First International Conference Bioprocess Cuba 2017

REPORT

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ABSTRACT

The first international conference BioProcess Cuba 2017, was celebrated on February 20 to 24, 2017, at the Santa Cecilia Convention Center in the city of Camagüey, Cuba. It addressed new developments, research updates and technological advancements in the field of biotechnology bioprocessing, mainly focused on biopharmaceuticals production. The conference was structured in five symposia, covering upstream and downstream bioprocessing, drug devlivery formulation for human and vereinary use, biocontrol formulations and quality assurance and control for bioprocess and regulatory affairs. New experiences and developments were shown by delegates from 11 countries, including Cuba. Plenary lectures, oral presentations and poster sessions aided on the celebration of useful expertise exchanges on this fundamental area for the scale up and optimization of industrial biotechnology processes. Developmental challenges and regulatory constraints and advances were also discussed.

Keywords: bioprocessing, upstream processing, downstream processing, drug delivery, biocontrol formulations, quality assurance, quality control, recombinant protein purification

Biotecnología Aplicada 2017;34:4501-4539

RESUMEN

Primer Congreso Internacional BioProcess Cuba 2017. El primer congreso internacional BioProcess Cuba 2017 se celebró del 20 al 24 de febrero de 2017 en el Centro de Convenciones Santa Cecilia, en la ciudad de Camagüey, Cuba. En el mismo se debatión sobre los nuevos desarrollos, actualizaciones investigativas y avances tecnológicos en el campo de los bioprocesos biotecnológicos, enfocado fundamentalmente a la obtención de productos biofarmacéuticos. El congreso se estructuró en cinco simposios dedicados al procesamiento inicial y posterior dentro de las etapas de los bioprocesos, las formulaciones para la administración de fármacos de uso humano y animal, las formulaciones para el biocontrol, así como el aseguramiento y el control de la calidadde los bioprocesos y sus aspectos regulatorios. Delegados de 11 países, incluida Cuba, mostraron nuevas experiencias y desarrollos en esta área fundamental para el escalado y la optimización de los procesos biotecnológicos a escala industrial, lo cual se evidenció en las conferencias plenarias, las presentaciones orales y las sesiones de trabajos en modalidad de cartel. También se abordaron los desafíos tecnológicos a enfrentar y las barreras regulatorias, así como los avances en su implementación.

Palabras clave: bioprocesos, procesamiento inicial, procesamiento posterior, liberación de fármacos, formulaciones para el biocontrol, aseguramiento de la calidad, control de la calidad, purificación de proteínas recombinantes

*I*ntroduction

The growing and vertiginous generation of new drugs using biotechnological techniques continously demands for an increasing development in production processes. To cope with such a challenge, the First Congress BioProcess Cuba 2017 was held from February 20 to 24, 2017, at the Santa Cecilia Convention Center in the city of Camagüey, Cuba. The conference organized by the Center for Genetic Engineering and Biotechnology, focused on on the latest developments in the field of bioprocessing. Up to 124 delegates from 11 countries attended the meeting, coming from Argentina, Austria, Chile, Colombia, Cuba, France, Germany, Mexico, Iran, Slovenia and United States of America. The Congress was divided into five symposia: Upstream, Downstream, New Drug Delivery Formulation for Human and Veterinary Products, Biocontrol Formulation, Quality Assurance and Quality Control for Bioprocess and Regulatory Affairs for Bioprocess. Six plenary lectures and 48 oral full and short presentations were delivered, and 78 posters were shown.

*S*ymposium 1. Upstream

The main global trends in cultivation and expression systems for different applications were discussed. Dr Parrish Galliher, Chief Technology Officer at General Electric Healthcare Life Sciences, USA, described the new trends in continuous processing at his conference "Points to consider for commercial continuous bioprocessing (CB)". Similarly, Dr. Ernesto Chico from the Center for Molecular Immunology, Cuba, explained the impact of the growing demand of antibodies for immunotherapies on the industry on his lecture entitled "New Trends in immunotherapy: future impact on biomanufacturing". There was emphazied the needs for increasing capacities and fundamentally the productivity of the production systems in their Conference.

In another lecture, Dr Renate Kunert from the University of Natural Resources and Life Sciences (BOKU), Austria, claimed for attention to the selection of production systems, specifying the benefits of expression in CHO cells on the lecture entitled "Decision finding for production systems and expectations for The CHO system ". Of special interest were the works of Dr. Rodolfo Valdés and Lázaro Hernández from the Center for Genetic Engineering and Biotechnology of Cuba. Dr, Valdés described a peculiar design of a bioreactor agitated by a disk simulating a fish tail, entitled "Mouse hybridoma cell culture in protein free medium using a bio - mimicking fish - tail disc stirred bioreactor". In the case of Dr. Hernández, he presented

a *Picha pastoris* expression system to produce an enzyme to transform sucrose for food purposes, on his work entitled "Production of sucrose - transforming enzymes in *P. pastoris* for the Usage in food industry ". Finally, Dr. Luís Ramos of the University of Camagüey described a kinetic model for the solid state culture of fungi to produce cellulases, on his lecture "Kinetic model for the production of Cellulases by a strain of Aspergillus niger in solid-state fermentation".

Symposium 2. Downstream

Current trends on recombinant protein purification stratgeies were discussed on this simposium. Dr. Ales Podgornik from the Centre of Excellence for Biosensors, Instrumentation and Process Control, Slovenia, talked about the advantages on the implementation of new monolithic matrixes and their measuring systems for protein purification, on the lectures "Downstream processing and PAT via chromatographic monoliths" and "What information can we get from pressure drop measurement?".

Similarly, Dr. Jin Seok HUR from Novasep, LLC, USA, delviered the talk "BioSC® and BioSC Predict®. Progress in continuous bio-manufacturing ", getting into the complexity and advantages of the multi-column sequential chromatography for continuous processing. Additionally, the direct processing of the cell culture supernatant was proposed by Dr. Alistair Hurst from Biotech, Germany, on his lecture "SMART Chromatography ™ - A new method for linearly scalable protein purification". The gain in specificity during purification of proteins of biopharmaceutical intsrest as the case of monoclonal antibodies was addressed by Dr Alex Xenopoulos of EMD, Millipore, USA on the lectures "Process intensification for bioprocessing of monoclonal antibodies" and "Platform filtration process for purification of virus like Particles". These two works emphazied on strategies for optimizing the purification based on bottleneck analysis in every purification step. There was also discussed the purification of different proteins with a mixed affinity systems approach by Dr. Xavier Santarelli from the Université de Bordeaux, France, on his lecture "Protein purification improvement by using new selectivity to Reduce steps purification, by analysis of different cation exchange matrice and different protein a format for antibody purification".

Systems specifically designed to purify particulated antigens for vaccine purposes were also addressed. In this particular, Dr. Seyed Nezamedin Hosseini from the Pasteur Institute of Iran described the use of antibody-loaded iron nanoparticles to purify the hepatitis B surface antigen (HbsAg) based on the increased surface area of the nanoparticles in comparison with other conventional supports. The lecture was entitled "Purification by antibody-coated supermagnetic nanoparticles". Two other lectures were also focused on the purification of the HbsAg. Leonardo Gómez Bayolo from the CIGB described the use of monolithic columns in the lecture "Immunoaffinity chromatography based on carboxymidazole-monolithic supports to purify hepatitis B surface antigen particles for human vaccination" and Dr. Miguel Castillo Ferrer also from the CIGB talked about the introduction of a precipitation step with PEG 4000 kDa to improve the previous purification process at the conference "Method to obtain the hepatitis b surface antigen for vaccine". Susana Miraidys Brito Molina from the National Center for Scientific Research of Cuba (CNIC) described the purification process of the human papillomavirus capsid L1 protein, expressed as inclusion bodies in *E. coli*.

From a theoretical and applied points of view, the phenomenological simulation of the purification process could provide insight to optimize and improve the purification process, as stressed by Dr. Gabriel Marquez, from the CIGB, Cuba. He used the process for obtaining the recombinant human epidermal growth factor as a case study on his talk entitled "Modeling and simulation for defining operational parameters landscape in a downstream process". The last work on this symposium was presented by Dr. Orestes Mayo Abad from the Havana Technology University (CUJAE), Cuba, on the hurdless and requirements of solution sterilization for parenteral use.

Symposium 3. New drug delivery formulation for human and veterinary products

In this symposium, different formulations strategies were presented. Dr. Gregor Cvec from The Advanced Treatments Institute, Gauting, and The Center for NanoScience / CeNS, Ludwig-Maximilians Universidad, Munich, Germany, delivered the Plenary lecture entitled "Some challenges and opportunities of formulating proteins in lipids containing products". He discussed on the challenges and opportunities offered by products based on proteins formulations containing lipids and how to design and prepare such formulations. Dr. Mario Pablo Estrada, Head of Ag-Biotech at the CIGB, Cuba, talked about molecular adjuvants and their application to improve the effectiveness of veterinary vaccines, on his lecture "Molecular adjuvants to increase the effectiveness in veterinary vaccines".

The advantages offered by immunization by mucosal route were remarked by Dr. Sthephane Ascarteil from SEPPIC, France, on his lecture "Polymer and microemulsion adjuvants enhance the immune response conferred by mucosal vaccines in mice and chicken". This was followed by the lecture "Stability and immunogenicity of a spray-dried bacteriophage l2 virus-like particles against human papillomavirus type 16" delivered by Dr. Ebenezer Tumban from the Michigan Technological University, US. The proposed virus-like particles were prepared as a stable formulation that, once reconstituted and intramuscularly administered, effectively protected the immunized animals from the experimental vaginal infection with the human papillomavirus type 16 pseudovirus.

Due to the relevance of biomodels for testing biotechnological formulations, a lecture was delivered by Ana Aguilera, M.Sc. from the CIGB, Cuba, on the development of a model for testing novel formulations against ulcerative colitis in rats, entitled "Epidermal growth factor-pellet for the treatment of ulcerative colitis". A positive therapeutic effect was demonstrated in the animals treated with the EGF pellet. Regarding immunotherapeutics, Professor Maria Eliana Lanio from

the Faculty of Biology at the University of Havana, Cuba, versed on the immunoenhancing potential of Stycholysins, the cytolysins proteins of the sea anemonae *Stichodactyla helianthus*, on her work entitled "Sticholysins, two pore-forming toxins (PFT) from an anemone, encapsulated into liposomes: a novel strategy for improving immune cellular". The results shown demonstrated the improved efficacy of the immune responses elicited against a model antigen by encapsulation it with Stycholysin into liposomes, especially the response mediated by cytotoxic T lymphocytes. In another work using lipid-based delivery systems, Professor Beatriz Tamargo, M.Sc., from the Faculty of Pharmacy and Food Science at the University of Havana, proposed the use of lipid nanoparticles as a promising candidate against Her1-positive epithelial neoplasias on his work "Lipid nanoparticles as delivery system and adjuvant for the DEC-Her1 tumor antigen".

Finally, Dr. Oriol Sunyer of the School of Veterinary Medicine University of Pennsylvania, USA delivered the conference "C5a functions as a molecular adjuvant in teleost fish", on the potentialities of C5a, the most potent anaphylatoxin generated during complement activation. He remarked that this molecule increases the antigen-specific response in mammals and can be used as molecular adjuvant in teleost fish, such as rainbow trout, thereby increasing the response to soluble antigens.

In the Poster session were presented several works on the development of vaccine candidates, antigen and adjuvants combinations, therapeutic formulations and immunoenhancing peptides, including: the THERAVAC® HIV therapeutic, Gavac® vaccine against cattle tick, Egf in microspheres, the gonadotropin-releasing hormone for cancer vaccination, the pneumococcal vaccine Quimi-Vio®, antimicrobial peptides as adjuvants in fish and a vaccine candidate against sea lice. A companion poster on the Stycholysins as immunoenhancers was also presented.

Symposium 4. Biocontrol formulation

The simposium on Biocontrol Formulation was chaired by Dr. Prem Warrior, Chief Executive Officer of Valagro, the leading company in the production and marketing of biostimulants and nutritional preparations for agricultural crops. He delivered a plenary lecture entitled "Biotechnology - the answer to global food insecurity?". He referred to the great challenges for the humanity in terms of food production and access, the marked differences in agricultural productivity between developed and underdeveloped countries. He also emphasized on the role that biotechnology could play on increasing production yields and to protect economically relevant crops from biotic and abiotic stress factors, also considering essential to get all agricultural producers access to most modern technologies.

Up to eight oral presentations and five posters were presented in this simposium. Oral presentations were delivered by researchers from the CIGB, Cuba, and the All the works were focused on the use of biocontrol organisms against relevant pests and diseases for plants, animals and humans. All presentations were conducted by researchers from the CIGB, Cuba, and the National Center for Agricultural Utilization Research (NCAUR), this institution adscribed to the United States Department of Agriculture (USDA). For the Cuban side, the three oral presentations were in charge of Dr. Rolando Morán, Dr. Idania Wong Padilla and Mr. Néstor Mora, respectively.

Dr. Morán referred to results obtained with a solid formulation of the bioproduct HeberNem®, which reduced the levels of crop damage due to the attack of phytonematodes. It also derived in the control of some pathogenic fungi of plants and in the stimulation of germination and plant growth. In the meantime, the presentation of Dr. Wong focused on the methods used to evaluate the nematicidal capacity of the HeberNem® product on its liquid standard formulation both in vitro and in vivo. This product was able to inhibit nematode egg hatching, increase larval mortality and locally produce hydrogen sulfur by biological processes in the nematode microenvironment of in vitro. These effects were also demonstrated in vivo by using the indicator plant root model in pots. Moreover, the work presented by Dr. Mora further complemented the view of the HeberNem® product, attending to its ability to also induce defensive mechanisms in plants against pathogens. This was assessed by evaluating the differential expression of the genes normally involved in this type of response, demonstrating that the underlying mechanism was regulated both locally and systemically.

The US works were presented by Dr. Robert Behle, Dr. Alejandro Rooney and Dr. David Schisler, Dr. Ephantus Juma-Muturi and Dr. Jose Ramirez, all of them from the National Center for Agricultural Utilization Research. Dr. Robert Behle disserted on the application of granulated formulations of the entomopathogenic fungus Metarhizium to control different types of arthropods either soil-isolated or ticks.

Dr. Rooney talked about the need to increase the consistency in the efficacy of biocontrol formulations against pests and diseases, in comparison to chemical formulations. He also emphasized on the importance of combining the availability of microorganisms collections with the scientific experience, in order to disvover new strains with potential for biocontrol, especially against insects. Dr. Schisler's work focused on methods to produce a solid formulation from different strains of Pseudomonas fluorescens as biocontrol for fungal diseases in potato, including dry rot, late blight, pink rot, among others. Dr Juma-Muturi made an interesting proposal to control mosquitoes as vectors of infectious diseases in humans, animals and wildlife, by using plant-derived products based on their intrinsic capacity to either attrack or repel. He suggested a a methodology that can be technologically improved and incorporated into the programs of integral control of mosquito control as vectors. On the other hand, Dr. Ramirez talked about the importance of the tripartite interaction between mosquitoes, their microbiota and the entomopathogenic biological agents for their control, with incidence in the effectiveness of the biological control approach of several mosquito species. Similarly, it was mentioned in this work some fungal candidates with mosquitocidal activity and their ability to produce bioactive molecules.

The poster session included works on the control of insect pests in avocado plantations using emulsified

formulations of *Beauveria bassiana* fungus, presented by Dr. Christopher Dunlap; on the isolation and identification of bacterial strains with the ability to control ticks and their potential to become a veterinary product, presented by B.Sc. Liszoe Gladós; the strategy for the isolation from different sources of bacterial strains with chitinolytic and protease activity as biocontrol agents of parasitic phytonematodes, by M.Sc. Ramón Franco; and the demonstration of in vitro and in vivo control of phytonematode eggs and larvae by a new isolated bacterial strainfrom the field, as well as the combination of selection strategies for the isolation and identification of new strains of bacteria with nematicidal activity, both by MCs. Ileana Sánchez.

Symposium 5. Quality assurance and quality control for bioprocess and regulatory affairs for bioprocess

Quality assurance, control and the regulatory environment for biotech products for human and agricultural use were presented at this simposium. Different essential methodologies such as scanning electron microscopy were analyzed for their application in quality control of active pharmaceutical substances by Dr Miran Čeh from the Jožef Stefan Institute, Slovenia, on his lecture "Morphology and Chemical composition investigations of pharmaceutical substances using scanning electron microscopy". Yumisley Alfonso Marín from the Cuban Regulatory Agency, summarized in detail the regulatory environment based on ICH guidelines of processes for the development of biopharmaceuticals, on the conference "Strategy for guidelines development for marketing authorization of biosimilars or biological known products in Cuba".

In a more practical approach, Zeila Santana Vázquez from CIGB analyzed the implementation of key regulatory aspects during the establishment of a fermentative process using *E. coli* as host strain for the manufacturing of granulocyte colony stimulating factor (GCS-F) as active pharmaceutical ingredient in a multipurpose plant. The lecture was entitled "Aspects to take into account in the development of fermentative processes for the production of biopharmaceuticals expressed in *E. coli*. Application of ICH Guides Q5, Q6, Q7, Q8, Q9 and Q10 ". Alex Xenopoulos from EMD Millipore proposed an analytical methodology for the characterization and quantification of the host contaminating proteins (HCP) during antibody purification. This procedure offers advantages over other previous techniques regarding its increased sensitivity and the imporved efficiency of the purification stages, despite the relative increase in the general cost of this methodology for batch release. Analytical methodologies were described by Seyed Mehdi Hassanzadeh from the Pasteur Institute of Iran for biopharmaceutical control, with a new MTT-based method for determining the viability of the BCG vaccine.Also, the relevance of accurate experimental design was further delineated as a useful tool for the optimization of analytical methods, as described by Lázara Muñoz, from the CIGB, Cuba.

As an endpoint key aspect in the production pipeline of every biotechnological product, regulatory aspects required to be followed to register and market biotech products were also addressed in two lectures, one focused on agricultural products, by Dr Jesús Mena Campos from CIGB , entitled "Registration of agricultural products: international standards and regulatory environment in Cuba", and the other regarding veterinary vaccines, delivered by Alain Moreira Rubio from CIGB, entitled "Regulatory standards for the registration and manufacture of veterinary vaccines".

Concluding remarks

In summary, the international conference BioProcess Cuba 2017 contributed to fruitful discussions and the necessary knowledge exchange among specialist in the area of bioprocessing, with an integrative view. On its first edition, it strengthened the colaboration among researchers with the focus put in the production of biopharmaceuticals for human and animal use mainly, and other products for field application in economically relevant crops. The contributions reinforced the excellence projection from laboratory science into robust development and production processes that will end up in more suitable biotechnological products in compliance with regulatory standards.

CONFERENCE ABSTRACTS

Plenary lectures

Algunos desafíos y oportunidades de formular proteínas en productos que contengan lípidos

Gregor Cevc

The Advanced Treatments Institute and The Center for NanoScience / CeNS, Germany

A pesar de su diferente composición, forma y tamaño, las proteínas y los lípidos fundamentalmente interactuan del mismo modo y todos dinámicamente: principalmente atraídos por van der Waals y electrostática fuerza "+-" y repelidos por hidratación y fuerza electrostática "--" o "++". Esto permite extrapolaciones e interpolaciones entre las dos clases. Sin embargo, a diferencia de propiedades de proteínas, que dependen principalmente de la estructura química y los enlaces químicos, las características de las supramoléculas lipídicas se rigen principalmente por fuerzas físicas. Por lo tanto, las supramoléculas lipídicas son más sensibles a los cambios en las condiciones de contorno. En consecuencia, la estructura y propiedades de agregados lipídicos dependen de la temperatura ambiente, pH, composición y concentración de electrolitos. La diversidad molecular impide definir una receta de formulación general, pero se pueden establecer principios generales para diseñar y elaborar formulaciones de proteínas y lípidos: (1) los lípidos se presentan a las proteínas principalmente como un sistema bidimensional; (2) las proteínas tienden a unirse a las capas de lípidos (y la mayoría de las otras superficies), pero deben penetrar barreras interfaciales para eso; esto influye

en la unión (específica) de proteínas a las interfaces; (3) barrera interfacial de una bicapa lipídica siempre aumenta con la fluidez molecular y la hidratación superficial; por lo tanto, la polaridad de lípidos, suavidad y espesor superficial, así como concentración interfacial de protones (H+) y de otros moléculas influyen interacción proteína-lípido; (4) a su vez, el aumento de la movilidad lipídica / exposición de las partes moleculares hidrofóbicas facilita y consolida unión no específica de las proteínas y las capas de lípidos. La interfase lípido-agua puede considerarse, fenomenológicamente, como un disolvente prótico con constante dieléctrica entre la de agua y la aceite (para los fosfolípidos alrededor de 30). Su espesor refleja la distribución, accesibilidad y movilidad de los grupos polares ('cabezas polares'). Esta es la razón principal por la que las constantes de unión (por ejemplo p*K*) medidas en solución difieren ($\leq$~10^4×) de los valores correspondientes que caracterizan la unión molecular a una capa lipídica. Los lípidos pueden afectar positivamente o negativamente la estabilidad (especialmente física) de la proteína. A la inversa, las proteínas pueden causar la agregación de los supramolecules lipídicos. Como resultado, lípidos (agregados) pueden servir como modificadores de la formulación proteica / portadores de proteínas.

Biotechnology – the answer to global food insecurity?

Prem Warrior

COO. Valagro SpA, Italy

Agriculture has been high on the global agenda for the past decade. Besides crop productivity, increased food prices, threat of climate change, population increase and the overall energy dependence are all linked to food security. In the next 20 years, we will need 50% more food, 30% more water and 50% more energy, in order to feed 1.2 billion more people in this world. While productivity has increased over the past three decades, approximately 75% of the world's poorest economies practicing smallholder farming face very low productivity. These regions are further constrained by lack of resources, poor policy environment, low capacity for R&D and low private sector interest. Undernutrition now affects 925 million people worldwide, causing 36 million deaths a year. Paradoxically, there are also 1.3 billion people who are obese and over-nourished, with over 29 million deaths a year caused by bad eating habits. The power of biotechnology can be harnessed for higher productivity in terms of abiotic stresses, IPM and postharvest management. Improving input efficiency through advances in formulations and delivery mechanisms is needed. Successful interventions adapted to the local contexts and better market access must be scaled up while balancing the limited natural resources to ensure sustainability. Technological solutions to address these issues exist. Through the advances in various cutting-edge technologies, harnessing the power of biotechnology, channeling information in an already-connected world and developing inclusive policies will finally deliver the much-needed solutions to address food security.

