applications, especially in the field of conversion of structural polysaccharides in bioenergy and bioproducts. In this study, a eukaryotic LPMO belonging to the family AA15 from the insect Coptotermes gestroi (named CgAA15) was cloned, successfully expressed in soluble form and purified by affinity chromatography from the periplasmic space of Escherichia coli. The structural stability of CgAA15 was characterized using biophysical techniques. Furthermore, the results have shown that the CgAA15 exhibited oxidative activity against chitin. These results should provide a useful basis for a better understanding of the mode of action of LPMOs from insects.

Keywords: lytic polysaccharides monooxygenases; insect; chitinase activity.

Financial support: Fundaçao de Amparo à Pesquisa do Estado de Sao Paulo (FAPESP) - 2017/17275-3.

Highly Sensitive Colorimetric Immunoassay Using Enzyme-Catalyzed Silver Growth on Gold Nanoparticle-Assembly Silica Nanostructure

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Abstract

Au nanoparticle (NP) based signal amplification of immunoassay has widely attracted because of the excellent characteristics of Au NP such as biocompatibility, chemical inertness and easy surface modification. However, the narrowness of dynamic linear range of

Au NP-based immunoassay limited the development of a highly sensitive and quantitative Au NP-based immunoassay. In this study, we developed a new method based on utilizing an alkaline phosphatase-catalyzed Ag growth on Au NP-assembled silica (SiO_2@Au@Ag). In the presence of antigen (IgG), the colorimetric signal of immunoassay was amplified by incorporating the reduction of 2-phospho-L-ascorbic acid to ascorbic acid under the catalysis of alkaline phosphatase (AP). Subsequently, the formation of ascorbic acid in solution convert Ag^+ ion to Ag making Ag shell on SiO_2@Au. As a result, the absorbance signal of SiO_2@Au@Ag increased with the target concentration. This method was demonstrated throughout the quantification of IgG as a target model based on the sandwich enzyme-linked immunosorbent assay (ELISA). This immunoassay with a detection limit of 1.4×10^{-13} M features hundred times higher dynamic range than that of conventional colorimetric immunoassays. Our signal-amplification method opens a new direction for highly sensitive in vitro diagnostics and detection of target proteins with the naked eye.

Biography

Eunil Hahm received the B.S. degree in Bioscience and Biotechnology from Konkuk university, Seoul, South Korea in 2014. He achieved his M.S degree in Bioscience and Biotechnology from Konkuk university, Seoul, South Korea in 08/2016. He is going to study Ph.D course from 2016 until now. His research interests include nanoparticles synthesis, Surface modification, Surface-enhanced Raman Spectroscopy (SERS) and their applications.

Genetic Diversity of Alfalfa Cultivars of Russian Breeding Revealed by Microsatellite Markers

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Federal Williams Research Center of Forage Production & Agroecology, Russia

Abstract

Cultivar differentiation using molecular markers to assess genetic variation may be of value for the future prospects of success in breeding programs and in protecting plant breeder's rights. The aim of this work were: (1) To study the genetic variability of alfalfa cultivars in a collection of forage crops from Federal Williams Research Center of Forage Production & Agroecology (FWRC) using SSR primers, (2) to select molecular markers that may be useful for identification and certification of selected varieties.

Materials and methods: Ten varieties of alfalfa developed by breeders of Federal Williams Research Center were analyzed. Besides, varieties: Sarga, Voronejskaya, Tambovchanka, Uzen from different geographical and climatic region of Russia were also included as controls. Genomic DNA was extracted from bulk-sample of 30 seedlings from each of 10 accessions, preliminary cultivated in Petri dishes during 7-days. We used the combined protocol for DNA-extraction: lysis buffer from SDS-method at the first step and the commercial Kit "DNA-extran-4" (Russian company "Syntol") at the next ones. PCRs were carried out using a "touchdown PCR" program with set of 6 microsatellite markers: RCS 2839, RCS 0004, RCS 4633, RCS 4982, RCS 4926, RCS 1303 (Sato et al., 2005). These SSR-markers were selected because the authors suggested the presence of macrosynteny between the genomes of red clover and the model legume M.truncatula. In turn M. truncatula and alfalfa exhibit nearly perfect macrosynteny between the two genomes and share highly conserved nucleotide sequences (Choi et al., 2004). Excel Microsatellite Toolkit v.3.1.1. (Kim K.S. and Sappington T.W., 2013) was used for the allele size estimation and for statistical treatment of the results.

