PATh/IL2BioPro/mVACCIL Workshop

June 2nd 2022

Leading strategies in biopharmaceuticals manufacturing

BOOK OF ABSTRACTS

Organizing Committee:

Ana Paula Tavares
Augusto Pedro
Francisca Silva
Mara Freire
Rita Teles

Program

The workshop will be held at *Sala de Atos Académicos* (UA Rectory) and virtually via Zoom platform

9:15 (PT) (5:15 - BR) – Opening session (*Augusto Pedro and Francisca Silva*)

Emerging and unconventional biomanufacturing technologies *(Chair Prof. João Coutinho)*

9:30 (PT) (5:30 – BR) – Invited Lecture I – Critical criteria senger RNA-based vaccines
Prof. Chantal Pichon, Center Molecular Biophysics, CNRS, Orléans, France

10:00 (PT) (6:00 – BR) – Discussion

10:10 (PT) (6:10 – BR) – Invited Lecture II – Shape-versatile biomaterials prepared at all-aqueous interfaces
Dr. Mariana Oliveira, Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal

10:30 (PT) (6:30 – BR) – Flash presentations
1 – **Dr. Matheus Pereira** (CICECO-UA) – Computer-aided identification of potential antiviral peptides from *Hancornia speciosa* Gomes leaf protein
2 – **MSc Leonor Castro** (CICECO-UA) – Application of ionic liquids as adjuvants in polymer-based aqueous biphasic systems to improve their purification performance
3 – **MSc Bojan Kopilovic** (CICECO-UA) – Gellable aqueous biphasic systems as a platform for biopharmaceuticals encapsulation
4 – **MSc Sónia Pedro** (CICECO-UA) – The successful partnership between deep eutectic solvent formulations and biopolymers in the transdermal delivery of NSAIDs

10:50 (PT) (6:50 – BR) – Discussion

11:00 – 11:30 (PT) (7:00 – 7:30; BR) – Coffee-break (*Sala de Senado*)

Bioprocessing strategies Part I – Downstream processing *(Chair Dr. Márcia Neves)*

11:30 (PT) (7:30 – BR) – Invited Lecture III – Amino acids as a powerful tool for DNA and RNA purification
Prof. Fani Sousa, CICS-UBI, Health Sciences research Centre, CICS-UBI, Covilhã, Portugal

12:00 (PT) (8:00 – BR) – Discussion

12:10 (PT) (8:10 – BR) – Invited Lecture IV – Biopharmaceuticals preparative chromatography – Biophysical online and in situ characterization of the adsorption process
Prof. Ana C. Dias-Cabral, CICS-UBI, Health Sciences research Centre, University of Beira Interior, Covilhã, Portugal

12:30 (PT) (8:30 – BR) – Flash Presentations
1 – **MSc Catarina Almeida** (CICECO-UA) – Bio-based excipients to enhance the stability of Avian immunoglobulin Y (IgY) antibodies

2 – **MSc Rita Carapito** (CICS-UBI) – Ionic liquid-based chromatographic supports for RNA purification

3 – **MSc João Nunes** (CICECO-UA) – Enzyme purification using silica-based supported ionic liquid-like phase materials

4 – **Dr. Sandra Bernardo** (CICS-UBI) – Novel purification platforms for pre-miRNAs by customized oligo-affinity chromatography

12:50 (PT) (8:50 – BR) – Discussion

13:00 – 14:45 (PT) (9:00 – 10:45; BR) – Lunch Break (*Restaurante Universitário*)

Enabling technologies for biopharmaceuticals manufacturing *(Chair Prof. Pedro Carvalho)*

14:45 (PT) (10:45 – BR) – Invited Lecture V – Pegylation as a strategy to improve biological drugs - *Online*
Prof. Carlota Rangel-Yagui, Laboratory of Nanobiotechnology – nanobio, School of Pharmaceutical Sciences, University of São Paulo

15:15 (PT) (11:15 – BR) – Discussion

15:25 (PT) (11:25 – BR) – Flash Presentations
1 – **Dr. Ana Maria Ferreira** (CICECO-UA) – Extraction of recombinant membrane proteins using alternative solvents

2 – **MSc Ana Rufino** (CICECO-UA) – Recovery and purification of antibodies using three-phase partitioning systems based on aqueous biphasic systems

3 – **MSc Ana Isabel Valente** (CICECO-UA) – Three-phase partitioning systems for the separation of DNA and DNAse I

4 – **MSc Marguerita Rosa** (CICECO-UA) – Can egg yolk antibodies (IgY) be used to avoid or fight bacterial infections in aquaculture systems?