Downstream processing and PAT via chromatographic monoliths

Ales Podgornik

Centre of Excellence for Biosensors, Instrumentation and Process Control, Slovenia

In last decade chromatographic monoliths are gaining on importance due to several interesting properties, which can be mainly summarized into: high porosity, transport based on convection, high capacity for extremely large molecules. All mentioned features are tightly related to the monolithic structure. High porosity results in low pressure drop on the column meaning milder purification conditions. Convection based transport, resulting in flow-unaffected properties, is an extremely important feature that accelerates separation and purification process, especially pronounced for large molecules. Pores in the monoliths are open and highly interconnected forming a network of channels. The mobile phase is forced to flow through them transporting the molecules to be separated onto the active (binding) sites, therefore transport is based on convection. Since there are normally no dead-end pores in the monoliths, there are also no stagnant regions and the mass transfer between stationary and mobile phase is extremely fast. This is especially beneficial for purification of very large molecules having small mobility like large proteins, polynucleotides or viruses. Furthermore, monoliths exhibit also very high binding capacity for extremely large molecules. This is because the entire accessible surface is actually wall of the interconnected channels through which the sample travels. In this work, the advantages of the monoliths over other (conventional) chromatographic stationary phases will be discussed demonstrating clearly implementation areas where monolith performance would be superior. Special emphasis will be given to the effect of the monolith structure on its chromatographic performance. Several examples of monolith implementation in up-stream and downstream, including monitoring through PAT, will be discussed. Most of examples described large macromolecules like large proteins, plasmid DNA and viruses, including phages but protein modifications such as PEGylation will be discussed too.

*S*ymposium 1. Upstream

Bioprocess development and bio-manufacturing with process simulation and production scheduling tools

Demetri Petrides

Intelligen Inc., USA

The successful scale up and commercialization of biopharmaceuticals is a challenging task that requires collaboration of professionals from many disciplines. Process simulators can facilitate this task by assisting scientists and engineers to answer the following and other related questions: What is the impact of product titer increase on the capacity load of the downstream section, the overall throughput of a plant, and the cost of goods? What changes are required in an existing

multi-product facility to accommodate the process of a new product? What is the range of variability that a process can accommodate if it operates under a tight cycle time? What is the impact of single-use systems on the demand for utilities, the environment and the cost of goods? Our experience in addressing the above questions will be presented using industrial examples in which we evaluated alternative technologies for producing therapeutic monoclonal antibodies and vaccines.

Escalado de fermentación de alta densidad en la producción de estreptoquinasa recombinante

Salvador Losada Nerey, Orestes Mayo Abad, Mercedes Rodríguez Edreira, Saily Martínez Diaz, Victoria Lugo Hernandez, Michel Diaz Martinez, Yanelys Pestana Vila, Jorge Valdés Hernández

Centro Nacional de Biopreparados, Cuba

Todos los servicios de salud a nivel primario en Cuba disponen del agente trombolítico estreptoquinasa recombinante (Skr) producido en el BioCen, utilizado en el tratamiento contra el infarto agudo de miocardio. Debido a esto, y también por la demanda internacional, se plantea como necesidad el aumento de la capacidad productiva del fármaco. La etapa de fermentación constituye un cuello de botella en el proceso, ya que su capacidad de producción se encuentra a un 70 % de la capacidad de procesamiento de la etapa de purificación, por lo que aumentar el volumen de producción de fermentación es importante para un aumento de la capacidad. En la Planta de Ingredientes Activos del BioCen se realizó un escalado desde la escala de banco en 5 L hacia un volumen de 300 L, a partir de resultados obtenidos en el departamento de desarrollo del CIGB. Se utilizó un nuevo método de fermentación de cultivo incrementado de alta densidad utilizando la cepa de *E. coli* W3110, donde se logra duplicar la producción. Como criterio de escalado se mantuvieron las variables intensivas del proceso constantes, el coeficiente de transferencia volumétrico de oxígeno (kLa), y las relaciones de flujo de alimentación y volumen de incremento. Se realizaron 3 lotes de fermentación a escala de 300 L obteniéndose 100 g/L de biomasa húmeda con más del 20 % de expresión, los lotes fueron procesados y analizados en las etapas de ruptura, precipitación y purificación según procedimientos establecidos. Como resultado del escalado se logró reproducir lo obtenido a nivel de banco y duplicar el volumen de producción en biomasa sin modificar el equipamiento existente. Se alcanzó el objetivo propuesto al cumplir con todos los requisitos de calidad de los productos intermedios en las diferentes etapas del proceso hasta la purificación final.

Uso alternativo del programa EROS para el control de la adición de metanol, durante la fermentación de *Pichia pastoris* En 300 L

Josué García Parrado

Centro de Ingeniería Genética y Biotecnología, Cuba

El uso de sistemas de supervisión y control de procesos, es algo muy utilizado en la industria biotecnológica, facilita a los operadores, ingenieros, supervisores y directivos operar y dirigir cualquier proceso con eficiencia y productividad. En el proceso productivo del IFA Gavac®, durante la fermentación de *Pichia pastoris* en 300 L, se realizó la evaluación de la utilización de manera alternativa del programa EROS, para el control de la adición de metanol. Se realizó el estudio en 8 fermentaciones, en ellas se chequeó el rendimiento de la biomasa lavada y concentrada, tiempo de cultivo, peso húmedo y presencia de la banda de Bm86 a la altura de 89 kDa. Las fermentaciones se realizaron según lo descrito en la documentación vigente y se trabajó con el programa EROS según procedimiento aprobado para el estudio. Los resultados obtenidos fueron sometidos a un análisis de varianza utilizando el programa STATGRAPHICS Centurion XV. Los tiempos de fermentación estuvieron por debajo de las 96 h, en todos los lotes se observó la banda de Bm86 a la altura de 89 kDa, se obtuvieron valores por encima a los especificados como característica de calidad en cuanto a peso húmedo ($\geq$ 420 g/L) y el rendimiento ($\geq$ 45 kg de biomasa húmeda). Se obtuvieron resultados satisfactorios con el uso del programa EROS para el control de la adición de metanol.

Decision finding for production systems and expectations for the CHO system

Renate Kunert, David Reinhart, Linda Schwaigerlehner, Philipp Mundsperger, Julia Hennicke, Wolfgang Sommeregger, Patrick Mayrhofer

University of Natural Resources and Life Sciences (BOKU), Austria

The discussion about the preferable protein expression system considering procaryotes and lower or higher eucaryotes as biological factory is lively continuing since decades. An objective comparison of process parameters like volumetric yields versus specific productivity, challenges during filtration or cell digestion and time scale for inoculum preparation are rarely found and will be discussed here. Besides, biological protein expression is consistently supported by chemical modification of the product during down-stream procedure like in case of insulin and will gain more and more interest with new and artificial therapeutics in the future. For mammalian expression systems, the traditional CHO cells are still the workhorse for protein production mainly because expression titers are escalating significantly due to process improvements including culture media and feed strategies. We have developed recombinant expression clones for various mAbs of different immunoglobulin subtypes (IgG, IgA, IgM) and formats (whole immunoglobulins, F(ab)s, scFv-Fc fragments) and analyzed the process parameters of different IgGs, investigated dimer and aggregate formation of IgA molecules and also compared isogenic clones of different scFv-Fc in an proteomic approach. The formation and optimal secretion of recombinant mAbs depend on commonly accepted factors like the host cell line and the regulatory components driving the transcription and translation of the genetic information. Process conditions have to be carefully selected depending on the demands of the final product. Fed-batch strategies are mainly used for high-yield production of IgGs since the micro-heterogeneity of the product often does not affect functionality as in case

of coagulation factors or cytokines. For highly complex IgAs and IgMs we found significant differences in product quality leading to the conclusion that individual products can be expressed from standardized expression platforms, but the process strategy including media formulation and harvest criteria are defined by the intrinsic demands of the protein.

Mouse hybridoma cell culture in protein free medium using a bio-mimicking fish-tail disc stirred bioreactor

Rodolfo Valdés, Hasel Aragón, Marcos González, Daily Hernández, Déborah Geada, David Goitozolo, Williams Ferro, Adelma Pérez, José García, Yordanka Masforrol, Tatiana González, Maylín La O, Yodelis Calvo, Alexander Hernández, Grechen Menéndez

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Due to the Lambda MINIFOR bioreactor provides a good mixing of cell culture, nutrient distribution and oxygen transference without damaging hydrodynamic forces by using a biomimicking "fish-tail" disc stirrer; it can be successfully applied for the cultivation of bacteria, yeasts, insect cells, plant cells, and mammalian cells. However, reports on its application in mouse hybridoma cell culture in protein free media is non-existent in the scientific literature yet. Therefore, this study describes preliminary findings of the Lambda MINIFOR bioreactor suitability in mouse hybridoma cell culture and antibody production using as models the SP2/O-Ag14-CB.Hep-1 mouse hybridoma cell and the protein free medium PFHM-II. Main results allowed to verify 2.45×10^6 viable cells/mL as the highest cell concentration, 86% as maximum cell viability, exponential growth rate (μ) = 0.0161, 42 h as doubling time, a stable phenotype measured by limiting dilution after 2.5 months, no antibiotic and antifoam requirements, 28.3 ± 19.0 µg IgG/mL, 34.18 ± 11.9 pg IgG/cell, 71.4% of IgG SDSPAGE purity in the cell culture supernatant, up to 99.5% of purity in purified sample (measured by SDS-PAGE and HPLC-GF) after an IgG capture step based on Protein A-Sepharose, low pH incubation and sizeexclusion chromatography, high specificity for the CKTCTT epitope (located in HBsAg "a" determinant), an IgG affinity constant equal to 1.11×10^{10} M^{-1}, and 10 pg DNA/mg of IgG. As conclusion, this study corroborated the Lambda MINIFOR bioreactor successful application in mouse hybridoma cell culture in protein free medium for research applications.

Antibody humanization strategies complemented with deimmunization for safe biotherapeutics

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One prerequisite for the introduction of new protein therapeutics is the development of safe products with high biological efficacy and quality. Modern and future biopharmaceuticals often contain protein sequences of non-human or artificial nature with the potential to trigger immunological reactions within the human body. Therefore, murine hybridoma-derived antibodies are often re-engineered to provide predominantly human frameworks (FR) but maintain full binding affinities defined by the original complementarity-determining regions (CDRs) and supporting FR residues. This is a first step to reduce precarious immunogenicity but remaining non-human sequences still possess residual immunogenic potential. Here we present how traditional humanization protocols can be combined with T-cell epitope prediction tools to analyze the expected immunogenicity of antibodies. Different humanization protocols were applied to a model antibody to design, express, purify and evaluate the binding affinity of CDR-grafted and superhumanized mAb variants. The generated sequences were also analyzed by freely available in-silico T-cell epitope prediction tools using the Immune Epitope Database (IEDB) to further assess the influence of specific protein sequence modifications. Selected antibody variants were then used to test an in-vitro assay for evaluation of the immunogenic potential of antibodies. Different humanized mAb variants were generated from our murine model mAb and additional mutations were rationally selected to restore binding affinity. Such mAb variants were analyzed for their potential immunogenic impact by immune epitope prediction tools. The in-silico prediction allows us to test and optimize in-vitro assays to evaluate immunogenic potentials in the wet lab. Our approach for the selection of a new mAb drug includes a holistic benchmark of multiple parameters such as binding affinity, expected immunogenicity and expression levels. We can conclude that although humanization strategies allow reduction of expected immunogenicity as a first rough step it can be complemented by novel T-cell epitope prediction tools to fine-tune the process of removing residual immunogenic sequences.

Production of sucrose-transforming enzymes in Pichia pastoris for the usage in food industry

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Yeasts are widely used host systems for heterologous protein production. They combine the ease of genetic manipulation and fermentation (rapid growth and high density cultures in simplified medium) of a microorganism with the capability to secrete and modify (processing of signal sequences, disulfide bridge formation, and both O- and N-linked glycosylation) foreign proteins according to a general eukaryotic scheme. Among yeast hosts, *Pichia* (*Komagataella*) *pastoris* has special attributes for the large scale industrial production and secretion of foreign proteins which include: (1) the availability of strong native promoters suited for the constitutive or controlled expression of foreign genes; (2) the correct processing of well-studied heterologous signal peptides that efficiently target

the recombinant protein into the secretory pathway; (3) A low level of endogenously secreted proteins that allows for the production of relatively pure recombinant secretory proteins; (4) the strong preference for respiratory growth, a key physiological trait that greatly facilitates its culturing at high-cell densities relative to fermentative yeasts. Due to its GRAS (Generally Regarded As Safe) status and the advantage of lacking endogenous saccharolytic activity, *P. pastoris* is a particularly appropriate host for the recombinant production of sucrose transforming enzymes with applications in the food industry. We will present, as way of examples, our group experience in using *P. pastoris* as a host system for the constitutive expression of fructosyltransferase, invertase and fructanase genes from different plant and bacterial sources. The recombinant enzymes allowed sucrose conversion into products of higher value, such as fructooligosacharides (FOS) and fructose-rich syrups, with diverse applications in the food industry.

Kinetic model for the production of cellulases by a strain of *Aspergillus niger* in Solid-State Fermentation (SOP)

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In this work a kinetic model's proposal is presented for escribing cellulose production in a of solid-state fermentation process of a strain of Aspergillus niger. The validation of the model was carried out at five temperatures: 20; 25; 30; 35 and 40°C. In the validation the characteristic parameters of the model were identified making use of dynamic data generated at constant temperature. The response variables described by the model are: the concentration of the biomass of the fungus measured as true protein, the total reducing sugars, the crude fiber, the FPase activity, the CMCase activity and the activity of proteases in the medium. For the identification of the arameters the pattern of the process was programmed in MATLAB and the adjusting non-linear procedure of its parameters. The results indicated the model is perfectly capable of describing the kinetics of cellulase production under these conditions. The temperatures where cellulase production was higher were 30°C and 35 °C. The experimental levels of FPase and CMCase are significant because maxima of FPase of 60 UI gDW^{-1} are reached and of CMCase of 50 UI gDW^{-1} at one time that it oscillates between 24 and 30 hours. The identified parameters are strongly associated to temperature for that it is planned in future works to identify auxiliary models to establish that dependence. The dependence of the kinetic constants with temperature was adjusted mainly by the models of Rosso and of Ratkowsky and, in only four cases, it was necessary to use polynomial models. The fitted model allowed estimating the optimal temperature and fermentation time for cellulose production: 32 °C and 20 hours respectively, giving an FPase activity of 64.1 UI gMS^{-1} and a productivity of 3.1 UI h^{-1} gMS^{-1}. This productivity was the highest value found in 20 scientific reports since 2004, which gives great interest to this solid culture for its production at industrial scale.

Measurement of heat capacity of concentrated culture media composed by yeast extract and sucrose

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This study aimed to obtain an equation for calculating the heat capacity of a concentrated culture medium composed by yeast extract and sucrose. This culture media is used in bionematicide HeberNem production. The experimental design allowed us to evaluate the influence in the heat capacity of the ratio of Yeast Extract/Sucrose, dry matter concentration and temperature. It was determined that the variables with most influence in the heat capacity are dry matter concentration and temperature. Obtained equation only applies to culture mediums with similar characteristics and within the levels evaluated in this study. Statistical analysis guarantees that equation obtained will help perform heat transfer calculations more reliable in the processes of scale up.

Oxicogenomics: the benefits and limitations

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Toxicogenomics, a burgeoning field that interconnects toxicology with genomic technology, may hold the promise of detecting changes in the expression of an animal´s genes if are exposed to toxicants. A quality risk management approach should be applied throughout the lifecycle of a medicinal product. As part of a quality risk management system, decisions on the scope and extent of qualification and validation should be based on a justified and documented risk assessment of the facilities, equipment, utilities and processes. Limits for the carryover of product residues should be based on a toxicological evaluation. As the technology develops and data become available, it is important to maintain discussion between scientists about the promises and limitations of this new field. The objective of our work is to discuss the potential applications and difficulties of this evolving branch of toxicology and its application in the establishing of limits for the carryover of product residues and the removal of any cleaning agents used. This kind of study include some potential benefits such as: accelerated discovery of gene polymorphisms associated with idiosyncratic toxicity, the identification of genetic markers for the prediction of adverse reactions to drugs, the definition of genetic markers for drug efficacy, identify biomarkers of incipient adverse effects, provide a rational basis for risk assessment, identify useful disease´s biomarkers to toxic substances, elucidate the molecular mechanisms of toxicity and create compendia on toxicologically important genes in animal models. However it has limitations contain difficulties in: analysis of high density data, integration of data obtained by different technologies, linking "omics" data to specific adverse effects, translation statistical assessments into biological understanding, to

complete functional annotation of genome data bases. In view of the previous discussion, we can conclude that the acceptance criteria should consider the potential cumulative effect of multiple items of equipment in the process equipment train. A quality risk management approach should be applied throughout the lifecycle of a medicinal product, for that reason the advances of the genetic bases for idiosyncratic responses to chemicals have practical applications in drug discovery, hazard identification and risk assessment and will more fundamentally contribute to our understanding of the health consequences of the interaction of xenobiotics with biological systems.

Caracterización de un clon estable para la producción del AcM CBHepB Natural utilizado en el diagnóstico del virus de la Hepatitis B

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La Hepatitis B es una enfermedad peligrosa que anualmente produce más de medio millón de muertes relacionadas a la infección por el virus en sus distintas formas de evolución. Para su diagnóstico se utiliza el ensayo UMELISA® HBsAg Plus fabricado por el Centro de Inmunoensayo y comercializado por TecnoSuma Internacional. Uno de los principales componentes de este juego diagnóstico es el anticuerpo monoclonal (AcM) CBHepBNatural producido por el CIGB Sancti Spíritus. La línea celular hibridoma utilizada en la producción de este anticuerpo mostró inestabilidad en su secreción lo que implicó un aumento de recursos y lotes para satisfacer la demanda creciente del cliente. El objetivo del trabajo consistió en obtener una línea celular estable del hibridoma CBHepBNatural. El clonaje se realizó por el método de dilución limitante, el ELISA de cuantificación de IgG total sirvió para el monitoreo de la presencia anticuerpos en las etapas del proceso; la selección del clon específico se realizó por el UMELISA® HBsAg Plus con la adición de un paso de inhibición. La purificación del AcM se realizó por matriz de nProtein A Sepharose 4 Fast Flow. Se seleccionó el clon CBHepBNatural 8/5/14 por ser el de mayores niveles de secreción y especificidad. El estudio en ratones mostró una concentración de proteínas específicas en el líquido ascítico seis veces superior al clon anterior y permitió obtener un purificado final con un 99,01 % de pureza. Con los resultados obtenidos en este trabajo se sustituyó el clon usado por el CIGB SS por el nuevo clon 8/5/14, con una disminución en un 15 % del costo de producción. El hecho de contar con un proceso productivo eficiente que permita obtener un AcM CBHepBNatural que cumple con los requisitos del cliente nos permite ser el proveedor de este reactivo para el ensayo diagnóstico UMELISA® HBsAg Plus.

Recuperación de la capacidad del hibridoma HI0844001 para secretar el anticuerpo monoclonal CBIFN alfa 2.3

Maylin Castellanos Cancio, Carlos Hernández Díaz, Yeleny Machín León, Emilio Carpio Muñoz, Rodolfo Valdés Véliz, Dayami Dorta Hernández, Reinaldo Blanco Aguila, Odalys Péres Cruz, Vladimir Leal Gómez, José Manuel Sanchez Ríos, Isi Veitía Cobas, Dany Daniel Miranda Espósito

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Los productos biofarmacéuticos son moléculas bioactivas que se utilizan con fines médicos. Su fabricación debe realizarse según Buenas Prácticas de Producción. Para el control de la calidad de estos productos se utilizan sistemas analíticos, generalmente de tipo inmunoensayo. Los interferones son glicoproteínas producidas por el sistema inmunitario. El Centro de Ingeniería Genética y Biotecnología (CIGB) de La Habana produce el Interferón alfa 2b humano (Heberon Alfa R®), que se cuantifica realizando un ELISA sándwich, utilizando una pareja de anticuerpos monoclonales (AcM) el CBIFN alfa 2.3, como recubrimiento y el otro como segundo anticuerpo conjugado a peroxidasa del rábano picante, CBSSIFN alfa 2.4 – HRP. En ELISA realizados se detectó que el hibridoma dejó de secretar el AcM de interés. Una posible solución al problema sería clonar nuevamente el hibridoma partiendo de un ámpula del banco maestro. Se seleccionó y caracterizó un hibridoma capaz de producir el AcM CBIFN alfa 2.3 utilizando un ELISA de inhibición. La purificación a partir de ascitis de ratón permitió obtener cantidades suficientes del AcM para su posterior análisis. La caracterización química e inmunoquímica del AcM obtenido por este método demostró que es de la misma clase y tipo que el que anteriormente se utilizaba. Mostró valores supriores al lote control, concentración óptima de recubrimiento determinada por ELISA de 5 a 15 μg/mL, afinidad de $1{,}67 \times 10^{10}$ y pureza de 94,27 %. El AcM CBIFN alfa 2.3 obtenido por este método permitió la cuantificación por ELISA de 17 lotes de IFA de Interferón α2b humano, en el año 2013. Demostrándose así de manera práctica la solución del problema tecnológico.

Establishment of a fermentation process at a bank scale to ob8tain the antitumor candidate CIGB 370

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Cancer has become one of the leading causes of death worldwide and the first in Cuba, therefore acquiring an increasing interest on drugs on antitumor effect has increased. The CIGB 370r polypeptide has been obtained at the Center for Genetic Engineering and Biotechnology (CIGB).This protein has a broad spectrum of action and selectivity on tumor cells. In the Technological Development Department, a fermentation

process has been developed to obtain this antitumor candidate, capable of guaranteeing the necessary quantities of product with the required quality for its injectable use in humans. The objective of this work is to improve the fermentation process increasing the expression levels and compliance with the regulatory standards. Thus, favorable conditions at the bank scale were established for the growth and expression of the heterologous protein by an experimental design of response surface with the application of statgraphics centurion XV software. The best results as fermentation parameters were 7.85 units of optical density and 11% of the CIGB 370r expression at 37 ºC, 700 rpm, pH 6 and 18 h of culture.

Steady states of metabolic networks in continuous cell cultures

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Biotechnological products are obtained by treating cells as little factories that transform substrates into products of interest. Cells must be grown and fed with nutrients inside a bioreactor. A major mode of cell culture is the continuous or perfusión mode, where a constant flow carrying fresh medium replaces culture fluid, cells, unused nutrients and secreted metabolites. By definition, a continuous cell culture ideally reaches an equilibrium state where conditions in the tank, including the cell population, remain constant. Industrial applications place demands on the equilibrium state, such as: high-cell density, stability, minimum waste byproduct accumulation, and efficient nutrient use. Due to the difficulty of simulating large genome-scale metabolic networks, mathematical models of cell metabolism in culture has hitherto focused on the dynamics of a few variables. We develop a framework to study the equilibrium states of continuous cell-culture considering genome-scale metabolic networks. The model is consistent with/explains the following experimental observations: 1) the multi-stability of this type of culture; 2) the disagreement between assessments of medium quality through batch experiments and the results obtained in perfusion; 3) the decaying stability of continuous cell culture with increasing cell density or dilution rate. Finally, we use the model to predict better medium formulations, allowing for stable cell cultures with higher cell densities at cheaper medium expenditure.