Results: Amplification of the 6 SSR primers in the 14 varieties of alfalfa resulted in a total of 19 alleles, ranging in size from 50 to 230 base pairs. The number of alleles per locus ranged from a minimum of two (RCS 2839, RCS 1303 and RCS 4926) to a maximum of 6 (RCS 4633), with a mean of 4.3 alleles per locus. Allele frequencies for all varieties by locus in average were 41. The expected heterozygosity level ranged from 0.6 (RCS 2839, RCS 4926, RCS 1303) to 0.8 (RCS 0004, RCS 4633, RCS 4982). Polymorphism Information Content (PIC) for each locus was calculated and compounds a minimum of 0.3 for RCS 2839 and a maximum of 0.7 for RCS 4633. As a whole, we observed a moderate differentiation between alfalfa varieties of FWRC for the analyzed loci that reflects their genetic relationships. It was determined the markers, generating the unique bands in some tested alfalfa varieties (Selena, Vega, Sarga, Mira).

Conclusion: The obtained results provide the value information for practical use, including identification and certification of alfalfa varieties and preservation and maintaining the forages grass collection, of the Federal Williams Research Center of Forage Production & Agroecology (Russia, Moscow region), that consists at present of more than 4.500 accessions.

Metagenomic Study of Water Kefir Grains for the Development of Starter Culture and the Isolation of Microorganisms with Probiotic Potential

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Abstract

Water Kefir Grains (WKG) are a microbial consortium consisting of lactic-acid bacteria, acid-acetic bacteria, bifidobacteria, and yeasts, embedded in an exopolysaccharide. WKG transforms sucrose aqueous solutions (such as Panela and Chancaca, autochtonous from Colombian and Chile) in fermented artisan beverages that currently the industry is trying to commercialize, therefore, standardizing the process is necessary, which is affected by the WKG origin and the substrate. Next-generation sequencing (NGS), in one hand, gives information about the stability of the microbial community, in the other hand, the isolation and identification of the WKG microbiota allow to know which microorganisms can be cultured and study their probiotic potential. For the genomic analysis, we used two different WKG (from Colombia and Chile) and Panela and Chancaca-like substrate, at concentrations of 80 g/L in 500 mL shaken flasks. Fermentations were conducted with both substrate at temperatures of 21°C and 37°C, we took samples for NGS. Isolation of microorganisms and identification was achieved via MALDI-TOF mass spectrometry. As results, the sequencing analysis showed twelve different bacteria species with predominant Lactobacilli and six different yeast species, some microorganisms either are absent depending on the fermentation conditions or are present in great abundance in all conditions (the case of Lactobacillus casei, Lactobacillus hilgardii, and Lactobacillus ghanensis). The isolated microorganisms identified by MALDI-TOF were Lactobacillus casei, Lactobacillus pasacasei, and Saccharomyces cerevisiae. This information shows that the community stability is defined by the fermentation conditions and can determine the most useful consortium as a starter culture in biotechnological processes.

Biography

Laura Munoz is a Biotechnology Ph.D. candidate at the Molecular Microbiology and Environmental Biotechnology Lab at Universidad Tecnica Federico Santa María (Valparaíso, Chile). She is a Food Engineer from Universidad de Antioquia (Colombia), with humanistic and research formation, whit a lot of experience in the field as advisor professional and technical assistance in food safety and nutrition government programs. Her principal passion is development functional food with added value, especially foods that help people with fewer resources. The Panela is a food resource produced by farmers in Colombia, and Laura hopes that her research will be an opportunity to improve their economy.

