

First International Conference Bioprocess Cuba 2017

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REPORT

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 delivery formulation for human and veterinary 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

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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 debatió 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 calidad de 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

Introduction

The growing and vertiginous generation of new drugs using biotechnological techniques continuously 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 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.

Symposium 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 emphasized 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 *Pichia 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 strategies were discussed on this symposium. 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, delivered 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 interest 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 emphasized 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 matrixes 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 hurdles 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 Universität, 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. Stéphane 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 I2 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 cytolytic proteins of the sea anemone *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 THE-RAVAC® 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 symposium 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 symposium. 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 discover 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 attract or repel. He suggested 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 strain from 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 M.Cs. 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 symposium. 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 improved 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 collaboration 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.

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