Fed-batch fermentation strategy for Hepatitis B Virus core antigen production

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Hepatitis B is an infectious disease of theliver characterized by the appearance of hepatocellular necrosis and inflammation, caused by the Hepatitis B Virus (HBV). This disease, which currently constitutes a public health problem worldwide, is responsible for the death of approximately 600000 persons per year. The Center for Genetic Engineering and Biotechnology (CIGB) is developing NASVAC, a therapeutic product against Hepatitis B whose main components are the surface and core antigens of HBV. NASVAC is administered nasally, it is used in Cuba, after the approval of the Center for State Control of Drugs, Medical Devices. At CGEB, HBV core antigen (HBcAg) is produced by recombinant DNA technology, using *Escherichia coli* strain W3110 transformed with an HBcAg-coding gene, which is grown in a batch fermentation process that employs a chemically defined medium. However, the yields of this process in terms of both biomass and expression levels are lower than those most commonly reported in the literature for this host. Therefore, in order to increase productivity, we designed a new process in which a constant flow fedbatch fermentation strategy is used together with a chemically defined medium containing, as main components, inorganic salts, trace salts, glucose, glycerol, isoleucine and valine. The new process yields 81.78 ± 1.31 g wet weight/L, expression levels of 13.01 ± 1.63% and a maximum productivity of 43.05 ± 0.61mg/Lh, which is 4-fold larger than that of the current process.

Symposium 2. Downstream

Evaluación de modificaciones en el paso de intercambio iónico de la producción de P64Kr

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La P64K es una proteína utilizada como carrier en la vacuna terapéutica contra el cáncer de pulmón Cimavax-EGF. Esta proteína se obtiene por vía recombinante a través de la fermentación de una cepa transformada de *E. coli*. El presente trabajo, estudia la influencia de cambios en pasos de su purificación de P64Kr. El primer ajuste está asociado a la etapa de resuspensión previo al paso de cromatografía de intercambio iónico, donde se demuestra que la sustitución del tampón de resuspensión por el tampón de equilibrio utilizado en la cromatografía de intercambio iónico, no afecta la operación cromatográfica. También se adiciona un lavado en la cromatografía de Intercambio iónico. Este cambio fue analizado previamente por un Análisis de Riesgo. A partir de estas modificaciones se realiza un balance de masa de los diferentes picos que salen del proceso de intercambio iónico para establecer las pérdidas del proceso instalado contra el proceso modificado. También se evalúa el cambio de tampón final utilizando un método de UF/DF respecto de una cromatografía de tamizado molecular. Como resultado de este trabajo se evidencia que no existen diferencias significativas en la pureza del producto en ambos procesos; para el caso del proceso modificado la media fue de 97,35 % por 97,31 % de los procesos sin cambio. Al realizar la prueba de significación de las medias para este parámetro el Valor-P obtenido fue de 0,80; superior a 0,05. Para el parámetro recobrado se obtuvieron diferencias significativas con un incremento a favor del proceso modificado de 8,81 % respecto del 7,08 % del proceso sin cambios. La prueba de significación de las medias el Valor-P = 0,0017 inferior a 0,05.

Optimización del bioproceso para la obtención del antígeno p26 recombinante del virus anemia infecciosa equina

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La obtención de la proteína p26 por vía recombinante (p26r) garantiza una fuente segura de antígeno para el desarrollo de medios diagnósticos para el Virus anemia infecciosa equina. El establecimiento del bioproceso, con la inclusión de los aspectos regulatorios, constituye una etapa necesaria para garantizar la pureza del producto, la actividad específica y las especificaciones de calidad que permitan el escalado a niveles de producción. El objetivo de nuestro trabajo fue establecer las condiciones óptimas para la expresión y purificación del antígeno empleando la cepa de *Escherichia coli* BL21 (DE3), transformada con el plásmido pET28-p26r. En este estudio se estableció un sistema de lote semilla para garantizar la estabilidad del banco maestro. Para el control de la calidad del banco se determinó la viabilidad celular, la pureza microbiana, la ausencia de bacteriófagos, la construcción por análisis de restricción enzimática y la secuencia nucleotídica del gen que codifica p26. Para establecer las condiciones óptimas de crecimiento las fermentaciones se realizaron en un fermentador Biostat B Plus en 2 L de medio de cultivo LB autoinducible con lactosa. Al final del cultivo se evaluó la estabilidad plasmídica, como parte de los requisitos de liberación por calidad de esta etapa del desarrollo del proceso. La optimización de las etapas de purificación de p26r, mediante cromatografía de afinidad por quelatos metálicos, permitió alcanzar una pureza superior al 87 %. Para el control de la calidad de la proteína purificada se estudió la secuencia aminoacídica por espectrometría de masas, empleando el programa de identificación por homología MSBlast y la actividad biológica mediante el reconocimiento inmunológico por inmunodifusión en gel agar y Western Blotting. Este estudio permitió establecer condiciones óptimas para cada etapa del bioproceso y especificaciones de calidad para la obtención del antígeno p26r.

Desempeño de la cromatografía de quelatos metálicos a escala industrial con modificaciones en las soluciones

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Este reporte muestra el desempeño del proceso de cromatografía de la eritropoyetina y el cumplimiento de los atributos críticos de calidad de la molécula en el ingrediente farmacéutico activo (IFA) al aplicarse a escala industrial la modificación de las soluciones de equilibrio y elución en la cromatografía de quelatos metálicos. El proceso modificado fue evaluado según las indicaciones de la ICH Q5E en cuanto a los parámetros de proceso y los controles de proceso críticos para cada etapa de la purificación comparándolos estadísticamente con lotes obtenidos sin efectuar la modificación. Los resultados del análisis de los controles de proceso (IPC) establecidos en el proceso de purificación demuestran que este proceso cumple consistentemente con cada uno de estos en cuanto a sus criterios de aceptación y se obtuvo en todos los casos una menor variabilidad que los lotes comparados del 2014 antes del cambio de soluciones en el paso del Quelato. Los lotes sometidos al cambio de estas soluciones del paso del Quelato a pesar de tener 2,6 veces menos la cantidad de lotes purificados se obtuvieron mayores rendimientos en el paso de Quelato con un promedio de 44,5 % comparado con 18,3 % sin el cambio, se obtuvo una mayor masa promedio obtenida en el paso final de Superdex de 9,1 g contra 6,4 g sin las modificaciones y una mayor cantidad de bulbos por litros de sobrenadante generados en el proceso de purificación de 434 g/L contra 316 g/L, solo la masa total acumulada fue menor debido a que fueron solo 23 lotes comparados con los 60 de la campaña del 2014. Se determinó que el nuevo proceso es capaz de cumplir con las especificaciones de calidad del IFA en cuanto a identidad, actividad biológica, concentración de proteínas contaminantes, endotoxinas y ADN provenientes del hospedero.

Mejoras tecnológicas aplicables al proceso de producción de factor de transferencia

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El Extracto Dializable de Leucocitos (EDL) con actividad de Factor de Transferencia (FT) es producido en el Centro de Ingeniería Genética y Biotecnología, es un hemoderivado obtenido de la ruptura de leucocitos de sangre humana, cuando los mismos son aislados y sometidos a un proceso de ruptura de sus membranas y posterior diálisis del extracto celular. La utilización del método de diálisis como mecanismo principal para la obtención y purificación de este producto, hace que el mismo no cuente con un proceso que cumpla con las exigentes regulaciones internacionales, debido a que, con éste método, el producto es objeto de una manipulación excesiva, aumentando el riesgo de contaminación y haciéndolo poco fiable para la remoción viral. Por todo lo anteriormente expuesto, el presente trabajo desarrolla, un nuevo proceso de purificación del Factor de Transferencia escalable, que permite obtener un Ingrediente Farmacéutico Activo (IFA) con todos los atributos de calidad requeridos para el Registro Sanitario y que posibilita obtener cantidades de proteínas por el método de ultrafiltración de un valor promedio de 1,83 g, mientras que por diálisis sólo se alcanza 1,23 g, se incrementa la productividad del proceso en 1,64 g/h, mayor que el alcanzado por el método de diálisis, 0,053 g/h. La productividad alcanzada

mediante la ultrafiltración es 33,4 veces superior a la del método de diálisis.

Comparación de los indicadores económicos de la operación de inversión de sacarosa por vía química y enzimática

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El proceso de obtención de glucosa y fructosa se basa en la inversión de la sacarosa. Estos productos son ampliamente utilizados por la industria alimenticia y aunque existen varias formas de obtenerlos se prefiere la vía enzimática por sus ventajas. En este trabajo se propone la obtención de los mismos mediante hidrólisis enzimática empleando un conjugado de Invertasa-quitosana inmovilizada sobre un soporte de quitina-carboximetilcelulosa sintetizado en nuestro laboratorio. Se toma como referencia el proceso tecnológico de la UEB Chiquitico Fabregat, Villa Clara. El biocatalizador sintetizado retiene un 50 % de la actividad al término de 170 días de operación. Se presentan los parámetros de diseño y condiciones de operación del reactor de lecho fijo propuesto. Los indicadores económicos obtenidos muestran que el valor de la producción de la glucosa enzimática es 2,4 veces mayor que el de la glucosa ácida, siendo el costo operacional/peso de la etapa de hidrólisis inferior (0,51) en la enzimática con respecto a la ácida (0,82). Se pudo determinar que es más factible realizar los cambios del biocatalizador empleado como lecho fijo al término de 40 días de operación. La valoración preliminar de la factibilidad del proceso enzimático demostro la superioridad técnica y económica del mismo.

Sustitución del paso de tamizado molecular por un proceso de ultrafiltración en la producción de P64kr

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El objetivo principal es la evaluación de un cambio en el proceso de purificación de la proteína P64kr (Ingrediente Farmacéutico Activo de la vacuna terapéutica contra el cáncer de pulmón CIMaVax-EGF), consistente en la sustitución del paso cromatográfico de tamizado molecular (G-25) por un paso de ultrafiltración (UF/DF) en la etapa de formulación. Se realiza un análisis básico de los principales aspectos que influyen en un proceso de UF/DF. Se caracteriza el paso de tamizado molecular que se estaba utilizando en la purificación y son identificados los principales riesgos en el proceso. Se realiza el diseño y ejecución de un proceso de UF/DF por casete Sartocon Slice (10 kDa), donde se determina el coeficiente de retención del producto de interés y los volúmenes de diafiltrado. Resultados y Discusión: El coeficiente de retención de P64kr durante el proceso de concentración-dialfiltración fue cercano a 1, obteniéndose recobrados cercano al 100 %. La conductividad del producto respecto a la del tampón se iguala entre 7 y 8 volúmenes de diafiltración. Se reduce la cantidad de reactivos utilizados así como el tiempo total del proceso a 1hora. Conclusiones: Se obtuvieron resultados satisfactorios con ahorros sustanciales de tiempo y reactivos en los 51 lotes de introducción realizados en el BioCen durante la transferencia tecnológica efectuada por el CIGB.

Chromatographic separation of full and empty AAV8 capsids

Blaž Goričar, Romina Žabar, Vid Skvarča, Tomas Kostelec, Maja Leskovec, Hana Jug, Lucija Batič, Mojca Tajnik, Sebastijan Peljhan, Aleš Štrancar

BIA Separations, Slovenia

Adeno-associated virus (AAV) vectors of various serotypes are considered to have high potential for gene therapy applications. Currently, manufacturing of AAV vectors faces the challenge of co-production of incompletely formed particles lacking a recombinant viral genome. Empty capsids increase the dose of total AAV administered for efficient transduction and are thought to cause unwanted immunological reactions against the virus. Removal of empty capsids during manufacturing, as well as analysis of empty/full AAV particle content is therefore a critical requirement for any AAV production process. This poster demonstrates how CIMmultus™ QA monolithic columns can be used to remove empty AAV capsids from the product chromatographically in a single step.

Optimizing a separation of IgG charge variants using weak cation-exchanging analytical monolithic column

Urh Černigoj, Sebastijan Peljhan, Aleš Štrancar

BIA Separations, Slovenia

The upstream and downstream monoclonal antibody (mAb) bioprocessing makes them susceptible to physical and chemical modifications. In the biotechnological production process of mAbs, structural variations may arise due to some enzymatic activity. Antibody charge variants have gained considerable attention in the biotechnology industry due to their potential influence on stability and biological activity and cation-exchange chromatography (CEX) is one of the typical approaches for mAb charge variant analyses. We tested several CEX columns under different conditions and the best column for isotype separation was weak cation-exchanging CIMac COOH chromatographic monolith in pH gradient. We have proven a flow independent separation of mAb charge variants and in this way, a resolution comparable to classical CEX particulate-based analytical columns was achieved in only 6 min analysis time.

Design and optimization of purification process of MY32Ls protein solubilizing inclusion bodies for a new vaccine against sea lice

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The sea lice is the most important marine pathogen in the salmon industry, affecting Europe and America. The most affected are genera: *Pseudocaligus*, *Caligus* and *Lepeophtheirus*. Over 305 million euros losses are estimated. Recent results have suggested that subolesin/akirin/my32 are good candidate antigens for the control of arthropod infestations, including sea lice. This study aimed to design and optimize the purification step of MY32Ls protein to obtain the API against sea lice. Non-chromatographic purification strategies were employed based on published works to establish rupture, washing, solubilization and refolding conditions. Disruption process using bead mill was established. Cell culture volume was flushed through the mill eight times. Four step proces for IBs washing was performed. Buffer Tris-HCl 50 mM first two washes were performed using Triton X-100, in the third wash triton X-100 was removed. In all cases time was set at one hour. The fourth washing at pH 10.5 for 20 minutes was set. The solubilization in alkaline conditions (pH 12.5) for 1.5 hours was performed. Refolding step in the Tween 80 as stabilizing additive was selected. The process allowed to obtain a yield of MY32Ls 0.0414 g/g of wet biomass (monomer) and 0.0557 g of MY32Ls/g of wet biomass (monomer and dimer). API obtained had a better immune response (IgG) to the positive control used, purified by affinity chromatography.

Design, evaluation and simulation of a purification process to obtention a new vaccine against sea lice, using metals affinity chromatography

Carlos Perez Heredia, Nemecio González Fernández, Licette León Barreras, Eladio Salazar Gomez, Rafael Pimentel-Pérez, Eulogio Pimentel Vázquez, Carmen García Molina, Yamila Carpio González, Mario P Estrada García, Miladys Limonta Fernández

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This work in the Technology Development Group of the Center for Genetic Engineering and Biotechnology of Camaguey was done. Its objective was to design stage MY32Ls protein purification for the active pharmaceutical ingredient (API) against sea lice, marine pathogen with important affection in the salmon industry in South America and Europe. A compact purification process based on the analysis of alternatives to establish the steps of rupture, chromatography and refolding was designed. Immunological techniques and protein quantification in the analysis of results were used. Taken into account of technical - economic advantages the chemical rupture process with 8 mol/L urea for 1 hour was stablished, with 20 % more expensive than mechanical rupture. For washing and elution of the protein in the chromatography step the pH and imidazole was evaluated. Washing and elution with imidazole was selected by technical - economic advantages existing. The costs of the selected variant were 12.33 % below to wash and elution using pH. In scale-up of the chromatographic step a new variant "economic" with modifications in urea concentration was evaluated, lowering the costs of the stage at 3.4%. This variant has positive impact in the reduction of volumes to be handled in the refolding step. refolding step in the Tween 80 was selected, which enabled to obtain the protein in solution. The IFA obtained showed higher humoral response to the pattern used, similarly obtained by chromatographic processes (3 fold average). IFA specifications obtained satisfy the requirements for the formulation of vaccine against sea lice.

Mejoramiento de la productividad del sistema de expresión de la estroptoquinasa recombinante mediante mutagénesis en regiones no traducibles del gen

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En el CIGB se produce el agente trombolítico Estreptoquinasa recombinante (SKr). El sistema de expresión se basa en el empleo del hospedero *E. coli* W3110 transformado con un plasmidio recombinante. Debido a la alta demanda de un nuevo producto para la trombosis hemorroidal y al aumento del rigor de las exigencias impuestas por las agencias que regulan todo lo relacionado con los biofármacos de uso en humanos, se hizo necesaria la elevación de la productividad del sistema y la inactivación del gen de la β-lactamasa. Nos propusimos los objetivos de mejorar el vector de expresión pEKG-3 sustituyendo el marcador de selección β-lactámico e introduciendo cambios para aumentar la productividad y estudiar la expresión del producto recombinante en una cepa con mejores potencialidades. Para elevar el nivel de expresión de la proteína SKr se modificaron, mediante mutagénesis por PCR, las secuencias de la señal de terminación traduccional y del RBS (contiguas a los extremos 3´ y 5´ del gen skc-2, respectivamente). Además, se introdujo en el plasmidio el gen Tn903, generándose el vector pEKOpK. Con este se transformó la cepa de *E. coli* BL21 (DE3), se preparó un banco de células y se realizaron ensayos de expresión en zaranda. Los estudios en zaranda arrojaron porcentajes de expresión de SKr de 30 a 40 % respecto a la proteína total del hospedero, el doble de los que resultan del sistema actual. La productividad promedio obtenida fue de aproximadamente 115 mg de SKr/Lh, mayor a la del sistema en explotación (38 mg de SKr/Lh). Se desarrolló un sistema vector - cepa - condiciones de cultivo con el que se consigue una productividad superior a la verificada para el sistema actual de producción de SKr, y que cumple con los estándares regulatorios respecto al no empleo de antibióticos β-lactámicos.

Obtención del factor de crecimiento epidérmico humano intacto activo, a partir de una proteína quimérica expresada en *Escherichia coli*

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El Factor de Crecimiento Epidérmico humano (hEGF) es una proteína asociada con la proliferación celular, la cual se produce en el CIGB de forma recombinante, a partir de una cepa de Sacharomyces cerevisiae, siendo una de sus indicaciones terapéuticas la de estimular la cicatrización de úlceras de miembros inferiores de pacientes diabéticos. Los altos niveles de expresión de hEGF activo en *Escherichia coli* no han tenido éxito debido a que la molécula contiene tres enlaces disulfuro intra-moleculares difíciles de formar en el entorno bacteriano. Para resolver este problema nos propusimos como objetivos: fusionar el gen hEGF con un gen modificador, expresar la proteína quimérica en una cepa modificada para la correcta formación de los puentes disulfuro, purificar y caracterizar la molécula obtenida. Se fusionó el gen hEGF (aislado por PCR) al gen SUMO del vector pSUMO, generándose el gen SUMO-hEGF. Con esta construcción genética se transformó la cepa de *E. coli* Origami (DE3). La proteína quimérica expresada se purificó por cromatografía de afinidad Ni-NTA y el hEGF se escindió por digestión con la proteasa ULP1. La proteína SUMO-hEGF se expresó soluble en *E. coli* Origami (DE3), con un nivel de 25 % respecto a la proteína total del hospedero. La pureza de la molécula hEGF escindida (verificado HPLC de fase reversa) fue mayor del 98 %. La estructura primaria del hEGF purificado se confirmó por espectrometría de masas, y la actividad biológica resultó comparable con la del hEGF comercial.

Purificación y caracterización de la proteína E2 del virus de la peste porcina clásica producida en nicotiana benthamiana

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La Peste Porcina Clásica (PPC) es una enfermedad viral, altamente contagiosa, producida por el virus de la peste porcina clásica (VPPC). Esta enfermedad ocasiona grandes daños a la industria porcina mundial, tanto desde el punto de vista económico como sanitario. La E2 es una glicoproteína estructural del virus que ha sido utilizada para la inducción de respuesta inmune contra esta enfermedad. El gen de E2hist se clonó en un vector binario que contiene las regiones 5´y 3´UTR del CPMV y diseñada para el almacenamiento del antígeno en el apoplasto de la célula. Cultivos de Agrobacterium tumefaciens con los genes E2hist y el supresor del silenciamiento P19 se combinaron para la agroinfiltración de hojas *Nicotiana benthamiana*. El extracto proteico de hojas agroinfiltradas se utilizó para purificar la E2hist por el método de IMAC. La expresión de la E2hist se analizó por Western blot y ELISA. La vacunación se realizó mediante la aplicación a los cerdos de dos dosis de 50 µg de E2hist purificada administrada por vía intramuscular. Los animales se retaron con una dosis de 104 PID50 de la cepa virulenta del VPPC "Margarita". Se obtuvieron altos niveles de acumulación de E2his de hasta 1,2 mg/g de hojas. La proteína se purificó con más de un 80% de pureza. Los animales inmunizados con E2hist y enfrentados al virus de la PPC no mostraron síntomas de la enfermedad. La sobrevivencia de los cerdos fue de un 100% con respecto a los controles no vacunados. Los cerdos inmunizados con E2hist producida en hojas sobrevivieron al reto frente a una cepa altamente patogénica de PPC. La agroinfiltración de *N. benthamiana* se presenta como una alternativa para la producción de antígenos veterinarios.

Introducción de mejoras en el proceso de purificación de la proteína CIGB 550E7

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The CIGB develops a project to obtain a vaccine candidate against cervical cancer and infections associated with HPV 16, which uses the recombinant protein CIGB 550E7, expressed in *Escherichia coli*, as drug substance. The purification process of CIGB 550E7 protein is divided into two fundamental stages: the first one is formed by cell disruption, washing and solubilization; the second consists of metal chelate affinity chromatography (IMAC) and a change of buffer by molecular exclusion chromatography. The yield in the cell disruption stage of the purification process is low, which adversely affects the overall yield. The process also has low capacity for the removal of contaminants like lipopolysaccharides (LPS). An experimental design was performed to evaluate the efficiency of the variables biomass concentration (w/v), pH and number of passes in the high-pressure homogenizer on the yield of the cellular disruption step. It was determined that working with a biomass concentration of 10% (w/v), pH 9 and carrying out three passes in the homogenizer, it is possible to increase 1.74 times the yield in the step. For the removal of LPS contaminants, the introduction of a wash step with Triton X-114 detergent was evaluated in IMAC chromatography, taking into account detergent concentration and column volumes (VC) to be applied. The results demonstrate that at a concentration of 1% of detergent and applying a wash with 20 VC, it is possible to remove 3.33 times the presence of LPS contaminants. The introduction of the improvements to the process of purification of the protein CIGB 550E7 induced an increase of 1.15 times the overall yield of the process and guarantees a product with less than 5 EU / dose, complying with the established by the regulatory authorities for injectable products.