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Impact of Genetics and Environmental Factors on Third Molar Agenesis

Giedre Trakiniene*, Dalia Smailiene, Kristina Lopatiene, Arunas Vasiliauskas, Antanas Sidlauskas and Tomas Trakinis
Lithuanian University of Health Sciences, Lithuania

Abstract

Aim: The purpose of this investigation was to determine the impact of genetics and environmental factors on third molar agenesis in twins.

Materials And Method: The study sample consisted of the same sex twin pairs with normal growth and development. Digital panoramic radiographs of the twins were evaluated by one orthodontist. Intra-examiner reliability was assessed by means of Kappa statistics, which showed perfect agreement (Kappa for all third molar agenesis was 1). The zygosity of the twins was established using 15 specific DNA markers.

Results: The study sample consisted of 284 same sex twins (172 monozygotic and 112 dizygotic), whose mean ages were 19.7±4.3 and 18.9±4.8 years, respectively. The monozygotic group consisted of 36.3% males and 63.7% females, while the dizygotic group consisted of 50.1% males and 49.9% females. The prevalence of third molar agenesis in monozygotic twins was 19.6%, which was higher than in the dizygotic twins group (15.50%) (p=0.004). In both groups, third molar agenesis was more frequent in the maxilla than in the mandible (p=0.000). Agenesis of the maxillary third molars was mostly affected by additive genetic factors (62-63%), with the common environment and the specific environment accounting for up to 25% and 13%, respectively. In contrast, agenesis of the lower third molars was associated with a higher additive genetic determination (81-83%), with the specific environment accounting for 17% to 19%.

Conclusions: the formation of the third molars follicle is strongly controlled by additive genetic factors.

A Next-Generation Sequencing Approach for Genetic Testing: Targeted Gene Panels

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Abstract

The introduction of next generation sequencing (NGS) has led to an exponential increase in number of reported disease-causing genes. It can be applied to the whole (genome or exome sequencing) or to selected parts of the genome (gene panels). In heterogeneous and complex diseases, not merely one gene but a number of genes are involved in a large phenotypic spectrum. Targeted sequencing of selected genes can significantly reduce costs and turn-around time for a genetic test while offering a higher coverage and more friendly analysis compared to WGS or WES.

We present a PCR-based primer panel to amplify and sequence coding exons of 15 PD-associated genes. Parallel sequencing using the PGM Ion Torrent platform was used to detect mutations in 92 blood DNA samples from PD patients. After bioinformatics analysis and filtering, 95.13% coverage of the targeted region was obtained at >40-fold mean coverage. The results revealed 44 previously documented variants in these 15 genes, with five revealed as pathogenic. We also discovered six novel variants, five of which had an in silico prediction of being pathogenic.

Using a next-generation sequencing panel of 15 Parkinson-associated genes can lower the medical costs greatly while it allows identifying both pathologic and new undescribed mutations.

Biography

The Genomics Platform (GP) offers a personalized Service to the scientific community, both to clinicians and researchers. Transcriptomics and genomics can be studied with microarray, qPCR or NGS techniques. Users of GP are advised for the appropriate methodology implementation of experiments. Gorostidi has been leading the GP of Biodonostia Health Research Institute (Donostia, Spain) for several years. She participates in national and international consortiums and collaborates in many research projects of different areas as neuroscience or oncology. She has been specialized in massive sequencing and gene expression analyses.