Ajuste de los parámetros de operación de las etapas de ruptura celular y cromatografía de IMAC del proceso de purificación de la proteína CIGB 550E7

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Cervical cancer is the second most common cancer disease in women. Half a million new cases occur annually, causing the death of 250 000 women. Most

of these are caused by infection with the human papilloma virus (HPV). There are two prophylactic vaccines approved by regulatory agencies but there is not any therapeutic vaccine to treat individuals previously infected. Researchers at CIGB are working to obtain a therapeutic candidate against cancer caused by HPV, where the active principle is based on the CIGB 550E7 protein. Previous studies have shown that protein has immunogenic and antitumor activity in animals, which is an endorsement to proceed with the development of a clinical trial in humans. A process for obtaining enough quantities for this purpose has been developed using *E. coli* as host. A fermentation stage and purification steps have been established to obtain a product with the required quality attributes. The adjustment of the operating parameters in the stages of cell disruption and metal chelate chromatography is the main objective of this work. Optimization of mechanical cell disruption and IMAC chromatography allow obtaining higher yield and recovery than current process. Chemical cell disruption is evaluated as an alternative of the cell disruption, obtaining similar yield than mechanical method, but it isn't economically feasible. The introduction of the proposed changes allowed obtaining an active pharmaceutical ingredient to fulfill quality requirements of regulatory authorities in terms of purity and endotoxins contaminants.

BioSC® and BioSC Predict®. Progress in continuous bio manufacturing

Jin Seok HUR

Novasep, LLC, USA

While continuous separation using Simulated Moving Bed (SMB) has been well accepted and established in Pharmaceutical and Food industries for the high productivity, it is relatively recent that Biopharmaceutical companies starts looking at and investigate the continuous technologies. The main driving force was initially high titers of upstream processes. So, the first approach was adding a productive downstream process to the existing batch platform in order to debottleneck in the overall process stream. However, the trend is now moving more toward complete continuous manufacturing platforms thanks to new and improved technologies such as perfusion bioreactors and single path membranes in addition to the sequential multi-column chromatography. This presentation will address the recent progress in bio manufacturing and benefits of a sequential multi-column chromatography, BioSC® and its simulation software BioSC Predict®.

SMART ChromatographyTM - A new method for linearly scalable protein purification

Alistair Hurst, Derek Levison, Rüdiger Welz, Franziska Meier-Hättig

Emp Biotech, Germany

SMART Chromatography™ has been designed to allow for rapid and cost-effective purification of biomolecules directly from the cell culture system. Designed to be truly linearly scalable, SMART Chromatography™ offers the purification scientist a familiar packed-bed chromatography column format that does not require any removal of cell mass (cells, cell debris, etc.) prior to application of the feed stream to the purification column. By exploiting the advantages of radial flow chromatography and combining it with ZetaCell solid phases, processes may be fully developed in the R&D laboratory before transfer to large-scale manufacturing without any modification to the process. Emp Biotech has developed the ZetaCell range of solid phases, which allow the user to select the correct resin for their application, be it ion-exchange (IEX), hydrophobic interaction (HIC) or immuno-affinity. We also offer an activated solid phase to allow the user to create their own functionalised solid phase. The company has developed SMART Chromatography™ to assist the purification scientist in developing and operating more efficient and cost-effective processes. We will present data demonstrating the efficiency of the system with real world applications on both bacterial and mammalian cell systems. When compared to traditional processes, purification times can be reduced from > 24 hours to as little as 2½ hours.

Process intensification for bioprocessing of monoclonal antibodies

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EMDMillipore, USA

Monoclonal antibodies (mAbs) are the workhorse of biotherapeutics and wellestablished, large-scale processes exist for their production and purification. In spite of those platform technologies, innovation continues to flourish given that more and more mAbs enter clinical studies. Changes in upstream titer and improved process efficiencies have reduced batch volumes, so that single use and other new technologies become possible. A key driver, especially in emerging economies, is the need to reduce cost and footprint and increase flexibility. Process intensification covers multiple approaches that range from optimization of individual unit operations to integrated templates that operate continuously. The extent of connectedness between steps, the level of continuous or semi-continuous operation and even the use of single use components can vary widely. In this presentation, we will present options for some of those approaches and highlight both operational advantages and cost savings. We will attempt to delineate the relative contributions of each factor in an effort to assist in process selection and decision making. A key focus will be on bottlenecks in current operation and facility considerations. Looking at individual unit operations, clarification can be improved through use of precipitants and specially designed filters that address the increased cell volume. Capture benefits from continuous, multi-column methodologies, while flow-through purification simplifies operation. We have successfully integrated these technologies in a continuous purification template with performance that matched or surpassed that of the traditional batch process. A staggered approach appears, however, the best way to move forward.

Protein purification improvement by using new selectivity to reduce steps purification, by analysis of different cation exchange matrice and different protein A format for antibody purification

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New selectivity was reach by using mixed mode chromatography. In order to develop a simplified bioprocess for producing recombinant human apolipoprotein A-I (rhApoA1) in its near-native form, rhApoA1 was expressed without the use of an affinity tag in view of its potential therapeutic applications. ApoA1 purification was scaled up to mixed-mode expanded bed adsorption chromatography to establish an '*on-line*' process for the efficient capture of rhApoA1 directly from the *P. pastoris* expression broth. A polishing step using anion exchange chromatography enabled the recovery of ApoA1 up to 96% purity. This two-step process would reduce processing times and therefore costs in comparison to the twelve-step procedure currently used for recovering rhApoA1 from *P. pastoris*. Analysis of different cation exchange matrice by systematic frontal analysis at different flow rate and selectivity determination at different flow rates, we suggested an independent evaluation of cation exchangers to facilitate media selection, and investigated the relationship between (i) surface modification and (ii) chromatographic performances. Structure-extended resins showed higher binding capacities compared to resins with conventional ligands directly attached to the matrix. Moreover, they maintained mainly high capacities even with extremely high flow velocities. Ligand accessibility was therefore largely enhanced, allowing proteins to interact and bind under harsh conditions. High throughput resins can be used for purification of high volume and high concentration feedstock in limited time. This results in higher productivity and could contribute to cost reduction. Different protein A formats for mAb purification (Axial, radial, membrane, monolith) protein A were investigated by using bind and elute conditions. Yield, purity, productivity, aggregates, DNA, HCP, protein A leakage showed that each formats have different qualities.

Recombinant HBsAg purification by antibody-coated supermagnetic nanoparticles

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Purification of Recombinant HBsAg expressed in *Pichia pastoris* is so complicated and time- consuming. Various chromatography steps are established to obtain r-HBsAg with purity more than 95%. Using Iron nanoparticles coated with antibody against Hepatitis surface antigen provided a specific method to separate HBsAg from cell free lysate of *Pichia pastoris*. Nanoparticles provided larger separation area than chromatography media as more antibodies could be conjugated on it. By increasing separation area, large amount of sample could be applied and separation process takes place faster and easier. The other advantage of this method to chromatography method is the stability of nanoparticles. Chromatography media is sensible to temperature and buffer conditions like high conductivity of buffer. The procedure of making nanoparticles and conjugation of these particles with antibody is much easier than immunoaffinity matrix production. Equilibration, sample application, washing and elution are done in immunoaffinity chromatography with appropriate process parameters like flow rate, pressure and time. Steps mentioned above were done with less consideration than chromatography method and elution took place by using a magnet to pull the nanoparticles away and supernatant was obtained by decanting the mixture in this method. The nanoparticles structural study was determined by TEM and FT-IR. Material balance was used to determine the amount of antibody attached on magnetic nanoparticles surface. By using this method the total yield of HBsAg desorbed from iron nanoparticles, finally reached to 85%.

Esterilización de soluciones para productos parenterales. Análisis de la problemática

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Las soluciones para la formulación de productos parenterales deben ser estériles antes de pasar al proceso aséptico de la formulación. Por esta sazón se analizan los diferentes métodos de esterilización referidos en la literatura. Se presentan los criterios termodinámicos que rigen la esterilización. Además, se analizan las experiencias anteriores en la esterilización de soluciones para la formulación de productos parenterales en una autoclave, los cuales muestran tiempos prolongados del proceso y solo se pueden manejar bajos volúmenes de solución. Usando tanques agitados enchaquetados para la esterilización pudieran resolver esta problemática y, por tanto, se muestran los criterios para el diseño de estos que permiten procesar altos volúmenes de solución para la formulación de productos parenterales.

What information can we get from pressure drop measurement?

Ales Podgornik

Centre of Excellence for Biosensors, Instrumentation and Process Control, Slovenia

Measurement of pressure drop is extensively implemented in many areas because of its importance in thermodynamic equilibria and as a driving force for liquid flow. In chromatography it is mainly used to monitor performance of chromatographic columns. In addition to this information, this data can provide also a valuable insight into a structure of chromatographic support packed in chromatographic column, especially interesting due to increasing variety of microstructures encountered in the monolithic chromatographic supports. In this work, we present methodology how pressure drop data can be used to obtain different type of above discussed information. By comparing pressure drop on methacrylate monolith and adequate packed bed, implementing hydrodynamic models

usually employed for prediction of pressure drop in packed beds, some interesting results were obtained. Pressure drop on methacrylate monolith was found to be approximately 50% lower than in an adequate packed bed of spheres having the same hydraulic radius. Results of simple mathematical model, confirmed recently also by CFD, indicates that this phenomenon seems to be caused by a parallel pore nonuniformity, not encountered in conventional packed beds. Furthermore, for monolithic beds having same micro-topology but differing in pore size distribution, pressure drop can be used to accurately predict dynamic binding capacity of such matrix. Finally, pressure drop can also provide an insight into adsorption phenomena, more precisely, giving estimation about thickness of layer adsorbed on a matrix pore surface. Based on recently developed mathematical formalism we are able to estimate thickness of adsorbed plasmid DNA molecules of different size and different bacteriophages but also proteins like IgG. This approach can also be used for determination of thickness of grafted layer and good correlation between graft thickness estimated from pressure drop data and dynamic binding capacity was found. As recent methodology enables estimation of layer thickness without detailed information of monolith micro-topology, this simple measurement can be used on any convective based chromatographic support, including pack beds comprising of non-porous particles.

Immunoaffinity chromatography based on carboxymidazole-monolithic supports to purify Hepatitis B surface antigen particles for human vaccination

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About of 360 million people are chronically infected and 750 000 deaths each year worldwide by Hepatitis B virus. The number of the cases with program of vaccination against Hepatitis B in Cuba decreased from 1992 to 2014 of (2194 - 19) in all population, in children the efficiency of the immunosorbent, monolith support has been used to replace the Agarose resin. Monoliths are enabling tools for purification of biomolecules in nanometer range (virus, plasmid DNA, phages, IgM, PEGylated proteins, etc.) that offer unprecedented improvement possibilities. However, unspecific interactions with support were previously demonstrated affecting the purity of the HBsAg eluted from the columns. Therefore, this study was performed to find experimental conditions where the unspecific interaction among proteins and support where eliminated. As experimental methodology, 31 experimental variants were assessed. As main results, the HBsAg purity increased from 75 % to 95 % using as adsorption buffer 20mM Tris/3mM EDTA/1M NaCl pH 7.2 and 0.1M Tris/0.5 M NaCl pH 8.5. As conclusion, the monolith support can replace the Agarose from CB.Hep-1 immunosorbents to eliminate unspecific interaction with proteins. Though, improvements in the DNA purification capacity have to be demonstrated and some experiment has to be done to increase the HBsAg recovery.

Platform filtration process for purification of Virus Like Particles

Alex Xenopoulos, Sofia Carvalho, Ricardo Silva, Mafalda Moleirinho, Paula Alves, Manuel Carrondo, Cristina Peixoto

EMDMillipore, USA

Virus-like particles (VLP) have become prime vaccine candidates because of their versatility, immunogenicity and safety profile. The diversity of surface epitopes contributes, however, to a variability in downstream purification, that could ultimately affect manufacturability. For baculovirus expression systems in particular, the similarity between residual baculovirus and VLP particles causes significant problems. For that purpose, we have undertaken an effort to develop platform processes for purification of VLPs. In one approach described here, we focus on size exclusion as the key mechanism of separation, with the ultimate goal of an all filtration purification process. The first step was to evaluate a legacy purification that was not robust or efficient and replace the ion exchange chromatography step with size exclusion chromatography. Performance of the SEC step will be described and the shortcomings of such a method for a scaled up, GMP process will be discussed. The proposed all-filtration process will then be described, employing either normal or tangential flow filtration for the clarification stage, followed by multiple ultrafiltration steps to achieve the needed concentration and diafiltration purity specifications. Efforts to clear nucleic acid without the use of an endonuclease digestion step will also be described. To show the potential for a universal, platform process, preliminary results will be presented on two insect cell systems producing two different VLPs.

Expresión y purificación de la L1 VPH 16 de un aislado clínico cubano para la obtención de un candidato vacunal contra el cáncer cérvico uterino

Susana Miraidys Brito Molina, Alina Falero Morejón, Danay González-Acevedo Forte, Yunier Serrano Rivero, Karen Marrero Domínguez, Rafael Fando Calzada

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La L1 es la proteína mayoritaria de la cápside del virus del papiloma humano (VPH). La proteína L1 de VPH 16 puede expresarse de manera recombinante en cepas de *Escherichia coli* y obtenerse en forma de cuerpos de inclusión (CI). Esta proteína es potencialmente útil como componente de las vacunas preventivas contra el cáncer cérvico uterino. En nuestro laboratorio se amplificó el gen l1 de VPH 16 a partir de ADN total purificado de una biopsia de una paciente de Camagüey con cáncer de cuello uterino. La L1 del VPH 16 se expresó en las cepas de *E. coli* Shuffle C3026 y Rosseta, transformadas con el plásmido pETHPV16l1-Myc-6xHis. Los niveles de expresión fueron mayores en la cepa *E. coli* Shuffle, por lo que el proceso de purificación se realizó utilizando esta cepa. Los CI se solubilizaron con 6M de cloruro de guanidinio y la proteína se purificó mediante cromatografía

de quelatos metálicos en un solo paso. Las muestras se evaluaron mediante electroforesis (SDSPAGE y nativa) y la proteína L1 se identificó mediante Western blotting con un anticuerpo específico anti-L1. La L1 del VPH 16 se purificó con más del 90% de pureza. La estrategia utilizada pudiera emplearse en la obtención de la L1, para el desarrollo futuro de un candidato vacunal cubano contra el cáncer cérvico uterino. Este proceso contribuiría al desarrollo de vacunas preventivas con un proceso de producción más económico y un precio más asequible a la población.

Modeling and simulation for defining operational parameters landscape in a downstream process

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Process modeling can be a useful tool to aid in process development, process optimization, and process scale-up. The theoretical understanding of chromatographic behavior can augment available experimental data and aid in the design of specific experiments to develop a more complete understanding of the behavior of a unit operation. The production process for parenteral human epidermal growth factor is prone to be improved. In this work it was used as a platform for exploring the power and possibilities involved with process modeling. It is advisable to make process improvements with a strong knowledge of the phenomenological process involve in whatever modification. In the present work it is shown two different proposals of changes to the current process with the model and the simulation of both of them. The excellent match of the simulation with real data shows the great value of the models to explain the steps involved. Finally a full spreadsheet simulation of the process is shown and the possibility of implementing parameters with its variability as input variable is explained.

New method to obtain the Hepatitis B surface antigen for vaccine

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In this research a new method for obtaining the recombinant Hepatitis B surface antigen (AgsHBr), used for the production of active pharmaceutical ingredient (API) Heberbiovac HB® vaccine was developed. The new method required the combination of established and innovative steps that give the innovative character in the field of recombinant protein production and monoclonal antibody (mAb) applications. The main steps were a relative short fermentation process (80 h) which was based on increasing biomass production and a constant kinetics of AgsHBr expression in the C-226 strain of *Pichia pastoris*, a new antigen precipitation method applying PEG 4000 in the supernatant of the acid precipitation, which allowed the antigen concentration-extraction with a purity greater than 80%; a lyophilized immunosorbent using as ligand the CB.Hep-1 mAb for the purification of AgsHBr without affecting the efficiency and consistency of the process. Moreover, the methodology used to estimate changes in the initial stages of the production process of Heberbiovac HB® API and its impact on the purification process proved to be effective based on the evaluation of each of changes in the immunoaffinity chromatography, which ensured a high selectivity and originality to the new method employed to obtain the AgsHBr. Finally, the assessment of the AgsHBr quality specifications purified by the new method evidence to be equal to Heberbiovac HB®, API produced by the established procedure, demonstrating the advantages of a process that combine the steps of fermentation, cell disruption, acid precipitation PEG-4000, immunoaffinity chromatography and size-exclusion chromatography, which led to a 50 % reduction in process operation time and an increased up to 22 % the total recovered from the process, something that will allow an increase of up to 62 million doses per year.

Symposium 3. New Drug Delivery Formulation for Human and Veterinary Products

Polymer and microemulsion adjuvants enhance the immune response conferred by mucosal vaccines in mice and chicken

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Mucosal administration of vaccines presents many advantages for the control of veterinary infectious diseases. This needle-free approach reduces the safety risks, may allow mass vaccination, and allows the establishment of local immune responses at mucosal surfaces, which constitute the principal entry gate for pathogens. The main issue with mucosal vaccines is that they are usually not as efficient as injectable vaccines. Thus, new adjuvants are needed to improve vaccine efficacy. Cholera toxin (CT) has been shown to be a efficient mucosal adjuvant, but is not adapted for large scale use in the field. In this study, Montanide™ adjuvants based on polymer or microemulsion technologies have been tested for mucosal application. In the mouse model, we showed that the intragastric administration of ovalbumin (ova) with Montanide™ Gel 01 (polymer) or Montanide™ IMS 1313 VG (microemulsion) induce strong specific IgG antibody titers, comparable to CT adjuvanted ova. In a poultry trial, Montanide™ IMS 1313 NVG (IMS 1313N) and Montanide™ Gel 01 ST (Gel 01) were used as diluents for a lyophilized live infectious bronchitis vaccine. Day old chickens were vaccinated by either intranasal or spray delivery. Antibody titers and protection against homologous challenge were measured. Both adjuvants induced strong responses compared with a commercial non adjuvanted formulation. IB antibody titers were significantly higher for adjuvanted formulations compared to non-adjuvanted vaccine when delivered intranasally. By intranasal route, we observed ten days after challenge a protection of 70% for Gel 01

and 80% for IMS 1313 N VG, compared to 30% for the non-adjuvanted vaccine. By spray delivery, the rate of protection reached 90% with the polymer adjuvant Gel 01 while the non-adjuvanted vaccine was not protective (10%). These results show that Montanide™ Gel and IMS adjuvants trigger a strong immune response when delivered by mucosal route that could allow the improvement of the protection conferred by mucosal vaccines in the field.

Epidermal growth factor-pellet for the treatment of ulcerative colitis

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Intralesional injection application of epidermal growth factor (EGF) in addition to standard treatment is able to achieve both partial and complete healing and prevent foot amputations in diabetic patient. The optimal treatment for UC includes corticosteroid which can increase blood glucose levels, there may be an increased risk of diabetes developing and there are currently no alternative therapies to corticosteroids available for the treatment of serious active UC symptoms, so other alternatives of drug should be develop for these patients. In this work the effect of a new formulation in the form of pellet containing EGF to be administered by the oral route in a biomodel of UC in rat was evaluated. Male Wistar rats were divided in: Group I: Control, Group II: Colitis. Group III: Colitis + EGF pellet. Group IV: Colitis + placebo pellet. Morphological indicators such as the severity and extent of inflammation, damage of the crypt and percent of tissue involved were evaluated. In animals with colitis and treated with EGF pellet recovery morphological changes were observed such as the reduction of the area affected, the severity and extent of inflammation and damage of the crypt, statistical difference were observed with the other groups. Pharmacogenomic evaluations were also made. This result corroborated the use of EGF in pellet may be effective in the treatment UC. According to this results EGF pellets could be use in diabetic patient for the treatment of UC.

Stability and immunogenicity of a spray-dried bacteriophage l2 Virus Like Particles against human papillomavirus Type 16

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Human papillomavirus (HPV) infections are associated with cervical cancer, penile cancer and anogenital warts. Three prophylactic vaccines have been approved to protect against HPV infections. However, the vaccines require continuous cold-chain storage and may not be suitable for third world countries, with less developed refrigeration facilities. We had previously developed a candidate vaccine against HPV infection by displaying a conserved epitope -- derived from the minor capsid protein (L2) of HPV -- on the surface of a nanoparticle known as bacteriophage MS2 virus-like particles (VLPs). Bacteriophage MS2 L2 VLPs elicited broadly protective antibodies against diverse HPV types. To enhance the thermostability of the L2 VLPs-based candidate HPV vaccine, we formulated the VLPs with excipients of sugars/amino acid, and we spray-dried the mixture into dry a powder. Spray-dried VLPs were stable at room temperature and at 37 °C for more than one year. More importantly, the sprayed-dried L2 VLPs, stored at the above conditions for more than 1 year, were immunogenic and protective against vaginal infection with HPV pseudovirus type 16 after reconstitution and intramuscular immunization.

Sticholysins, two pore-forming toxins (PFT) from a sea anemone, encapsulated into liposomes: a novel strategy for improving immune cellular

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Sticholysins I and II (StI/II, Sts), two pore-forming proteins produced by a seaanemone, are highly hemolytic cysteineless proteins exhibiting a preference for sphingomyelin-containing membranes. Different strategies employing bacterial PFT have been used to improve the antigen-specific cytotoxic T CD8+ lymphocyte (CTL) response. Furthermore, liposomes have been used as adjuvants due to their ability to improve antigen uptake by antigen presenting cells. In this work we studied the enhancement of antigen-specific CTL-mediated immune esponse by liposomes with a specific lipid composition and co- encapsulating Sts with ovalbumin as model antigen LP/OVA+St). Immunization of mice with LP/OVA+St induced an OVA-specific CD8+ T-cell expansion superior to that observed with LP/OVA without Sts, suggesting potentialities of these proteins to mediate the antigen cross-presentation. In agreement, Lp/OVA+St significantly enhanced activation in vitro of the OVA-specific B3Z CD8 T cells as a consequence of antigen cross-presentation by macrophages but not by dendritic cells (DCs). Interestingly, Lp/OVA/StII-induced activation was inhibited by cathepsin general inhibitor and lysosome inhibitor, but not proteasome inhibitor indicating that StII induces antigen cross-presentation by vacuolar pathway but not cytosolic pathway. Additionally, the formulation LP/OVA+StII enhanced the OVA-specific CTL response in vivo in comparison with LP/OVA and also conferred a higher protection to mice challenged with OVA-expressing tumor cells. CTL activity induced by LP/OVA+StII was independent of CD4+T-cells, while anti-tumor response wasstrongly affected by CD8+ T-cells depletion. Interestingly, free-Sts were able of inducing

activation of DCs and it was dependent of TLR-4 and MyD88, suggesting that the effect of these proteins on the cellular immune response could be beyond their pore-forming ability. The antigen-specific CTL immune response enhanced by immunization of wild type mice with LP/OVA+StII was significant reduced in TLR-4 knockout mice. Our results suggest the potentialities of sticholysins encapsulated into liposomes as adjuvant for enhancing effective CTL mediated immune responses.

C5a functions as a molecular adjuvant in teleost fish

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C5a, the most potent anaphylatoxin generated during complement activation, has important pro-inflammatory actions and has also been shown to enhance antigen-specific antibody response in mammals, thereby acting as a molecular adjuvant. In rainbow trout, C5a has been shown to have a chemoattractant ability and its receptor has also been found on potential APCs. In this study, we tested the possible role of trout C5a as a molecular adjuvant. We demonstrated the presence of native C5a in trout serum using the antibody generated by recombinant trout C5a, and then we generated recombinant infectious hematopoietic necrosis virus glycoprotein (G), and a G-C5a fusion protein to test the adjuvant activity of trout C5a. Recombinant G-C5a displayed a potent chemoattractant activity in contrast to G alone, indicating that the C5a portion of the fusion protein was functional. Thereafter, G-C5a, partially emulsified in a small quantity of API, was injected into one group of trout, while the other group of trout was inoculated with the same dose of recombinant G. At four to sixteen weeks post-injection, the serum IgM antibody levels of the fish injected with recombinant G-C5a were obviously higher than those injected with G protein alone. Thus, these results suggest, for the first time, that C5a acts as molecular adjuvant in teleost fish by enhancing antibody response to a soluble antigen.

Nanopartículas lipídicas como sistemas de liberación y adyuvante del antígeno tumoral DEC-Her1

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La nanotecnología proporciona múltiples plataformas para el desarrollo de nuevos adyuvantes; los cuales, son estudiados para reducir las dosis necesarias, así como mejorar la estabilidad, seguridad y función terapéutica de las vacunas. Las vacunas terapéuticas, basadas en Antígenos Asociados a Tumores y potentes adyuvantes inmunológicos, han mostrado resultados alentadores en este campo; donde el receptor del factor de crecimiento epidérmico (Her1/ErbB/EGFR, del inglés Human Epidermal Growth Factor Receptor) se considera un blanco atractivo para la inmunoterapia dirigida a tumores de origen epitelial y su diseminación metastásica. Por otra parte, las formulaciones IFAL-Finlay, constituyen una novedosa plataforma de adyuvantes nano-particulados de naturaleza lipídica; que actúan combinando los mecanismos de liberación controlada del antígeno y el direccionamiento de la respuesta inmune. Se prepararon diferentes formulaciones en forma de nanopartículas, las cuales se evaluaron utilizando diferentes técnicas físico-químicas, tales como la ECF, e inmunoquímicas. Se realizó el ensayo de HSR para determinar la capacidad de activar la rama humoral y celular de la respuesta inmune por parte de las variantes de formulaciones del adyuvante en forma de nano-liposomas y nano-cocleatos, conteniendo el dominio extracelular de Her1 en el modelo de ratón Balb/c. Se evidenció el potencial inmunogénico de estas variantes y se demuestra que los anticuerpos generados por la inmunización, pueden reconocer a Her1 en una línea tumoral humana; así como la capacidad de bloquear la activación de este receptor. No se encontraron efectos adversos graves. La menor incidencia de eventos adversos manifestada por los animales tratados con la formulación denominada DEC-Her1-AIF-3 con respecto al resto de los grupos, unido a la capacidad inmunogénica mostrada por esta formulación, sugieren su futura aplicación como candidato prometedor para la inmunoterapia activa de neoplasias de origen epitelial Her1 positivas.

Development of a therapeutic vaccine candidate against HIV (TERAVAC HIV)

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The CR3 recombinant protein is made up of fragments of proteins present in HIV. Studies in mice have shown that the CR3 protein alone is not able to induce a Th1-like response or stimulate TCD8 lymphocytes, only when it is formulated together with the structural antigens (Ags) of hepatitis B virus (HBV) which provide an adjuvant effect that manifests itself after mucosal and parenteral immunizations. For the development of the vaccine candidated: definition of the main degradation products and formulation buffer. The pH, ionic strength and stability of HBsAg and AcHB were evaluated in addition to the excipients were performed. Once the best conditions were defined, different formulation variants were evaluated, performing studies at 37ºC and real stability for 12 months. The main degradation products of the CR3 protein were determined by the mass spectrometry method, obtaining a profile characterized by a main peak where eluates the

protein of interest and a hydrophilic peak eluates fundamental degradation. The influence of pH and ionic strength as well as different buffers were evaluated, demonstrating that the protein and HBV antigens are more stable at pH 5, in 50 mM sodium acetate. To stabilize the suspension and ensure an isotonic formulation the best additive used was glycine 20 mg/mL. Accelerated studies in different formulation showed that the best formulation was lyophilized. The best formulation was the lyophilized preparation, stabilized with 20 mg/mL Glycine in 50 mM acetate buffer, pH 5. Its stability was demonstrated for 12 months from 2 to 8 ºC. This result allowed the authorization of Regulatory Authority to the beginning the Phase I Clinical Trials recently concluded.

A new adjuvant based on liposomes encapsulating Sticholysin II with ability to enhance CTL-mediated immune response against tumor

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Cytotoxic CD8+ T lymphocytes (CTL) are crucial in the host defense against tumors. Vaccine adjuvants addressed to enhance CTL-mediated immune responses remain a current challenge. Liposomes encapsulating bacterial pore-forming proteins (PFPs) have been employed as adjuvants to improve CTL responses. In this work, we studied the ability of Sticholysin II (StII), a PFP from the anemone *Stichodactyla helianthus* encapsulated into liposomes, for enhancing an ovalbumin (OVA)-specific CTL-mediated immune response with antitumor functionality. C57BL/6 mice adoptively transferred with CD8+ T-cells from OT-1 mice were immunized with OVA-containing liposomes co-encapsulating or not StII (Lp/OVA/StII and Lp/OVA, respectively). Lp/OVA/StII induced an OVA-specific CD8+T-cell expansion significantly superior to that observed with Lp/OVA, and a higher percentage of the memory CD8+ Tlymphocytes (CD62LloCD44hi). In agreement, immunization with Lp/OVA/StII enhanced an OVA-specific CTL response in comparison with Lp/OVA and also conferred a higher protection to mice challenged with OVA-expressing tumor cells. Besides, CTL activity induced by Lp/OVA/StII was independent of CD4+ T-cells, while antitumor response was strongly affected by CD8+ T-cells depletion. Lp/OVA/StII vaccination was also more effective than Lp/OVA in tumor-bearing mice. Additionally, in vitro studies with mouse bone marrow dendritic cells (BMDCs) showed that StII induces BMDCs maturation and OVA-cross presentation when the PFP is mixed with this antigen. These data suggest the immunomodulatory properties of StII and its potentialities to mediate the antigen cytosolic delivery to APC. In general, our results support the use of liposomes encapsulating StII as promising adjuvant for enhancing CTL-mediated immune responses and their effectiveness against tumors.

Using Montanide™ ISA 50 V2 as adjuvant for the formulation of the anti-tick immunogen

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The cattle tick (*Rhipicephalus microplus*) infestations have an economic impact on cattle production by reducing weight gain and milk production. An alternative for tick control in cattle is the use of Bm86, the active pharmaceutical ingredient of Gavac™ immunogen. In this paper, the use of Montanide™ ISA 50 V2 as new adjuvant was evaluated to homogenize the formulation process. Physicochemical characterizations of both the current adjuvant and the proposed Montanide™ ISA 50 V2 as well as of the emulsion formed were performed. The results confirmed that both adjuvants were physicochemically similar and that the derived emulsions exhibited better characteristics when using Montanide™ ISA 50 V2. The three batches studied for stability during 24 months showed results analytically consistent with the expected ones, confirming that Gavac™ immunogen is stable for 2 years. To date, with this new adjuvant change proposal 9 batches were obtained, which is equivalent to 1.5 million doses. These batches were compared with historical data of previous batches. It was observed they were similar in terms of qualitative characteristics as well as in biological activity. It was demonstrated that the use of Montanide™ ISA 50 V2 ensures better qualitative characteristics for the Gavac™ Immunogen.

Estudio de reformulación del inmunógeno Gavac

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En el año 2007 la firma comercializadora SEPPIC, decidió reemplazar del mercado el adyuvante Montanide 888 de origen animal, el cual forma parte de

la formulación del inmunógeno Gavac, por un nuevo Montanide 888 VG de origen vegetal. En este trabajo se realizó un estudio de reformulación del inmunógeno. Se produjeron 20L con la formulación actual y 20 L con el nuevo adyuvante, ambos divididos en tres sublotes y analizados por estabilidad mecánica, térmica, tamaño de gotas, viscosidad, potencia e inocuidad. Se compararon los resultados de ambas formulaciones. Para el análisis de los resultados se utilizó el paquete estadístico Statgraphics Plus 5.1. Se apreció un incremento en la viscosidad de la nueva formulación en 1,7 veces con respecto a la formulación actual, aunque los valores obtenidos siempre estuvieron por debajo de la especificación establecida. Se demostró mediante un estudio de estabilidad acelerada que la nueva formulación es estable desde el punto de vista gravitacional 5 días a 55 °C y 15 días a 37 °C. Se comprobó que la sustitución del Montanide 888 por el Montanide 888 VG, no varía el costo del inmunógeno Gavac, por lo que resulta económicamente factible su uso en la formulación del inmunógeno. Se obtuvo una formulación que cumple con las características de calidad vigentes para el inmunógeno Gavac.

The influence of different peptide combinations to increase the immunogenicity of the gonadotrophin releasing hormone vaccine for treatment of prostate cancer

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Therapeutic vaccines, specifically the Gonodotrophin Releasing Hormone (GnRH) vaccine, are considered as an additional therapeutic option for the treatment of prostate cancer in advanced stage. Our work showed amplification of the immune response obtained when combining two peptides with and without. Very Small Size Proteoliposomes (VSSP) as enhanced immuno response. The test was carried out in Copenhagen rats as animal model. The use of both peptides and their combination with VSSP generated a statistically superior response, in term of generating anti GnRH antibody and effects on target organs, when it was compared with the effects which occurs with independent peptides and with and without VSSP. These results can find application in the development of GnRH vaccine candidates and in peptide based vaccine strategies. The vaccine proved to be safe and effective in the 4 dose levels. A total of 252 adverse events were reported, of very probable causality 69%, seven of these events led to the definite lifting of the treatment, wile 62.4% remained at the time of computing these data. However these said events are not only expected, but also desired events expected and desired events. Since only the 4.4% of them came to be of grade III.

Heberprovac, a GnRH based vaccine to treat advanced prostate cancer

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GnRH-based vaccines represent a promising antihormonal treatment alternative in prostate cancer, because these can reduce serum testosterone to castrating levels, avoid the "hot flushes" produced by GnRH analogues and can be administered in acute and complicated forms of prostate cancer. The present study assesses the application of Heberprovac as a vaccine candidate for patients suffering from prostate adenocarcinoma. The main objective of the trial were to evaluate the safe application of Heberpovac at 4 levels of dosage both from the local point of view and from the systemic one enabling thus the identification of some effects evidences of the product in the treated patients. To this aim, 56 patients affected by prostate adenocarcinome diagnosed by the biopsy were included. Since the first immunizations, the patients exhibited clinical improvement, signaled by the decrease of both the number of obstructive symptoms as well as also the severity of the most frequent symptoms. There was also a significant reduction of the PSA and Testosterone values for all groups under treatment often week seven of immunizations. This prevailed after the radiotherapy showing also as congruence between the response variable in an individual way. On the other hand there was evidence of an overall significant correlation between the prostate gland size, the testosterone levels and the PSA. This latter, inversely proportional to the antibodies anti GnRH titers.

Synthesis and characterization of the aluminum phosphate adjuvant used in the formulation of the Cuban pneumococcal vaccine: Quimi-Vio

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Most vaccine manufacturers prefer to synthesize the aluminum phosphate adjuvant (AlPO4) to use it fresh, because it offers better adsorptive properties and guarantees adequate stimulation of the immune system. This work focuses on the synthesis and characterization of $AlPO_4$ used in the formulation of the Cuban pneumococcal vaccine: Quimi-Vio. Synthesis of the adjuvant was performed in a reactor by mixing $NaH_2PO_4 \cdot 2H_2O$ and $AlCl_3 \cdot 6H_2O$, at constant pH. A pH study of 2-9 was performed, the stirring speed (100, 150, 250, 300 rpm) was studied and the washes were optimized. The production lots were synthesized with the established conditions and the stability was evaluated. The adjuvants were determined to be aluminum, phosphorus, chloride, % Adjuvant with Quimi-Vio, particle size (tp) and analyzed by IR Spectroscopy, Transmission Electron Microscopy (TEM), X-Ray Diffraction (XRD and NMR-Al^{27}. Adjuvants synthesized at pH 4-6; adequately sedimented.

The velocities from 200 to 150 rpm, ensured an adequate pH control and the latter allowed to obtain $AlPO_4$ with the highest% of adjuvant (97%). The 5 washes with 0.9% NaCl guaranteed a chloride content of 0.99%. Adjuvants were classified as amorphous aluminum (IR) hydroxyphosphate (DRx), with a primary tp < 50 nm (TEM) and the NMR - Al^{27} showed tetrahedral and octahedral coordinated aluminum with neighboring phosphate groups. The secondary tp was 4.9 μm ± 0.33. The percent of adjuvant with Quimi-Vio was ≥ 80% in all cases. The adjuvants were stable for one year and after resterilization. It was possible to synthesize an $AlPO_4$ with properties suitable to be a vaccine adjuvant. It proved to be stable for one year and after resterilization.

Epidermal growth factor (EGF) loaded polymeric microspheres: preparation, characterization and release studies

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Epidermal Growth Factor (EGF) is a therapeutic peptide used in wound healing and more recently for preventing the amputation of lower limbs in diabetic patients having chronic ulcers. A novel delivery system based on microspheres containing EGF could offer some advantages over an immediate release formulation. This work was aimed to obtain polymeric microspheres of biologically active EGF having a potential healing effect. EGF loaded microspheres were prepared by the double emulsion-solvent evaporation and spray drying technologies using copolymer of lactic and glycolic acid as polymeric matrix. Spherical particles with a smooth surface having randomly distributed pores were obtained. A quantitative extraction procedure wasdesigned for evaluating the properties of encapsulated EGF. Several experimental samples were analyzed using such procedure and in all of them the extracted EGF exhibited their initial properties. The release profile of encapsulated EGF showed a two-stage pattern with a burst release of about 30% followed by a slow release stage. A study performed in vivo in a model of full-thickness wounds in rats showed evidences (capillarogenesis and maturation of collagen fibers) of the potential healing effect of EGF released from polymeric microspheres. The results presented here support the idea that these microspheres could be a good approach for designing novel EGF delivery systems with potential applicability to the treatment of diabetic foot ulcers.

Antimicrobial peptides isolated from tilapia: a promissory alternative as therapeutic agents and molecular adjuvants

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Antimicrobial peptides constitute an important component of the innate immune system. They play an essential role in host defense against pathogens, either because of its antimicrobial direct action or due to their immunomodulatory properties. Modern vaccines based on purified recombinant antigens have improved their safety; however they induce a suboptimal immune response without the help of adjuvants. Consequently, the development of new adjuvants to enhance the immunogenicity of purified subunit antigens and modulate resulting immune responses is of great interest. In the present study, we isolated three antimicrobial peptides from tilapia (*Oreochromis niloticus*). These peptides were named Oreochromicins (Oreoch-1, Oreoch-2 and Oreoch-3). Oreochromicins were characterized according to their antimicrobial and cytotoxic activities, as well as their lipopolysaccharide binding properties. Moreover, we evaluated the ability of these antimicrobial peptides to enhance adaptative immune responses in mice and tilapia. When co-administrated with ovalbumin in mice, Oreochromicin-1 induced a humoral immune response while Oreochromicin-2 and 3 induce a cellular immune response characterized by the induction of interferon-γ in a dose depend manner. Additionally,co-administration of Oreochromicin-1 with the sea lice my32 from *Lepeophtheirus salmonis* antigen (my32-Ls) increases the humoral immune response in mice and tilapia. We also tested different combinations of these Oreochromicins with the sea lice antigen my32-Ls in mice. Humoral and cellular responses were enhanced by co-administration of my32-Ls/Oreochromicin-3 and the combination my32-Ls/Oreochromicin-2/3. In summary, the results showed that tilapia antimicrobial peptides Oreochromicins are able to boost immune response in mammals and fish, encouraging their use as molecular adjuvants to subunit antigens. This study provides important insights for the use of these peptides as molecular adjuvants and for treating and/or preventing microbial infections in fish and mammals.

Novel candidate vaccine against sea lice based on ribosomal P0 antigen and a SEPPIC "water in oil" adjuvant

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Infestations with sea lice (*Copepoda*, *Caligidae*) have grown to become the greatest challenge in salmon aquaculture. Treatment-resistant lice, and the necessity to reduce costs and threats to the environment, highlight the importance of developing new methods such as vaccines for parasite control. To date, there are no commercial vaccines available against sea lice. The P0 protein is a structural component of the ribosome of all organisms. Recently, our group demonstrated high vaccine efficacy against the ticks, *Rhipicephalus sanguineus* and *Rhipicephalus Boophilus microplus* in rabbits and cows respectively, using a peptide of 20 amino acids derived from the ribosomal protein P0 of *R. sanguineus*. In

this connection, we have identified an immunogenic region of the ribosomal protein P0 from *Caligus rogercresseyi* and *Lepeophtheirus salmonis*, the two more important sea lice species affecting salmon aquaculture. This fragment is not very conserved compared to Salmo salar P0. We developed several vaccine candidates based on this peptide, produced them in *E. coli* and purified by metal affinity chromatography. We also evaluated and compared different Seppic adjuvants in tilapia as teleost fish model. As results, these purified antigens were able to elicit a high specific IgM antibody response after intraperitoneal immunization in tilapia in the formulation containing the antigen adjuvated in Montanide ISA 50 V2 (W/O). Taking into account that the efficacy of vaccination has been often attributed with specific antibody levels present on immunized fish, these results are promising. These findings will be finally validated in an immunization-challenge trial in the vaccine´s target specie *S. salar*.

Symposium 4. Biocontrol Formulation

Formulation studies of synthetic compounds that stimulate plant defense

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Protection of plants against infectious microorganisms depends on both constitutive and induced defense mechanisms. Induction of disease resistance is a method of great importance and interest at present, which allows the use of biochemical and molecular mechanisms that already exist in the plant for use in disease control. The defense of plants to disease comprises a series of events related to the recognition, signaling and response defined as innate immunity in plants. In our work we use different synthetic compounds for stimulating the natural defense and induction of resistance plant diseases. We studied the formulation of these molecules to improve the activity and persistence of the active ingredient in *Arabidopsis* plant´s leaves. In this regard we have found that combination of DMF and PEG (1500) with neutral pH was the best variant which allowed an enhance response of relative expression in some defense related genes. Finally, the successful use of any active ingredient depends on its correct formulation into a preparation which can be applied for crop protection safely and with low risk to those applying the material, to non-target species and to the environment in general.

A foam formulation of entompathogens for control of boring beetles in avocado orchards

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A foam formulation of *Beauveria bassiana* was adapted to control boring beetles in avocado orchards. The two geographically independent avocado growing areas in the United States are threatened by emerging diseases vectored by boring beetles. In the California growing region, Fusarium dieback is vectored by the Tea shot hole borer (*Euwallacea fornicatus*) which has a symbiosis with a plant pathogenic *Fusarium* sp. In the Florida growing region, Laurel wilt is vectored by the red bay ambrosia beetle (*Xyleborus glabratus*), which has a symbiosis with the plant pathogen *Raffaelea lauricola*. The current study evaluates the effectiveness of this novel application strategy and formulation to control the insects under orchard conditions. Preliminary results show some potential in controlling some species of boring beetles in avocados.

Producción de xilanasas por *Bacillus subtilis* E44 en condiciones de fermentación en fase sólida

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Las xilanasas son enzimas que catalizan la hidrólisis de los enlaces β-(1,4) del xilano, componente mayoritario de la hemicelulosa presente en la pared celular de las plantas. Estas enzimas han sido ampliamente utilizadas en la industria alimenticia, del papel y cosmética. La obtención de esta proteína a partir de microrganismos empleando residuos agroindustriales como sustratos para la fermentación, disminuye los costos de producción de las mismas, aumentan el valor agregado de estas importantes fuentes renovables y además constituye una alternativa ecológicamente viable. El objetivo de este trabajo es evaluar la producción de xilanasas por *Bacillus subtilis* E44 empleando residuos agroindustriales en condiciones de fermentación en fase sólida. Se evaluó la actividad enzimática cualitativa en placas empleando como única fuente de carbono el xilano de haya. La cepa mostró un halo de hidrólisis, lo cual demostró su capacidad de expresar la enzima. De los residuos agroindustriales evaluados bajo condiciones de fermentación en fase sólida, el afrecho de trigo mostró los mejores valores de actividad xilanasa (2,613 UI/g), seguido de la avena (1,082 UI/g) y el bagazo de caña de azúcar (0,981 UI/g). El resto de los sustratos también mostraron actividad de la enzima. Estos resultados indican que es posible emplear desechos agroindustriales para la obtención de xilanasas a partir de *Bacillus subtilis* E44.

Screening method for selection of bacteria for biological control of *Meloidogyne incognita*

Ramón Franco, Liszoe Galdós Betártez, Laritza Domínguez Rabilero, Arletys Mogena Verdecia, Nestor Mora González

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The total global agricultural damage caused by plant-parasitic nematodes is estimated to be $100 billion USD per year, among which the most important

are root-knot nematodes (RKNs) *Meloidogyne* spp. Biological control provides an efficient alternative to control RKNs with no or little hazard to the soil environment. Some bacteria shows nematicidal activity against RKNs, but little is known about the mechanisms of bacteria pathogenicity. Understanding nematophagous bacterial populations and their mechanisms of action against nematodes provide a basis for developing novel isolation strategies. Therefore, the purpose of this study was to describe a screening methodology based on the production of extracellular enzymes by rhizobateria as mode of action against RKNs, *Meloidogyne* spp. We selected three sources of isolation; they were Tomato (*Solanum lycopersicum*) roots with rhizosphere soil, eggs and females of *Meloidogyne* spp. from a plant-parasitic nematode infested greenhouse. This method allowed to process large number of samples and the obtaining of several bacterial strains with high chitinolytic and protease activities.