New Episomal Expression Vector for Protein Directed Evolution in Yeast Pichia pastoris

Alexander Schmidt[1,2*], Yury Kil[1], Vladimir Sergeev[1,3] and Marina Surzhik[1]
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[2]Kurchatov Institute, Russia
[3]Peter the Great Saint-Petersburg Polytechnic University, Russia

Abstract

Directed evolution has proved to be an effective strategy for improving stability and activity of proteins for industrial and therapeutic applications. This technique requires efficient methods for creating DNA libraries of mutant genes, an expression system that is suitable for the protein under study, and a reliable and fast method for clone selection. Pichia pastoris is well established as a host for high-level recombinant protein biosynthesis. This methylotrophic yeast has many of the advantages of higher eukaryotic expression systems such as protein folding and posttranslational modification. Moreover, because it is a simple microbe, high-throughput laboratory protocols involving P. pastoris can take advantage of the robotics and other automation equipment.However, most P. pastoris expression plasmids are designed to integrate into the host chromosome, which results in primary tranformants with heterogeneous expression levels of the gene of interest and hence are not as amenable to high-throughput screening projects.

We have designed new episomal expression vector pPEHa. This vector contains the alpha-factor signal sequence that ensured the secretion of protein of interest into the culture medium. The S. cerevisiae HIS4 gene allowed selection of transformants on the basis of histidine independence, rather than zeocin resistance, which significantly reduced the cost of screening. A high expression level of the protein of interest was driven by the strong AOX1 promoter. Key characteristics of plasmid such as copy number, stability and transformation efficiency were determined. The utility of the vector was tested by screening a mutant gene library of a glucoamylase enzyme.

Scale Up of Live Biotherapautics Production for Reproductive Health in South Africa

Marijke Fagan-Endres[*], Obakeng Jona, Jo-Ann Passmore and Sue Harrison
University of Cape Town, South Africa

Abstract

Bacterial vaginosis is a highly prevalent inflammatory condition, characterized by the absence of protective Lactobacilli commensal species and their replacement by vaginal pathogens such as Gardnerella vaginalis. It is known to increase HIV infection rates and thus presents a significant health riskto South African women. Traditional treatment is with antibiotics, but this does not support reestablishment of the natural protective vaginal species and thus recurrence rates of the condition are high.

Treatment with probiotics that are endemic to South African women is proposed as an alternative to antibiotics. Screening of over 100 vaginal clinical isolates has been performed to identify the most promising Lactobacilli species. Desired characteristics included ability to adhere to vaginal epithelial cells, production of lactic acid [D and L] and H_2O_2, ability to lowerpH, and inhibition of common vaginal pathogengrowth. The top ten vaginal isolates included six L. crispatus, two L. gasseri and two L. jensenii strains.

Production of a multi-strain probiotic at large scale requires design of a suitable suspended liquid media culturing system. Growth of the isolates was tested in 100 mL serum bottles containing MRS media (37°C, 140 rpm incubator agitation) where the measured variables were pH and optical density. L. jensenii 92.1 PA and L. crispatus 100.16 a had the best performance with respect to maximum specific growth rate, yield and ability to lower pH. Design of the growth medium and culturing conditions in 300 mL Sixfors batch bioreactors has been commenced using these strains.

Biography

Marijke Fagan-Endres is a Senior Lecturer in the Department of Chemical Engineering at the University of Cape Town (UCT) and

researcher at the Centre for Bioprocess Engineering Research (CeBER). She holds an undergraduate degree in Chemical Engineering (BSc, first class honours) from UCT and a PhD in Chemical Engineering from Cambridge University. Her PhD and subsequent work has focussed on biohydrometallurgical processes such as heap bioleaching and bioflotation, and development of tomographic methods for their study (MRI and X-ray μCT). Her research portfolio has recently expanded to include production and formulation of probiotics and extraction of microbially produced pigments.