"In vivo" and "in vitro" nematicidal activity of *Sphingobacterium* sp CIGBTb

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The effectiveness of *Sphingobacterium* sp CIGBTb (1, 2) for controlling Meloidogyne spp was evaluated in vitro and in pot trials. 50 ml de bacterial cell cultures (*Sphingobacterium* sp CIGBTb) at concentrations of 10^6 ufc/mL were applied to the soil surface infested with 1500 eggs of Meloidogyne spp 5 days before. There were 10 replicates for treatment. After 7 days, *L. esculentum* UC-8213 were transplanted in each pot. *Tsukamurella paurometabola* C924 was the positive control and Triptone Soy Broth was the negative control. The gall index, shoot length, plant weight, and the number of egg per mass were determined at 40 days. Also the hatching inhibition of *Meloidogyne* eggs was evaluated "in vitro". In vitro the treatment with *Sphingobacterium* sp CIGBTb at concentrations of 2×10^8 ufc/mL resulted in 87% inhibition of *Meloidogyne* sp egg hatching. In pots Sphingobacterium sp CIGBTb reduced significantly ($P < 0.05$) the gall index from 5.3 to 2.9 and *Tsukamurella paurometabola* C924 (positive control) to 2.6. In addition *Sphingobacterium* sp CIGBTb diminished significantly in 58% the number of nodules in the roots of *Lycopersicon esculentum* and C924 in 70%. However *Sphingobacterium* sp Tb reduced the number of egg per mass (in a 53%) while the controls did not. *Sphingobacterium* sp CIGBTb at this concentration also promoted the growth of *Lycopersicon esculentum*. Plant weight of this treatment increased over the control in 0.59 times. This strain can allow the development of a biological nematicide with a different action mechanism.

Combination of selection strategies for screening of bacteria as biocontrol of phytonematodes

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Many bacterial isolates have been evaluated as potential biocontrol agents against nematodes. However, few of them were successful after evaluation in field trials due to an inadequate selection strategy. Three selection strategies applied in CIGB of Camagüey were compared. In the first case, 158 isolations from banano suppressive rizosferic soil for nematode were obtained on complete medium (LB agar). After a large " in vitro" and in pot selection process, C-924 and C-926 strains controlled *Meloidogyne* sp, Tricostrongilus sp and *Radopholus* sp with a technical effectiveness over 80% in field trials. C-924 became in the active agent of the nematicide bioproduct Hebernem. In the second methodology, seven parasitic bacteria were isolated on LB agar from disinfected eggs, larvae and adults of *Radopholus* sp, *Haemonchus* sp and *Tricostrongilus* sp with morphological alterations determined by specialists in Nematology. The "in vitro" evaluation on *Haemonchus* sp eggs showed inhibition of hatching in over 75% with CIGBG1, CIGBTb and CIGBR23. In pot trials CIGBTb was the most effective treatment and it reduced significantly the infestation index in *Cucurbita maxima* and *Lycopersicon esculentum*. The last strategy combined several criteria: the pre-enrichment of suppressive rizosferic soil in Minimal medium (M9) with nematode eggs as only carbon source with the selection by enzymatic hydrolysis (of chitin and haemolysin), "in vitro" assay and the pot trials. It was obtained 140 strains that degraded an structural component of nematode. The five non hemolytic bacteria that were proven "in vitro" (H16, H23, H32, Q23 and Q24) inhibited the hatching of *Meloidogyne* sp. eggs over the 75%. H32, a promissory biocontrol bacteria of quick growth and few nutritional requirements reduced significantly the infestation index of *Meloidogyne* sp. in pot trials. The combination of these selection strategies guaranteed the obtaining of bigger number of bacteria with potential as biocontrol.

Assessment of bacterial selection strategies for biological control of *Rhipicephalus microplus*

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Rhipicephalus microplus are obligated blood-feeding arthropods that are distributed worldwide and denote a serious hazard to both human health and animal production. *R. microplus* are controlled at present mostly by chemical acaricides. However, biological control is becoming an increasingly attractive approach to tick management. The entomopathogenic fungi are good candidates as biocontrol agents but they have some disadvantages. Some bacteria show pathogenicity to ticks but further researches are required to elucidate the mechanism of bacterial pathogenicity. This study aimed to describe methodologies for obtaining tick antagonist bacteria. We selected several sources of isolation from: entomopathogenic nematodes, dead ticks, ticks killed by entomopathogenic nematode and dead beetles. We isolated several bacterial strains as possible tick controllers. Batches of larvae ticks were immersed in a suspension of 10^9 CFU/mL of each strain. All treatment and control groups were observed for 30 days, and the larval mortality was

assessed. The effect of the isolates from entomopathogenic nematode tested herein on larvae of *R. microplus* showed a significantly higher mortality than those of the control groups ($p < 0.05$). This study demonstrates that we possess potential strains of interest as a biological agent against ticks.

Developing *Metarhizium* granules for control of soil-dwelling arthropod pests

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Entomopathogenic fungus in the genera *Metarhizium* have been developed into successful biological pesticides. Recent production advancements are capable of producing large quantities of microsclerotia using liquid fermentation. These sclerotia are fungal propagules that are well suited for processing into granular products for control of soil dwelling pests. When applied to the moist soil environment, the microsclerotia produce conidia, which subsequently infect target pests. Initial research has evaluated robust granule production and product storage characteristics in an effort to identify boundaries of microbial survival. Additionally, potential target pests including ground dwelling beetles and ticks were challenged under laboratory and field conditions with formulated fungal granules to establish control efficacy data

Leveraging culture collections for the discovery and development of microbial biological control agents

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The incorporation of living microbial biological control agents into integrated pest management programs is highly desirable because it reduces the use of chemical insecticides harmful to livestock, humans and the environment. In addition, it provides an alternative means to combat resistance to chemical insecticides. However, the commercial development of living microbial agents for insect pest control has lagged far behind in comparison to the development of chemical pesticides. Limited success in commercializing living microbial agents is due in large part to the lack of effective strains to produce consistent pest control under field conditions. One possible solution is to genetically improve existing production strains, but this could eliminate the desired property of an agent being regarded as "natural". One alternative is to conduct large scale screening studies of samples from environmental and other sources. Unfortunately, there is a tendency in some circles to label such studies as "fishing expeditions", although they need not be if care is taken to design them in a rationale way based on clear and focused objective that attack a well-defined question or hypothesis. For that purpose, utilization of culture collection resources and scientific expertise is an important strategy. In this presentation, an overview is given of how culture collections can be leveraged to develop new microbial biocontrol agents.

Formation of dry gram-negative bacteria biocontrol products and small pilot tests against potato dry rot

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Pseudomonas fluorescens strains S11:P:12, P22:Y:05, and S22:T:04 reduce important potato maladies in storage including dry rot, late blight, pink rot, and sprouting. Experiments were conducted to identify methods for producing a dried, efficacious biological control product from one or more of these strains. Isolates were grown in a liquid medium for 24 h, amended with differing amounts of carbohydrate-based osmoprotectants, and assessed individually for viability after drying using a high-throughput microtiter plate assay. Fructose and trehalose at 20g/L were the most effective at maintaining the viability of the strains. High titer suspensions of washed cells of each strain then were combined with different grades of diatomaceous earth (DE), perlite, fumed silica and clay and dried for 18-22 h in a controlled RH atmosphere. Several DE products were superior in maintaining cell viability. Combining individual strains suspended in osmoprotectant with DE resulted in dried products with up to 10× higher cell survival. In laboratory assays, the majority of dried products containing only P22:Y:05 or S22:T:04 reduced dry rot by more than 50% ($P < 0.05$, FPLSD) but dried products containing only S11:P:12 were less effective. Fresh, three-strain co-cultures of these strains have enhanced efficacy and consistency of biocontrol compared to the individual strains or blends of these strains. Trehalose and fructose enhanced the survival of each component strain of co-cultures at 1 and 7 days after drying ($P \leq 0.05$). These co-cultured products reduced dry rot by 74% and 25% in laboratory assays, respectively. Cells survived drying better when combined with DE versus perlite ($P \leq 0.05$). All combinations of carrier, osmoprotectant and co-cultured cells reduced dry rot by 65-83% when applied to tubers seven days after product drying. In a small pilot-scale test, dry formulations of these co-cultured strains and the fungicide Stadium ™ reduced dry rot decay by approximately 20% and 55%, respectively ($P \leq 0.05$).

Local and systemic effect of Hebernem®-S on markers of the immune system of tomato (*Solanum lycopersicum* L.)

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Interactions between plants and beneficial soil microorganisms are known to promote plant growth and help plants to protect against abiotic and biotic stresses. Experimental evidence supports a major role of plant defenses in the observed protection. Knowledge of the complex network of interactions between plants and microorganisms could lead to the development and improvement of novel biological

products for agriculture. The aim of the present study was to evaluate the ability of HeberNem®-S, a bionematicide for agricultural use, to modulate the immune response in tomato (*Solanum lycopersicum* L.). One experimental group of plants were treated with a single dose of HeberNem®-S while other group received placebo. Samples were taken on one, three and ten days after treatment. Relative expression of defense genes in leaves and roots of tomato plants was quantified using real-time reverse transcription-PCR analysis. The relative specific activity of defense enzymes was also quantified. The analyses of our data revealed that in the roots of plants treated with HeberNem®-S the expression of phenylalanine-ammonium lyase, glutathione peroxidase, osmotin, chitinase, and hydroperoxide lyase genes decreased, as well as specific enzymatic activity relative of phenylalanine-ammonium lyase, catalase, superoxide dismutase and polyphenol oxidase, suggesting a repression of the immune response in plant roots, in spite of a preferential activation of allene oxide synthase and PR-1 genes. A decrease in the expression of hydroperoxide lyase and allene oxide synthase genes was observed in leaves, whilst overall the expression of genes PR-1, phenylalanine-ammonium lyase, chitinase, osmotin and glutathione peroxidase increased; resembling a pattern of predominance of salicylic acid on jasmonic acid. The activity of all enzymes assayed was also furthered in leaves. Our data demonstrate that HeberNem®-S is able to modulate the immunological status at local and systemic level in tomato plants.

Plant-based strategies for mosquito control

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Mosquitoes transmit some of the most devastating emerging infectious diseases of humans, domestic animals, and wildlife. Although vector control by use of chemical insecticides has played an important role in prevention and management of these diseases, their sustained use remains questionable due to evolution of insecticide resistance and public concerns regarding their harmful effects on humans and the environment. These concerns have led to a growing need to develop safe and effective alternatives to chemical insecticides. Plant-based biopesticides are favored over chemical insecticides because they are rapidly degraded and lack persistence and bioaccumulation in the environment. However, despite their proven ability to control mosquitoes and other biting insecticides, their application in mosquito control is limited. In this talk, I will discuss a simple and naturally occurring plant-based strategy for mosquito control that can be improved technologically and implemented as an integral component of integrated vector management program.

The interplay between mosquitoes, entomopathogens and symbiotic microbes: a niche for the development of novel microbial-derived vector control strategies

Jose Luis Ramirez

National Center for Agricultural Utilization Research, USA

The current outbreak of Zika virus in the Americas has highlighted the need for improved methods of control. This concern is exacerbated if we consider that all three major arboviruses (Zika, dengue and chikungunya virus) are transmitted efficiently by two wide spread mosquito vectors: *Aedes aegypti* and *Ae. albopictus*. These vectors are widely distributed throughout the Americas and present varying degrees of insecticide resistance, further hampering control efforts. Hence, microbe-derived biopesticides provide an alternative method of vector control. An important component defining the effectiveness of biopesticide control of mosquitoes depends as much on the mosquito and its microbiota as it depends on the entomopathogenic agent used to control it. This talk will discuss the importance of such tripartite interaction on the effectiveness of a biocontrol approach. Additional discussions will include information on fungal candidates with mosquitocidal activity and on their ability to produce bioactive molecules with microbicidal activity.

Methods for testing the nematode control activity of biological products

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Among the main pests and diseases affecting crops in Protected and Semi-protected Cultivation houses are nematodes of the genus *Meloidogyne*. An alternative for the biological control of these nematodes is the bioproduct HeberNem®, widely applied since several years ago to different crops in our country. The present work shows a procedure including three different methods to evaluate the nematicidal activity of HeberNem® bioproduct formulations against eggs and larvae of *Meloidogyne* spp. They are useful tools to determine the nematicidal effect of formulations of bioproducts composed by microorganisms affecting the life cycle of nematodes by different mechanisms such as the degradation of the chitin cuticle, production of hydrogen sulfur and extracellular proteases. The first in vitro method determines the activity against nematode eggs and larvae by means of calculation of the egg inhibition hatching percentage as well as the larval survival percentage. The second method indicates the concentration of microorganisms contained in every bioproduct able to produce hydrogen sulfur. And the third method consists of a test in pots with indicator plants to evaluate the technical effectiveness of the product against nematode infection. At 35 days after sowing, the plants are removed, the roots are washed and the degree of infestation in each group is measured as Root Gall Index (RGI) by the scale of Bridge and Page (1980). The necessary conditions of time, temperature and means of incubation of the samples were established to obtain the best results in each test. Tested HeberNem batches showed greater than 90% egg hatching inhibition and 52% larval survival reduction for the 5E06 cfu/mL cell concentration. In the pot trial, the technical effectiveness shown was greater than 50% with respect to the control.

Biological activity against phytonematodes, fungus affecting plants as well as plant growth promoting effects of several solid formulations of the bioproduct HeberNem®-S

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HeberNem-S (HN-S) is a registered bioproduct with proven phytonematode control activity. It also exhibits control against some fungus affecting plants as well as plant growth promoting effects. In the way to improve the properties of the product to facilitate its application and to increase its efficacy, works focused to obtain formulations with low wettability times have been carried out. As result, a group of formulations containing the bacterium with different components were obtained by spray drying. The present work describes the evaluation of the effectiveness on nematode control, fungal control and seed germination of the best formulations selected in terms of the wettability time requirements. Several experiments in pots and protected crop houses have been performed. Tomato plants were used as indicators of nematode attack. Root damages were evaluated and statistically compared according to the Bridge and Page Root Gall Index (RGI) reported scale after 35 days of interactions between plants, nematodes and different HN-S formulations. In the case of the experiments under greenhouse controlled conditions, yields were also evaluated and compared. Fungal control was "in vitro" evaluated measuring the size of the fungus growth inhibition halos in agar plates. Seed germination and plant height treated with different product formulations were evaluated. All formulations under study showed similar biological activity effects, comparable with the product batch used as positive control and at the same time with statistically significant differences regarding the untreated control plants. These results suggest that product composition used with the aim to low the wettability time of HN-S does not affect its useful properties against nematodes, fungus and plant growth promoting.

*S*ymposium 5. Quality Assurance and Quality Control for Bioprocess and Regulatory Affairs for Bioprocess

Mejoras en el proceso productivo del ingrediente farmacéutico activo de los extractos alergénicos en el BIOCEN

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La obtención del Ingrediente Farmacéutico Activo (IFA) de los extractos alergénicos se realiza en la Planta de Ingredientes Activos desde el 2008. Durante estos años se han recibido varias inspecciones por el Centro Estatal de Control de Medicamentos y Equipos Médicos (CECMED), sin embargo, no se ha podido obtener el certificado de Buenas Prácticas de Fabricación (BPF) que permita la introducción del producto en otros países. Los aspectos regulatorios y las tendencias actuales relacionadas con las BPF de productos biológicos y específicamente los extractos alergénicos exigen requisitos, principios y elementos esenciales cuyo cumplimiento garantiza la máxima calidad de los procesos productivos. El presente trabajo tuvo como objetivo general Implementar mejoras en los parámetros operacionales y en los métodos de control del proceso productivo del IFA de los extractos alergénicos, cumpliendo las BPF. Para ello se ejecutó un diagnóstico de la situación del proceso productivo del IFA de los extractos alergénicos mediante técnicas y herramientas de calidad, que demostraron incumplimientos de aspectos regulatorios para las BPF. Para trabajar en la eliminación de los incumplimientos fue establecido un plan con siete acciones, cada una con fechas de cumplimiento y establecimiento de responsabilidades. Los principales cambios identificados e implementados en el proceso productivo se relacionan con la sustitución del material de envase, la introducción de un sistema de filtración cerrado, la determinación de la biocarga y del límite microbiano; lo que unido a otras acciones permitieron la obtención del certificado de BPF.

Establecimiento y validación de una metodología para la determinación de la actividad biológica de la estreptoquinasa recombinante, necesaria para el control de la calidad del producto PROCTOKINASA®

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En el CIGB de la Habana, se desarrolló un producto novedoso, PROCTOKINASA®, formulación en forma de supositorio, cuyo principio activo es la Estreptoquinasa recombinante, la cual ha demostrado ser eficaz y segura en el tratamiento de la crisis hemorroidal aguda. Uno de los requisitos de calidad especificados para el producto terminado es la actividad biológica, por lo que fue necesario validar una metodología para este fin. En el siguiente trabajo se presentan los resultados de la validación de la metodología analítica propuesta. Materiales y Métodos. La proteína fue extraída de la fase sólida sometiendo el supositorio a temperatura de 37 ºC durante 30 min, posteriormente una separación la fase acuosa, por centrifugación y finalmente una filtración por membrana. La actividad biológica de la proteína extraída fue determinada por el método del sustrato cromogénico. Resultados: El método resultó ser específico para la cuantificación de la estreptoquinasa, sin interferencias de los componentes del placebo ($t_{experimental}$ (1,53) ≤ $t_{crítica}$ (2,36)) y capaz de variar ante la aparición de degradaciones de la molécula [$t_{experimental} \geq t_{crítica}$ (2,78)]. La curva de calibración resultó ser lineal en el rango de 600-200 UI/mL ($R^2 \geq 0,98$; ANOVA regresión $P \leq 0,01$ y prueba de falta de ajuste $P \geq 0,05$). Las muestras evidenciaron un comportamiento lineal $C.V_{global} = 6$ %, ANOVA ($P = 0,16$) ≥ 0,05). La metodología de extracción y el método en cuestión resultaron ser exactos, obteniéndose porcientos de recuperación estadísticamente iguales al 100 %, 95 ± 6,4 %: adición de material de referencia y 96 ± 5,4 %: adición de estreptoquinasa, al placebo y 96 ± 8,1 % adicionando material de referencia al producto terminando. La repetibilidad: C.V ≤ 10 y 5 % respectivamente, precisión intermedia: C.V ≤ 15 y 10 % respectivamente, reproducibilidad: C.V ≤

20 %, $F_{experimental}$ (0,5 y 0,4) ≤ $F_{crítica}$ (3,9) en el ANOVA. El método evidenció ser robusto ante discretos cambios en el tiempo de incubación con el plasma [(P = 0,27) ≥ 0,05 en el ANOVA], y la aplicación de las muestras en el centro, borde inferior y superior de la placa respectivamente [texperimental (0,28 y 1,23) ≤ tcrítica (2,78)]. Las muestras extraídas son estables a temperatura de 2 a 8 ºC y -20 ºC durante el 15 días [(P = 0,7 y 0,3) ≥ 0,05 en el ANOVA de significación de la pendiente].

Incorporación del producto HeberNem-L al sistema de gestión de la calidad en el CIGB Camagüey

Diasmarys Salinas, Alain Moreira Rubio, Madelyn Sardiñas Padrón, Manuel Expósito Negrin, Eikel Pérez González, Irian Mendoza Rodríguez, Rutdali Segura Silva, Dayamí Santiesteban Pérez, Jesús Zamora Sánchez

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HeberNem-L es un producto ecológico desarrollado por el CIGB Camagüey, que ha demostrado ser efectivo para el control de plagas de nemátodos en la agricultura, en este trabajo describimos las estrategias y acciones ejecutadas para la incorporación del producto HeberNem-L al sistema de gestión de la calidad (SGC) del CIGB Camagüey. Para lograr este objetivo se diagnosticaron mediante revisión completa del proceso productivo y de los elementos del sistema de aseguramiento de la calidad. Para la revisión se utilizaron listas de chequeo, datos históricos del desempeño y mediante análisis de causas, se tomaron acciones para la solución de las no conformidades. Transcurrido un año se realizó un segundo diagnóstico, se consideró como avance si la evaluación mejoraba en al menos 2 puntos. El procesamiento gráfico de los datos se realizó mediante el programa Microsoft Excel 2003. Las estrategias y acciones ejecutadas permitieron la introducción el producto HeberNem-L en el SGC con resultados positivos, los nuevos enfoques y la elevación de los estándares en el mercado impusieron nuevos retos y permitió el rescate y aceptación de la imagen del producto ante los clientes ampliando su aplicación en el territorio nacional.

Establecimiento y validación un método para cuantificar ADNg de hospedero en ingredientes farmacéuticos activos obtenidos en la bacteria *Escherichia coli*

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La cuantificación de ADN genómico (ADNg) de hospedero, en productos biofarmacéuticos, es un requisito establecido en regulaciones vigentes para el control de la calidad de los mismos. En el CIGB se emplea con este objetivo un método semi-cuantitativo, lento y de engorrosa ejecución, basado en la hibridación de ADN. El PCR en tiempo real cuantitativo es una de las técnicas de mayor novedad recomendadas con este propósito para el análisis de muestras de proteínas recombinantes. Nos propusimos como objetivo establecer y validar un PCR en tiempo real para cuantificar trazas de ADNg de *E. coli* en muestras de proteínas obtenidas en la bacteria. Se empleó ADNg de referencia preparado a partir de la cepa de *E. coli* W3110 y cebadores específicos para el gen rRNA 16S de la bacteria. Las curvas de calibración del ADNg de 10 ng a 1 pg fueron preparadas mediante diluciones en agua para inyección. Las proteínas recombinantes se digirieron con Proteinasa K, y el ADNg se concentró por precipitación alcohólica. Los PCRs en tiempo real se realizaron en un sistema Rotor Gene durante 40 ciclos. Se desarrolló un nuevo método para la cuantificación de ADNg. Durante la validación el mismo exhibió una respuesta lineal en el rango establecido, con coeficiente de determinación (R^2) ≥ 0,98 y una pendiente significativamente diferente de cero (P ≤ 0,01). La repetibilidad y precisión intermedia indicaron que el método es preciso, con coeficiente de variación (CV) ≤ 20% y exacto en su aplicación a las muestras de estudio. Quedó establecido y validado un método analítico novedoso, recomendado en regulaciones vigentes, específico, lineal, preciso y exacto, aplicable para todas las cepas de *E. coli*, con un límite de cuantificación de 1 pg, válido para la cuantificación de ADNg en productos farmacéuticos obtenidos en esta bacteria.

ISO 9001:2008 - quality management for agricultural biotechnology at CIGB

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Los proyectos desarrollados en el área de Investigaciones Agropecuarias (IAP) del CIGB se caracterizan por su alto rigor científico y el impacto de sus resultados, en nuestra sociedad. Sin embargo, es necesario continuar trabajando por una mejor sistematización de los proyectos a partir de datos confiables, reproducibles, auditables y publicables. Objetivo: Incorporar y actualizar el Sistema de Gestión de la Calidad (SGC), basado en las normas ISO 9001:2008, para una mayor calidad de los productos biotecnológicos y el desarrollo de procesos más eficientes. Materiales y Métodos: Se aplicó por primera vez los manuales de OMS, ICH Q10, la Norma ISO 9001:2008 y el Manual de calidad del CIGB. La certificación al área de Investigaciones Agropecuarias contribuyó a mejorar la organización del trabajo en los laboratorios, ahorrar en los recursos empleados y, en conjunto, a mejorar la gestión de los proyectos. Se logró además intensificar el comercio de productos (vacunas veterinarias y bioproductos agrícolas), los negocios de proyectos y las transferencias de tecnologías. Con los beneficios reportados se crean las bases para la implementación de las BPL y las normas ISO en todos los laboratorios de Investigaciones Agropecuarias del país.

Desarrollo de un ensayo en ratones como método alternativo a la prueba de potencia del candidato vacunal E2CD-154 contra la peste porcina clásica (PPC)

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The difficulty of counting seronegative animals for classical swine fever virus (CPVV) and the high cost of immunogenicity tests in their natural host, the pig, suggest the need for an alternative method to evaluate the potency of vaccines used for the control and eradication of this disease. This paper proposes an animal model laboratory test to evaluate the potency of the vaccine candidate E2CD-154. For this, it was necessary to standardize the doseresponse curve in mice immunized with a previously approved batch at the time of manufacture, with demonstrated efficacy and safety in pigs, as well as studies of realtime and accelerated stability. This lot will be considered hereinafter a reference preparation to approve the new batches produced. To characterize the immune response, several groups of mice were immunized under a biphasic scheme with decreasing doses of the vaccine preparation ranging from 1 to 0.016 μg. The results obtained allowed to determine the linear region of the dose-response curve and to calculate the effective dose for which 50% of the mice in the study (ED50) were seroconverted. The potency of the reference lot was established and the relative potency of two other batches produced was determined by a parallelism study. The results obtained in mice are still preliminary to replace the potency test in pigs, so that the immediate steps of the study are aimed at demonstrating the concordance of the tests between both species and the validation of the technique. The proposed methodology contributes to improving the animal welfare of mice and pigs according to the 3 R´s principle and also to reduce the cost of vaccine release trials.

Seguimiento de los trabajadores expuestos en bioprocesos que implican un riesgo biológico. Experiencia del Centro de Ingeniería Genética y Biotecnología

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El desarrollo de candidatos vacunales incluye la manipulación de virus a nivel de laboratorio o ensayos clínicos. Por ello se hace necesario, como parte del proceso de mejora del sistema de bioseguridad, el establecimiento de nuevas medidas que permitan alcanzar un nivel más alto de gestión. En este trabajo se establece una estrategia de chequeo para el control y seguimiento de los parámetros de salud a estos trabajadores y se describe la documentación y logística seguida con la seroteca. La evaluación de marcadores virales, como parte de las pruebas de salud, incluyó a todos los trabajadores que manipulan virus, sueros de pacientes, animales y otros materiales biológicos procedentes de ensayos clínicos o preclínicos como: dengue, hepatitis B y C. Algunos de los trabajadores expuestos que habían sido inmunizados no presentaron anticuerpos contra marcadores específicos por lo que fueron declarados como no protegidos y a los cuales se les aplicó una dosis de recobrado. En otros casos, personas que por su edad no habían sido incluidos en el esquema de inmunización de nuestro sistema de salud tenían un nivel de anticuerpos que los declaraba como protegidos, lo que evidencia que en algún momento estuvieron expuestos al patógeno. La inclusión de la evaluación de marcadores virales al personal expuesto y el manejo de la seroteca donde se incluye una documentación completa de cada trabajador y una encuesta para el examen clínico. La estrategia de mejora permitió integrar acciones de calidad, bioseguridad y seguridad y salud para el manejo del riesgo biológico de personal expuesto asociado al desarrollo de candidatos vacunales.

Determinación de indicadores de desempeño ambiental en el Centro de Ingeniería Genética y Biotecnología

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El desempeño ambiental de las empresas está determinado por diferentes aspectos que incluye tanto controles operacionales para la determinación de los consumos y los costes de materia prima, el potencial contaminante de sus operaciones, la obligación de cumplimiento a requisitos legales, el ahorro que conlleva una correcta gestión económica ambiental y los aspectos preventivos relacionados con la gestión de riesgo. El objetivo de este trabajo es determinar la cantidad mínima de indicadores que se requieren para medir de forma sistemática del desempeño de la organización, enfrascada en la implementación de un sistema de gestión ISO 14001:2015. Para dar cumplimiento a este objetivo se realizó un análisis de los requisitos legales aplicables a los Centros de Ingeniería Genética y Biotecnología y la forma de gestión por área de trabajo teniendo como marco teórico la revisión ambiental inicial, el diagnóstico de la gestión y los requisitos de la norma ISO 14001:2015. Para la definición de indicadores se realizaron inspecciones ambientales en toda la organización incluyendo aquellas áreas de apoyo que no están directamente asociadas a las producciones. Como resultados se definieron tres grupos de indicadores de desempeño, los relacionados con el consumo de agua y combustible, los relacionados con la prevención de la contaminación y los que están asociados al manejo de los riesgos biológicos químico y radiológico. La evaluación de los indicadores nos permitió definir el comportamiento de la entidad en los años 2014, 2015 y el primer semestre del 2016. Estos al ser integrados con herramientas que apoyan la fiabilidad, reproducibilidad y trazabilidad de los datos obtenidos constituye uno de los pasos imprescindibles para la alineación del sistema de gestión

ambiental con los sistemas de seguridad y salud del trabajo y el sistema de gestión de la calidad primera etapa para la integración de los sistemas.

Evaluación de bacteriófagos como control de pureza de los bancos de *Escherichia coli* de interés biotecnológico

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El control de calidad de bancos de las cepas de Escherichia coli utilizadas en las metodologías de la Biotecnología moderna incluye la verificación de contaminantes de bacterias ambientales. Sin embargo, uno de los problemas de contaminación más difíciles de evaluar en la industria biotecnológica, que ha recibido mucha atención en los últimos años, es la contaminación por bacteriófagos. Para certificar que un banco está libre de estos virus bacterianos hay que establecer metodologías microbiológicas donde se induzca la fase del ciclo lítico, lo cual depende de la disponibilidad de nutrientes en los medios de cultivo. En este trabajo nos propusimos evaluar medios de cultivos elaborados con bases de las casas comerciales Oxoid, Biocen y Biolife para la detección de bacteriófagos contaminantes en bancos de cepas de *E. coli*. Para ello utilizamos un aislado de bacteriófago ambiental y la cepa sensible a la infección por fago LE392. Después de estandarizar la metodología de ensayo se evaluó la habilidad de formar placas de lisis de la preparación concentrada del aislado ambiental en los medios elaborados con reactivos de las tres casas comerciales. Los mejores resultados se obtuvieron con bases provenientes del Biocen donde se detectó el mayor conteo con 1×10^5 ufp/ml, el medio donde menos placas de lisis observamos fue en el Biolife donde se obtuvo 2 órdenes de magnitud por debajo de este valor.

Diseño estratégico para la implementación de un sistema de gestión ambiental ISO 14001:2015 en el Centro de Ingeniería Genética y Biotecnología

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La velocidad con que se producen los cambios en los entornos tecnológico, económico, político-legal y sociocultural supera la capacidad de reacción de las organizaciones. Estos cambios representan para el entorno empresarial riesgos y oportunidades que pueden definir el futuro de una organización. Ante esta problemática, las empresas competitivas estudian el contexto donde operan para aprovechar o minimizar los impactos. El Centro de Ingeniería Genética y Biotecnología es una gran empresa con una variedad de productos y servicios que se comercializan en más de 50 países. La creación del Grupo Empresarial BioCubaFarma implicó la formulación de objetivos comunes al sector como la implementación de Sistemas de Gestión Ambiental (SGA). Esto ocurre en un contexto complejo debido a las particularidades de la industria, la amplia gama de regulaciones ambientales nacionales, los cambios en estructuras reguladoras cubanas, la política económica y social del país. Esta investigación se planteó como objetivo diseñar una estrategia para la implementación del SGA según los requisitos de la NC ISO 14001:2015 en el Centro de Ingeniería Genética y Biotecnología. Para ello se diagnosticó la gestión ambiental y se evaluó su impacto sobre el desempeño ambiental. Las debilidades y fortalezas fueron analizadas incluyendo la influencia de amenazas y oportunidades. Con los resultados se formularon 14 objetivos estratégicos que se incluyeron en un Cuadro de Mando Integral. Para cumplir estos objetivos, bajo el enfoque de la ISO 14005:2010, se elaboró un Plan de Implementación en tres fases con un total de 27 acciones a ejecutar en 371 días. Para facilitar la implementación se propusieron 11 ayudas prácticas con referencia en la ISO 14004:2016, se identificaron elementos de gastos relacionados con la estrategia diseñada. El diseño propuesto puede ser aplicado con adecuaciones a otras empresas del sector según la misión corporativa.

Establishment and validation of two analytical chromatography techniques for the quality control and characterization of pegylated interferon Alpha-2b (PEG-HEBERON)

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Chronic hepatitis C is considered one of the major causes of chronic liver disease and cirrhosis, and the most common reason for liver transplant. Interferon-α2b combination therapy with ribavirin is the current standard for the treatment of this infection. Interferons displays a wide range of antiviral, antiproliferative and immunomodulatory activities but present a several problems in humans: a high clearance rate, low stability and short plasma half-time. Pegylation, is a well-developed process and can be applied to therapeutic proteins resulting in a number of potential clinical advantages such as enhanced pharmacologic-activity, increased half-time, improved safety/tolerability, reduced immunogenicity, antigenicity and toxicity, leading to increased patient compliance and quality of life. The objective of this work is the establishment and validation of two chromatographic techniques for PEG-IFNα2b: Size-Exclusion Chromatography-HPLC (SE-HPLC) and Ionic-Exchange Chromatography-HPLC (IEC-HPLC). Two type of samples were used, API- 40-kDaPEG-IFN α2b and DS- PEGHeberon (CIGB). SE-HPLC was performed with a Merck Hitachi HPLC system equipped with an Amersham-Biosciences Superdex 200, 10 mm × 30cm column, flow rate 0.4 mL/min and 280 nm, room temperature, 100 µg of sample. IECHPLC was performed using an analytical strong-cation exchange column, TOSOHBIOSEP, SP-5PW, 7.5 mm × 7.5 cm, flow rate 0.7 mL/min and 218 nm, room temperature, 200 µg of sample. We carried out a stress temperature study with the objective to determine any modification on the molecule. Those samples were analyzed by SE-HPLC and some product related impurities

were detected: aggregates and others. Coefficient of variation obtained from validation parameters were ≤ 2 %. When 40-kDa PEG-IFN-α2b was analyzed by IECHPLC, four major fractions were identified. All chromatography profiles obtained were similar for the samples studied. Coefficients of variation obtained from validation parameters were alike to those reported in the literature, which allows concluding that the established and validated methods were appropriated for the analytical control and characterization of 40-kDa PEG-IFNα2b.

Consistencia del proceso de fabricación de HEBERPROT-P

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Introducción: Heberprot-P es un medicamento novedoso y único prescrito para la terapia de la úlcera del pie diabético, basado en el Factor de Crecimiento Humano recombinante. Materiales y métodos: La unidad productiva fue construida de acuerdo a las exigencias para este tipo de instalaciones y cuenta con el equipamiento adecuado para realizar este tipo de procesos. La fabricación del IFA de FCEhr está basada en la tecnología del ADN recombinante teniendo como hospedero la levadura Sacchromyces Cerevisiae. Resultados: La producción de Heberprot-P comenzó en el año 2006. Han sido fabricados más de 867 lotes de IFA y 293 lotes de Heberprot-P. Los lotes han sido fabricados acorde a las especificaciones de calidad del producto y el estado del arte internacional. Se han logrado obtener consistentemente valores cercanos al gramo y valores de Pureza por RP-HPLC superiores al 97 % en la mayoría de las campañas. La Planta de producción está certificada por CECMED (Autoridad Reguladora Nacional de Cuba) y otras autoridades regulatorias internacionales. Conclusiones: Durante más de 10 años de intenso trabajo se ha logrado producir de manera consistente un producto de una elevada calidad, capaz de satisfacer no solo la demanda del sistema nacional de salud; sino también la creciente demanda internacional existente por el Heberprot-P. En Cuba se han distribuidos más de 484 000 viales y más de 2 210 000 se han exportados a otros países. Esto ha hecho posible el tratamiento de más de 243 000 pacientes. Heberprot-P ha sido registrado en 21 países en 10 años.

Análisis de tendencia del control microbiológico ambiental de un área de filtración final de Ingrediente Farmacéutico Activo

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El análisis de tendencia es un procedimiento para detectar el cambio en los patrones de las observaciones realizadas a lo largo de un período. Se aplica para detectar la pérdida gradual del control, que pudiera no ser detectada por el enfoque de la ventana móvil, así como una pérdida súbita del control. Objetivo de este trabajo es presentar los resultados obtenidos según el análisis realizado a los parámetros microbiológicos de control ambiental del local donde se realiza la filtración final estéril del Ingrediente Farmacéutico Activo (IFA). El área fue monitoreada por microbiología del aire, las superficies y el personal. En la Microbiología del aire se muestrearon los métodos Volumétrico y de sedimentación por Placa Expuesta, en las superficies: las paredes, los pisos, las cortinas, los estantes, las mesas de trabajo y otros, del personal: el vestuario, donde los puntos a muestrear fueron los guantes, los antebrazos, el frente y el gorro del uniforme. Otro método utilizado fue el monitoreo de Partículas Totales. Como resultado del Análisis de Tendencia realizado encontramos que los representantes microbianos con mayores porcientos de caracterización fueron *Staphylococcus*, *Micrococcus*, *Bacillus*, diplococos gram positivos, con respecto al tipo de bacterias aisladas, se refleja predominio de bacterias gram positivas habituales en el ambiente de áreas limpias. El género bacteriano *Micrococcus*, es de origen humano pues forman parte de la microbiota de la piel. El género más identificado fue el *Staphylococcus* y cocos gram positivos. Los resultados del monitoreo mantuvieron un comportamiento satisfactorio en la microbiología del aire, las superficies y el conteo de partículas. Los microorganismos aislados correspondieron con la microbiota habitual del área. El área de filtración final estudiada mantuvo las condiciones ambientales microbiológicas y físicas adecuadas para garantizar la seguridad ambiental durante el proceso de filtración final estéril de las IFAs producidas.

Aplicación del análisis de riesgo a la producción de anticuerpos monoclonales en el CIGB Sancti-Spiritus

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La gestión del riesgo permite identificar y valorar las fuentes de no conformidades, optimizando el empleo de recursos. Tal enfoque precisa del conocimiento de aquellos factores que afectan la estabilidad de los procesos y la calidad del producto final, con una valoración de su mayor o menor nivel de incidencia. Resulta de gran importancia producir los anticuerpos monoclonales con las especificaciones de calidad establecidas. El objetivo del siguiente trabajo será aplicar el análisis de riesgo, empleando la metodología de análisis de modos y efectos de fallas (AMEF), a los procesos de producción de los anticuerpos monoclonales. Se seleccionó un grupo de expertos y se realizó la descripción de las etapas del proceso de producción mediante un diagrama de flujo. Se identificaron los modos de fallo potenciales en cada etapa del proceso y se realizó un diagrama causa-efecto. Se realizó la selección de los principales modos de falla, efectos de la falla y

causas potenciales, evaluándose la severidad, probabilidad de ocurrencia y probabilidad de detección de las mismas. Se calculó el número de prioridad de riesgo para diferenciar y categorizar el nivel de criticidad de cada causa de fallo. Las causas que tienen mayor influencia en la calidad del proceso de producción de anticuerpos monoclonales son: Aparición de variantes del hibridoma no secretoras, Exceso de cargas de impurezas en la ascitis, Incorrecto empaque de la columna cromatográfica, Exceso de carga de Anticuerpos en la matriz cromatográfica e Insuficiente tiempo de residencia en la columna. Se proponen las acciones prioritarias para minimizar el riesgo. La aplicación del análisis de modos y efectos de fallas, como herramienta del análisis de riesgo, permitió jerarquizar las causas potenciales de riesgo en el proceso de producción de Anticuerpos Monoclonales.

Estabilidad genética del banco de células primario que expresa la proteína recombinante MY32/Ls activa contra los piojos de mar en salmones

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La proteína MY32/Ls activa contra los piojos de mar *Lepeophtherius salmonis* es producida a partir de la fermentación del sistema de expresión *E. coli* BL21(DE3)-pET28a-my32/Ls que se encuentra en conservación por congelación a –70 ºC en un Banco de Células Primario (BCP) puro y homogéneo. El objetivo del presente trabajo es determinar la estabilidad genética del BCP mediante la obtención de células con elevado número de generaciones y su influencia en el crecimiento y la expresión de la proteína. Se diseñó el estudio y estimó el número de generaciones de la cepa *E. coli* BL21(DE3)-pET28a-my32/Ls y se determinó su influencia en el crecimiento bacteriano. Al cultivo con elevado número de generaciones se determinó estabilidad plasmídica, análisis de expresión de la proteína, se realizó digestión enzimática y secuenciación al gen de interés. Se estima que transcurran 46 generaciones aproximadamente durante el proceso productivo en un fermentador de 200 L. El número de generaciones no afectó la velocidad de crecimiento (μ) ni el tiempo de duplicación (td) obteniéndose durante la Fermentación1 $\mu = 0{,}78\ h^{-1}$ y td = 53 minutos, mientras que durante la Fermentación3 $\mu = 0{,}81\ h^{-1}$ y td = 51 minutos. No se afectó la estabilidad plasmídica ni la expresión de la proteína y se obtuvo un % de homología entre las secuencias del gen MY32/Ls comparadas. Se obtuvo una banda de 5,25 kb del vector y una banda de 535 pb que coincide con el fragmento del gen de interés. Se concluyó que el número de generaciones no afecta la estabilidad genética del banco primario.

Utilización del ensayo de determinación de proteínas totales, Lowry; en las muestras de filtrado no estéril, en el proceso productivo de IFA Gavac

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Determinar la concentración de proteínas en una muestra biológica es una técnica de rutina básica cuando se aborda un esquema de purificación de una proteína concreta. El método de Lowry es un ensayo bioquímico para la determinación de proteínas en una disolución y tiene la ventaja de ser un método extremadamente sensible. El objetivo de este estudio es la obtención de resultados que avalen el cambio del ensayo de determinación de proteínas totales por Coomassie por el método de Lowry, en la muestra del filtrado no estéril del Ingrediente Farmacéutico Activo (IFA) del inmunógeno Gavac®, para que nos permita llegar al paso de filtración esterilizante con una mayor certeza del rendimiento del lote en cuanto a dosis equivalentes y gramos de proteína Bm86. Para este estudio se realizó la determinación de concentración de proteínas a 21 lotes por ambos métodos. Los valores obtenidos fueron comparados con el límite establecido para esta característica y posteriormente se realizó una comparación estadística mediante prueba-t Student, utilizando el programa Statgraphics Centurion XV. En el análisis estadístico realizado se pudo observar que en los 21 lotes hubo diferencias estadísticamente significativas entre las medias. Los mejores resultados fueron obtenidos con el método de Lowry, obteniéndose como promedio un incremento de 1,057 mg/mL por encima de los resultados obtenidos con el método de Coomassie. Como conclusión se obtuvieron los resultados que avalan la sustitución del método de determinación de proteínas totales Coomassie por el método de Lowry en la muestra de filtrado no estéril.

Validación del llenado aséptico en la planta de productos parenterales 3

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Como parte del proceso de mejora continua, se construyó en el Centro Nacional de Biopreparados, una nueva planta de productos parenterales (PPP3). La validación del proceso de llenado aséptico es un elemento clave a realizar antes de comenzar la fabricación de productos. Por lo tanto, este trabajo se realiza con el objetivo de obtener una evidencia documentada de que todas las operaciones se lleven a cabo según las regulaciones de Buenas Prácticas de Fabricación de productos líquidos y liofilizados estériles en la Planta de Productos Parenterales 3. El diseño del estudio se

elaboró para productos liofilizados, con conexión del colector (manifold) de la bomba peristáltica y de las cuatro bombas de pistón rotatorio. Además, se planificaron durante la operación de llenado cuatro intervenciones. Para efectuar esta validación, se efectuaron tres lotes consecutivos de llenado con medio. En el primer lote procesado no se obtuvo ninguna unidad contaminada, pero en el segundo y en el tercero se obtuvo una unidad contaminada, por lo que se realizó una investigación para conocer la causa de la contaminación. Finalmente, con este trabajo se obtuvo la condición de validado, según las regulaciones de Buenas Prácticas de Fabricación de productos líquidos y liofilizados estériles del proceso de llenado aséptico en la Planta de Productos Parenterales 3.

Validation Lowry technique for quantifying total proteins of core antigen Hepatitis B Virus in substance and product drug

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The core antigen of Hepatitis B Virus (CAgHB) is a protein that is used as active ingredient in the vaccine for the treatment of chronic disease Hepatitis B. In the analytical laboratory it was established and validated the Lowry method for the quantification of total protein for Active Pharmaceutical Ingredient (API) and Finished Product (FP) of the (CAgHB). The validated parameters were: specificity, linearity, range, accuracy, precision and robustness. The method proved to be specific for quantifying protein without interference buffer or placebo of the samples. The system complied with the requirements of linearity of calibration curve in the working range 10-100 µg/mL, which proved to be accurate and precise. The accuracy of the method was demonstrated (100 ± 10% recovery). The evaluation of the system met the precision acceptance criteria: repeatability (CV ≤ 10%) and intermediate precision (CV ≤ 15%). The technique showed to be robust against all variations studied in the trial: Incubation condition, temperature, reaction with Folin- Ciocalteu and Alkaline Copper. It was shown that the samples can be stored at temperatures between 2-8 °C during the time set in the laboratory for conservation (10 days) without affecting your concentration value. This result allows us to comply with the requirements of regulatory agencies Product.

Unidad de servicio de pesaje de CIGB, conciliación de materias primas por unidades productivas

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El presente trabajo tiene como objetivo principal exponer la conciliación de materias primas empleando un sistema digital que además controla las materias primas desde su solicitud al almacén central hasta el suministro en las unidades productivas 3, 4, 5, 6, 7, 18 y la Unidad de Desarrollo del CIGB. Se evaluaron los elementos considerados como indispensables en la puesta en marcha de una base digital para el procesamiento de datos, la conciliación, trazabilidad y estado de las materias primas en un área dedicada al pesaje y distribución de estas, utilizadas en la obtención de Ingredientes Farmacéuticos Activos con alto valor agregado producidos en el Centro de Ingeniería Genética y Biotecnología. Se elaboraron y aprobaron los procedimientos y registros necesarios para el funcionamiento de esta base de datos, se entrenó, adiestró al personal que labora en esta área y a los que se les dará servicio en el desempeño de las operaciones básicas de pesaje de precisión, recepción y despacho de materias primas pesadas, con el objetivo de minimizar cualquier error humano y dar solución de información inmediata ante cualquier dato necesario. Se logró un control preciso del consumo de materias primas por plantas así como la garantía de que las plantas reciban sus materias primas seguras, registradas y limpias. La aprobación de este sistema de conciliación de materias primas tuvo gran impacto económico, organizativo y regulatorio en la Planta de Producción del CIGB, garantizando de manera segura la trazabilidad de cada materia prima empleada en las plantas de producción desde su salida del almacén central. Esta Unidad de Servicio de Pesaje ha sido objeto de inspecciones por diversas entidades regulatorias, nacionales e internacionales como CEDMED, TRILLIUM, ANVISA entre otras, obteniendo excelentes resultados en la evaluación integral.