Insightful Understanding of Osteoarthritis Joint Pain and Disease-Specific Drug Target

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Abstract

Chronic or episodic pain is the most prominent symptom of OA which leads to psychological distress and impaired quality of life; and is a key reason to seek medical assistance, yet there is no effective way to relieve OA pain. A key clinical paradox in OA is that the severity of joint pain doesn't strictly correlate with radiographic and histological defects in joint tissues, raising a fundamental question: "Why is there no close correlation between the degree of cartilage degeneration and the degree of pain sensation?" Some OA patients suffer more pain than others and often unrelated to the degree of radiographic evidence of joint degeneration. The mechanisms by which OA develop from an asymptomatic condition to a painful disease is a critical hiatus in our knowledge. Our data demonstrate that: Mice with ablation of pkcd gene are strikingly resistant to cartilage degeneration but loss of pkcd exacerbates OA-associated pain, suggesting that OA pain can develop independently of progressive cartilage degeneration, and that severity of joint pain is due to cellular and molecular plasticity in the sensory neurons of the innervating dorsal root ganglion (DRG) and pathological changes in synovium (e.g., synovitis, angiogenesis). Our results from Tg mouse models and pharmacologic studies suggest that augmentation of NGF/TrkA in the joint synovium and the sensory neurons (peripheral level) is the key determinant that facilitates pro-nociception by which OA develops from an asymptomatic joint-degeneration to a painful disease condition. causing OA hyperalgesia independently of cartilage preservation.

Tailor-Made "Smart" Capsules Based on Biopolymer: Towards a New Generation of Drug Delivery System

Sabrina Belbekhouche
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Abstract

Recent progress in supramolecular chemistry leads to unparalleled control over the composition and shape factor of colloidal systems. Among them, the design of capsules is a new expanding area of physical-chemical research.[1,2] Here, we report on the development of tailor made polymer capsules for potential applications in biomedical field. The primary focus is to enhance the loading/release of therapeutic agents. The implemented strategy is mainly based on colloidal templating and self-assembly.[3]Size, dispersity and concentration of the nanocapsules are easily fixed by the initial nanoparticle template, while wall thickness is dependent on the number of layers. The present strategy is advantageous in comparison with other synthetic routes because at all steps, only water is used as a solvent and not organic one. The possibility to control the mechanical property of the capsules brings new promising property which will be evidenced during the talk. The benefits of using these capsules will be presented in numerous biological applications.

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Biography

Sabrina Belbekhouche is an associate professor (East Paris Institute of Chemistry and Materials Science/ University of Paris, France). Her core expertise is in polymer science, macromolecular assembly and surface modification. This includes the polymer synthesis; the study of the physical chemistry of surfaces/interfaces; and the use of controlled assembly at the sub-micrometer scale (nanoparticle,

nanocapsule...) as well as stimuli-responsive systems. Current applications of her research are mainly for biological application.

Production of Biochar Material using Supercritical Alcoholand Thermal Carbonization Technology from Raw Kitchen Waste

Je Lueng Shie*, Tian-Hui Liau and Yi-Ru Liau
National I-Lan University, Taiwan

Abstract

Although the kitchen waste is used as a pig feed, if the kitchen waste is not fully sterilized, it may cause the epidemic of the pig, and there is a high degree of doubt in the prevention of the African swine fever. The raw kitchen waste has a higher water content. If the incineration treatment causes an increase in the treatment cost, composting is usually used, and the kitchen waste composting has mature technology, but the traditional composting technology takes a long time, deriving odor, and wastewater problem.

Supercritical fluids can change their physicochemical properties, such as viscosity, density and diffusion coefficient, by pressure and temperature. Traditional hydrothermal carbonization technology uses water to carbonize biomass under critical conditions. The temperature and pressure requirements under water critical conditions are quite high, which is corrosive to equipment. The use of alcohol solvents as reactants requires lower critical temperature and pressure conditions and lower equipment manufacturing costs. This study mainly evaluates the production of bio-char form raw kitchen waste using supercritical alcohol thermal carbonization technology. In addition to feeding pigs and fermented compost of kitchen waste, the purpose of this study is to propose other feasible options.

Biography

Je-Lueng Shie now is a distinguished professor at the Department of Environmental Engineering, National I-Lan University, Taiwan. His study fields are focusing on 1) thermal biotechnology; 2) material and catalyst applications; 3) waste biomass refinery for advanced biofuels and biomaterials, and 4) environmental pollution and greenhouse gas control.

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