Evaluación de riesgos en la etapa de intercambio iónico del proceso de producción de la proteína P64k recombinante (P64Kr)

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La proteína P64Kr obtenida en CIGB se usa como una de las materias primas biológicas (MPB) de la vacuna terapéutica producida en CIM, CIMAvax-EGFhrec contra el cáncer de pulmón. En la Revisión Periódica de Calidad del Producto 2015 se observó una tendencia a la disminución de la pureza por lo que se propone realizar un Análisis de Riesgo para identificar sus causas. Finalmente, se recomiendan acciones para solucionar este problema en función del número de prioridad de riesgo (NPR). Por parte de un grupo multidisciplinario se realizó una tormenta de ideas, se elaboró el Árbol de Fallas y se aplicó Método de Análisis Modal de Fallos y Efectos (AMFE). Se establecieron los aspectos para la valoración de la severidad, ocurrencia y detección, en una escala de 1 a 5 para el cálculo del NPR siendo 125 el máximo y 90 % la confiabilidad estadística. Se determinó que la etapa cromatográfica de Intercambio Iónico (II) sería el objeto de este estudio. Se identificaron los efectos de fallo: Alto contenido de impurezas en el producto final y Disminución del recobrado de la etapa. Se identificaron 5 Modos de Fallo asociados a ambos efectos de fallo y uno solamente relacionado con el alto contenido de impurezas. De las 12 causas raíces identificadas las más importantes fueron: el fallo del software del programa Biocrom y la inadecuada ejecución de la etapa Ruptura/Precipitación asociadas al

fallo "Alto contenido de impurezas en el producto final". Se proponen 4 acciones relacionadas con ajustes y nuevos controles de proceso. La aplicación combinada de diferentes métodos, basado en un enfoque de riesgos permitió identificar las principales causas del inadecuado funcionamiento de la etapa de II que impactan directamente en los resultados de pureza proponiéndose un plan de acciones para reducir los efectos del riesgo.

Establecimiento y validación de un método por HPLC para la cuantificación de los contraiones acetato y trifluoroacetato en el péptido sintético CIGB-300

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El CIGB-300 es un péptido sintético que constituye un novedoso medicamento con acción antitumoral destinado al tratamiento de cáncer en el útero y otras localizaciones. En este trabajo se presenta el diseño, establecimiento y validación de un método de cromatografía Líquida de alta resolución en Fase Reversa para la separación y cuantificación de los contra-iones acetato y trifluoroacetato en muestras de Ingredientes Farmacéutico Activo (IFA) del péptido sintético CIGB-300 producido en el CIGB. Se utilizó un sistema de HPLC (Young Lin Instrument, Corea) (Interface, bomba, detector PDA, horno, Autosampler), una columna RP C18 Nucleosil (4,6 × 250 mm, 5 µm, 100 Å), con un flujo de trabajo 1 mL/min y la detección a 205 nm, como fase móvil se preparó una solución de dihidrógeno fosfato de sodio 0,115 M pH 3,5. Se utilizó el software: YL-Clarity versión 3.0.2.244. El método fue capaz de separar los contra-iones acetato y trifluoroacetato con una resolución mayor de 3. Se verificó la linealidad en el intervalo estudiado para cada contra-ion con coeficientes de correlación mayores de 0,999 en ambos casos. El límite de cuantificación para el trifluoroacetato fue de 1,5 µg/mL y el de detección fue 1,25 µg/mL. La repetibilidad de la determinación para el trifluoroacetato fue menor del 7 % y la del acetato por debajo del 3 %. La precisión intermedia mostró valores de coeficiente de variación menores del 10 % para ambos contra iones. Los resultados obtenidos muestran que la validación de la técnica resultó satisfactoria. Esto permitió el control del TFAc– como impureza del proceso y del Ac- como componente del IFA de los lotes de CIGB-300. Con este ensayo se completó la especificación de calidad del producto, para cumplir con el marco regulatorio internacional vigente.

Impacto microbiológico del nuevo método de limpieza en las áreas limpias GRADO A y GRADO B, evaluación de la efectividad de los Higienizantes

Biunayki Reyes Díaz, Caridad Gasmuri Gonzalez, Yenay Díaz leon, Alejandro Beldarrain Iznaga, Amaury Hierrezuelo, Noelia Baltrell, Dorelis Ortiz

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One of the main challenges facing the biopharmaceutical industry is compliance with environmental quality standards for work areas, particularly in clean rooms. This work aims to demonstrate the efficacy of three disinfectants: Surfalyse, Bacteranios and Peroxide 6%, when applied to define cleaning procedures and reducing microbial load on the surface of floors, walls, tables and equipment. The study areas were a total of 5 vaccine production plants. Nonparametric method was used in the statistical analysis of the results. As a result of the evaluation of the effectiveness of disinfection and respecting the acceptance criteria for the sampled points it indicates that the bactericidal and fungicidal disinfectant was achieved by reducing the load to acceptable levels in areas with adequate control of microbiological quality environmental. For sampling the study area, in the 3 sampling times were satisfactory showing results have disinfected the area. Also, the effectiveness of disinfectants in situ after 15 minutes and / or time of application, a low microbiological level remained.

PATfixTM - At-line monitoring of impurities and critical quality attributes in biopharmaceutical up- and downstream processes using

Blaž Goričar, Sebastijan Peljhan, Romina Žabar, Vid Skvarča, Tomas Kostelec, Valentin Steinwandter, Patrick Sagmeister, Aleš Štrancar

BIA Separations, Slovenia

Production of high value biological therapeutics usually involves complex manufacturing processes with high process variability. Additionally, development of robust and reliable bioprocesses can be challenging. PAT aims to enhance bioprocess understanding and implies a holistic approach to ensure that quality is built into products by design. Efficient PAT therefore calls for fast and robust analytical techniques which enables to asses high quality information about critical quality attributes and key performance indicators as parallel as possible to the manufacturing process. PATfix™ is unique HPLC system for routine gradient separations that enables every analytical task. Equipped with bioinert ceramic pump heads is deliberately tailored to meet the demands of analytical applications covering wide range of biomolecules. Highly sensitive and fast multi-wavelength detector enables to detect component peaks even in very fast gradients.

In-process control of pDNA production on CIMacTM pDNA analytical column

Urh Černigoj, Hana Jug, Sebastijan Peljhan, Aleš Štrancar

BIA Separations, Slovenia

As the demand for plasmid DNA (pDNA) based gene therapy and vaccines increases, large scale, cost effective, and reproducible pDNA production will be required. The key to success is a real time in-process control method that ensures a high percentage of supercoiled pDNA in the final product. CIMac™ pDNA Analytical Column allows the monitoring of degradation products (open circular and linear pDNA), the

removal of impurities (RNA), and ensures that each production step is yielding the amount of supercoiled pDNA anticipated. An example of a pDNA purification processs (Table 1) based on our CIM HIP2 Plasmid Process PackTM with in-process control steps is shown in Figure 1. The final product composition is confirmed by Agarose Gel Electrophoresis in Figure 2. In Figure 3, the complete separation of all three pDNA conformations from a test solution within 10 minutes on the CIMac™ pDNA Analytical Column shows the versatility of the column.

Using fingerprint HPLC method to develop and control IgG (IVIG) from Cohn (I+II+III) paste production process

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The demand for human immunoglobulin is invariably increasing on an annual basis. To satisfy demands, different manufacturing processes are used to isolate immunoglobulins from human plasma. A quest for alternative paths in manufacturing not only requires development of the most economical manufacturing process, but also a rapid method development and development of reliable analytics for manufacturing monitoring. For an efficient improvement of the purification methods as well as for in-process control during manufacturing stage, the usage of reliable and fast analytical techniques are of crucial importance. Fast and reliable fingerprint-based method for characterization of immunoglobulin G (IgG) prepared from Cohn I+II+III paste in two chromatographic steps is presented. The fingerprint method bases on partial separation of proteins in linear gradient on CIMac QA 0.1 mL column. Partial separation of proteins does not allow simple quantitative analysis of the samples during the IgG production from Cohn I + II + III paste, however, a very accurate qualitative information about the composition of the sample can be obtained in less than 5 minutes.

Uso alternativo del 2-Mercaptoetanol calidad biología molecular, en la producción de ingrediente farmacéutico activo del inmunógeno Gavac®

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Gestionar y asegurar las materias primas en calidad, cantidad, lugar y momento para las producciones biotecnológicas, es cada día un reto mayor. Para la producción del Ingrediente Farmacéutico Activo (IFA) Gavac en el año 2010, no existía disponibilidad suficiente del 2-mercaptoetanol NP 232, calidad cultivo. Demostrar que el uso del 2-mercaptoetanol calidad Biología Molecular, no tiene impacto negativo en la calidad del filtrado No Estéril y estéril (IFA), ni en el desempeño del proceso productivo en la etapa de recobrado de la proteína Bm86, fue el objetivo de este trabajo. Durante la producción del IFA realizada a inicios del 2011, fueron utilizadas las dos calidades diferentes del 2-mercaptoetanol, en la etapa de recobrado de la proteína Bm86. La evaluación se realizó en sublotes a escala productiva. Los resultados de la determinación de la concentración de proteínas totales en la etapa de Recobrado y obtención del IFA, se analizaron con el software STATGRAPHICS Centurion XV y se compararon los resultados alcanzados en los sublotes en estudio con las especificaciones de calidad de los productos intermedios, mediante un análisis de varianza. Como resultado del estudio se obtuvo que el 2-mercaptoetanol calidad Biología Molecular utilizado en la etapa de recobrado de la proteína Bm86, no tiene impacto negativo en la calidad del filtrado No Estéril y del IFA Gavac y su desempeño en el proceso productivo fue adecuado, por lo que puede ser utilizado en la etapa de Recobrado de la proteína Bm86.

Extensión del tiempo de vigencia del IFA Gavac®

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En la actualidad los estudios de estabilidad térmica constituyen etapas fundamentales dentro del desarrollo de procesos biotecnológicos y farmacéuticos, ya que mediante ellos se determinan las condiciones óptimas a tomar en cuenta durante el manejo y almacenamiento de los diferentes productos obtenidos. En el presente trabajo se llevan a cabo 2 estudios de estabilidad térmica sobre el Ingrediente Farmacéutico Activo (IFA) de la vacuna Gavac®, relacionados con: 1) Incremento del tiempo de vigencia del IFA hasta un período de 30 días a una temperatura de 2 – 8 ºC, y 2) Establecimiento el número de veces que el IFA se puede congelar y descongelar por un período de 90 días a una temperatura de – 20 ºC, todo ello sin afectar los parámetros de calidad establecidos. De acuerdo con los resultados obtenidos se puede concluir que el IFA es estable por un período de 30 días a una temperatura de 2–8 ºC, y puede ser sometido a 5 congelaciones y descongelaciones por un período de tiempo de 90 días a – 20 ºC sin que se afecten negativamente sus principales parámetros de calidad.

HPLC fingerprinting approach for increased speed and flexibility of PAT

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Process analytical technology (PAT) is a useful instrument to design, control and analyse manufacturing processes through measurement of critical process parameters. Compared to traditional chemical and pharmaceutical industries, where PAT has been

used for decades to ensure process reproducibility, implementation of PAT to biopharmaceutical manufacturing is much more challenging due to complexity of biomolecules and batch to batch variability resulting from slight process variations during the production. Methods able to provide nearly real-time data about the production process are highly desired for an efficient process monitoring. Since traditional biological assays for analytics of biopharmaceuticals are usually labour intensive and time consuming, chromatographic analytical methods are excellent alternative due their speed, accuracy and reliability. HPLC columns that would be able to handle samples from different feed streams and determine the amount of the target molecule and impurity profile in nearly real time should be highly efficient and selective for large biomolecules and nanoparticles. High complexity of the samples also reflects in complex chromatograms what makes their analysis particularly challenging. Modern computational algorithms enable us to overcome the obstacles of analysing complex sets of data efficiently and enable us to extract the patterns which are intricately masked in the abundance of chromatographic fingerprints. In this presentation, examples of fingerprint analysis by utilizing inCyght software on simple and complex examples will be presented.

Morphology and chemical composition investigations of pharmaceutical substances using scanning electron microscopy

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Scanning electron microscopy (SEM) with accompanying spectroscopic methods (EDXS, WDXS) is an indispensable analytical method to assess information on surface morphology and chemical composition of inorganic and organic materials. It is reasonable to expect that investigations of organic compounds by SEM, such as pharmaceutical substances, may be problematic due to induced beam damage of organic compounds and/or charging effects during observation. However, modern field-emission-gun (FEG) microscopes which enable low-voltage imaging with high-spatial resolution as well as low-vacuum columns designs (LVSEM) largely eliminate these problems. Consequently, the organic materials sample preparation procedures may well be the deciding parameter for obtaining high-quality analytical results. In present work we investigated morphology and chemical composition of various pharmaceutical substances in the form of powders, pellets and microcapsules. The surface corrosion of vials after being exposed to liquid formulations was also investigated. The powder substances were prepared for SEM observation by direct deposition of powders on carbon conductive tape or were deposited on polished aluminium holders from diluted suspensions. The pellets and microcapsules were prepared for cross-section investigation by different cutting techniques in order to distinguish the core and the shell structures. The interior surfaces of vials were obtained by cutting the vials with diamond wire saw. All specimens were either uncoated or coated with a thin amorphous carbon layer or gold layer prior to microscopic investigation. Our investigations showed that by optimization of experimental microscopic parameters, such as voltage, probe current, aperture size, frame speeds and working distance it was possible to observe all powder formulations, even with high resolution. The EDXS mappings and EDXS linescans across microcapsules cross-sections were also acquired with not substantial beam damage. Finally, corrosion investigations of inner vials surface clearly showed the size and morphology of corrosion pits on the glass surface.

Experiment Design, tool for the optimization of analytical tests

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Design of experiments is a well-proven characterization approach within product and process development and a key aspect of quality by design. Recently, more attention has been placed on applying Design of experiments to analytical methods, which has proven to be a powerful, but underutilized, development tool for method characterization and method validation. Analytical professionals need to be comfortable using it to characterize and optimize the analytical method. The ELISA method is one of the most complex and the most time consuming, so that, whenever possible, optimization is necessary. This work presents the results of the application of a multifactor design of experimental applied for the optimization of incubation times, the blocking system and the pH of the coating buffer in a sandwich ELISA established for the specific quantification of the CIGB 166A protein. The application of this DOE applied to the selection of the best combination of factors, taking as a response variable the signal-to-noise ratio of the background, allowed to arrive at coherent results from the immunochemical point of view, saving time and expenses. The selection of the appropriate response variable was the determining factor for this. The strategy adopted in this work for the selection of the best conditions of the trial could be generalized to later works.

Aspectos a tener en cuenta en el desarrollo de procesos fermentativos para la obtención de biofármacos expresados en *E. Coli*. Aplicación de las guías ICH Q5, Q6, Q7, Q8, Q9 Y Q10

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El desarrollo de la Ingeniería Genética y la Biotecnología ha propiciado la obtención de un gran número de moléculas novedosas que han sido expresadas

mediante la tecnología del Ácido Desoxirribonucleico recombinante en *Escherichia coli*. Entre los aspectos importantes a tener en cuenta en el diseño del proceso se encuentran: la selección de la cepa productora, la optimización de la composición del medio de cultivo y los parámetros de operación; así como la elaboración de los bancos de células que garanticen estabilidad de la información genética. Una vez establecido el proceso productivo para la obtención del ingrediente farmacéutico activo, que cumpla con los atributos de calidad requeridos; deben realizarse estudios no clínicos para demostrar la prueba de concepto y seguridad. Posteriormente es necesario realizar la evaluación clínica en seres humanos, por lo que el proceso debe cumplir con las Buenas Prácticas de Fabricación (BPF), así como guías y normativas para garantizar la calidad del producto y la seguridad del paciente. En este trabajo se muestran aspectos generales de la aplicación del espacio de diseño y herramientas de calidad a lo largo del ciclo de vida del producto con vistas a desarrollar procesos eficientes que cumplan con las exigencias regulatorias, así como prevenir causas potenciales de falla y favorecer por lo tanto la consistencia del proceso. Esta metodología interrelaciona las Guías ICH Q5, Q6, Q7, Q8, Q9 y Q10.

Targeting individual host cell proteins to improve efficiency of downstream purification

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Host cell protein (HCP) impurity removal is an important objective of most DSP processes. ELISA assays, either commercial or custom, are the current method of choice for quantifying low levels of HCPs. In spite of their widespread use, ELISAs have at least two issues: they recognize a subset of HCPs based on their immunogenicity instead of quantity and different ELISAs may recognize different subsets of HCPs, resulting in large differences between assays. To address these challenges, we developed analytical tools for identification and quantification of HCPs, focusing on antibody processes and Protein A eluates. Our immediate goal was to confirm the expectation that there is significant consistency of key HCPs across various processes, with the ultimate goal to generate immunoassay kits containing corresponding antibodies to the key HCPs. Multiple CHO cell lines, clones and protein A purification media were used to produce sample diversity. Samples were separated using 2D RP UPLC methods and samples were analyzed with a QTOF mass spectrometer. This separation and mass spectrometry method detects 2-20 fold higher levels of HCPs compared to ELISA assays. Four HCPs are found in all samples and a set of 20 high-abundance HCPs describe more than 70% of HCP concentration in all our samples. Most of the identified CHO HCPs could be matched to commercially available antibodies, however, demonstrating reactivity proved to be more difficult. While not currently required by regulators, identification and quantification of specific HCPs would be a useful tool for process developers, leading to a more rational approach to chromatography media selection and development. Furthermore, process to process comparisons would be facilitated, with such methods particularly applicable to biosimilar and biobetter development. Smart bioprocessing based on a better understanding of impurities rather than trial and error appears like a promising future avenue.

Aseguramiento regulatorio en el proceso de manufactura del ingrediente farmacéutico activo del producto Hebervital

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El factor estimulador de colonias de granulocitos (G-CSF) se utiliza como Ingrediente Farmacéutico Activo en la producción del Hebervital, producto farmacéutico manufacturado en el Centro de Ingeniería Genética y Biotecnología desde el año 2000, de administración en forma de inyectable. Indicado para aumentar el recuento de granulocitos neutrófilos en casos como: terapia citostática, radioterapia, terapia inmunosupresora. Reduce la incidencia y duración de infecciones, en pacientes con neutropenia febril o con neutropenias graves, y en pacientes con antecedentes de infecciones graves o recurrentes. Reduce el uso de antibióticos y la duración y frecuencia de la hospitalización. La patente del producto innovador expiró en el año 2008. Como resultado pudiera ser considerado como "biosimilar", del medicamento biológico original Neupogem. Oportunidad importante, si es producido de acuerdo con las exigencias establecidas por la Agencia Europea del Medicamento (EMA) en cuanto a calidad, eficacia y seguridad. El paradigma "el proceso es el producto" es el más utilizado en el entorno de los medicamentos biológicos, debido al complejo proceso, de fases irreproducibles, al que son sometidas diferentes líneas celulares, donde pueden surgir dudas acerca de si la eficacia es realmente comparable. El proceso de Producción fue validado en el año 2009, según lo establecido en el protocolo PVB- 001, el producto ha sido comparado estructuralmente y en ensayos clínicos con el de la competencia. El objetivo de este trabajo es mostrar a través de los resultados de análisis del desempeño de las campañas realizadas y los resultados de validación/revalidación, que el Proceso está funcionando como se diseñó y que el producto, comparado con el de la competencia mantiene la calidad, se evidencia que la producción del IFA de GCSF muestra la consistencia adecuada.

Registration of agricultural products: international standards and regulatory environment in Cuba

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El registro es el procedimiento destinado a verificar la calidad, eficacia e inocuidad de un producto mediante la evaluación y reconocimiento de sus antecedentes. Éste constituye el último paso indispensable para la autorización de uso y comercialización

de cualquier tipo de producto. Para el proceso de Investigación-Desarrollo (I+D) de un bioproducto, nmunógeno, aditivo nutricional, sistema de diagnóstico, etc., en primera instancia es necesario tener en cuenta desde el inicio, los estándares y regulaciones nacionales e internacionales. Para el caso específico de los productos agropecuarios del CIGB, tanto los comerciales como los que aún se encuentran en I+D, no existe un órgano único regulatorio y de registros tanto nacional como internacionalmente, esto se debe a la diversidad de los campos que abordan nuestras investigaciones, que contemplan la salud animal, la sanidad vegetal, la acuicultura y la producción de alimentos (para animales y humanos). En el ámbito nacional existen seis órganos u oficinas que establecen los requerimientos y regulaciones para el registro de los productos agropecuarios ya incluidos en la cartera de negocios del CIGB o aún en I+D. Estos órganos se rigen por los estándares internacionales establecidos por la OIE, la IPPC y el CODEX Alimentarios. El CIGB también proyecta directamente sus estándares sobre estas instituciones internacionales, paralelamente se tienen muy en cuenta otras entidades regionales e internacionales (ej: CAMEVET, VICH y EMA), así como las específicas de países que constituyen grandes mercados (ej: ICAMA y APHIS). El objetivo de este trabajo es esclarecer el complejo ámbito regulatorio y los diversos estándares que deben cumplir los bioproductos de uso agropecuario, producidos por el CIGB.

Global regulatory standards for the registration and manufacture of veterinary vaccines

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At the global level, harmonization of standards for veterinary biologicals is of great help to veterinary agencies, which, following instructions from the World Organization for Animal Health (OIE), are applicable to all international trade. This tool is intended to simplify and facilitate the marketing of the products. There are conditions for the international harmonization of national standards in the case of veterinary vaccines, particularly in Europe (VICH and EMEA, now EMA) and in America (FDA, CAMEVET), which have encouraged vaccination as one of the most effective tools to prevent animal diseases and to promote the health and welfare of animals, safe food production and public health. Veterinary vaccines play an important role in the protection of animal health through the prevention and control of serious epizootic diseases. They also have an impact on human health by ensuring the safe supply of food and preventing the transmission of infectious diseases from animal to human. The objective of this work is to clarify the complex regulatory environment in Europe, the United States, Canada and the different standards that veterinary biological products must meet for their manufacture and registration.