15:45 (PT) (11:45 – BR) – Discussion

15:55 – 16:25 (PT) (11:55 – 12:25; BR) – Coffee-Break (*Sala de Senado*)

Bioprocessing strategies Part II - Bioproduction *(Chair Dr. Pedro Madeira)*

16:25 (PT) (12:25 – BR) – Invited Lecture VI – Microbial Cell Factories for High Quality Biopharmaceuticals Production
Prof. Luís Passarinha, UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, Nova School of Science and Technology, Universidade Nova de Lisboa, Caparica, Lisboa (i4HB – Institute for Health and Bioeconomy/CICS-UBI – Health Sciences Research Centre/Laboratório de Fármaco-Toxicologia – UBIMEDICAL)

16:45 (PT) (12:45 – BR) – Invited Lecture VII – *Aliivibrio fischeri* L-Asparaginase production in *Bacillus subtilis*: from orbital shaker to stirred tank bioreactor - *Online*
Prof. Valéria Ebinuma, Department Bioprocess Engineering and Biotechnology, School of Pharmaceutical Sciences, São Paulo State University (UNESP)

17:05 (PT) (13:05 – BR) – Discussion

17:15 (PT) (13:20 – BR) – Flash Presentations
1 – **MSc Gabriela Paiva** (UNESP) – Development of L-asparaginase protein releasing systems for recombinant *Bacillus subtilis* - *Online*

2 – **MSc Diana Gomes** (CICS-UBI/UCIBIO/i4HB) – Recombinant E6 protein biosynthesis and purification from *Escherichia coli* cells: a target for cervical cancer

3 – **MSc Flávia Magalhães** (CICECO-UA) – Recombinant laccase production by *Komagataella pastoris*

4 – **Ana Andrade** (CICECO-UA) – Bioprocess integration of interferon alfa 2b using Pluronics®

17:35 (PT) (13:35 – BR) – Discussion

17:45 (PT) (13:50 – BR) – Final Remarks/Closing Session (Ana P. Tavares, Augusto Pedro, and Francisca Silva)

ACKNOWLEDGEMENTS

Workshop held within the framework of R&D Projects:

IL2BioPro (PTDC/BII-BBF/30840/2017, Towards the improvements of recombinant proteins bioprocessing) funded by FEDER, through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTE.

mVACCIL (EXPL/BII-BTI/0731/2021, Improving the manufacturing of mRNA vaccines applying ionic liquids and integrated production-clarification processes), financially supported by national funds (OE), through FCT/MCTES.

PATh/IL2BioPro/mVACCIL Workshop Participants

Path members (CICECO - *University of Aveiro, Portugal*)

João A. P. Coutinho	Full Professor	Leonor Castro	PhD student
Mara G. Freire	Research Coordinator	Leonardo Mesquita	PhD student
Pedro Carvalho	Assistant Professor	Leticia Contieri	PhD student
Ana Maria Ferreira	Researcher	Mahtab Moradi	PhD student
Ana Paula Tavares	Researcher	Marguerita Rosa	PhD student
Augusto Pedro	Researcher	Maria Mendes	PhD student
Catarina Seiça Neves	Researcher	Mariam Kholany	PhD student
Francisca Silva	Researcher	Raul Gonzalez	Visiting PhD student
Filipe Sosa	Researcher	Ricardo Pais	PhD student
German Perez Sanchez	Researcher	Sónia Pedro	PhD student
Helena Passos	Researcher	Telma Veloso	PhD student
Matheus Pereira	Researcher	Maria Inês Sousa	MSc scholarship
Márcia Neves	Researcher	Ana Sofia Marques	MSc student
Meena Bisht	Researcher	Ana Sofia Pinto	MSc student
Mónia Martins	Visiting Researcher	André Nogueira	MSc student
Nicolas Schaeffer	Researcher	Catarina Paixão	MSc student
Pedro Madeira	Researcher	João Fernandes	MSc student
Ana Catarina Almeida	PhD student	Mariana Aguiar	MSc student
Ana Filipa Pereira	PhD student	Mariana Castro	MSc student
Ana Filipa Rufino	PhD student	Sara Ribeiro	MSc student
Ana Isabel Valente	PhD student	Tatiana Ferreira	MSc student
Ana Rita Futre	PhD student	Ana Andrade	BSc student
Bárbara Vaz	PhD student	Catarina Caramelo	BSc student
Bojan Kopilovic	PhD student	Cecília Roque	BSc student
Bruna Soares	PhD student	Eduarda Carmo	BSc student
Diogo Barros	PhD student	Francisco Rocha	BSc student
Flávia Magalhães	PhD student	Margarida Costa	BSc student
Gabriel Teixeira	PhD student	Maria Clara Souza	BSc student
Inês Cardoso	PhD student	Maria Sofia Leite	BSc student
Inês Macário	PhD student	Patrícia Damião	BSc student
Jordana Benfica	PhD student	Rita Neves	BSc student
João Nunes	PhD student	Ana Rita Teles	Lab Technician

Guests

Name	*Afilliation*
Ana Cristina Dias-Cabral	CICS-UBI – Health Sciences Research Centre, University of Beira Interior
Carlota Rangel-Yagui	Laboratory of Nanobiotechnology – nanobio, School of Pharmaceutical Sciences, University of São Paulo
Chantal Pichon	Center Molecular Biophysics, CNRS
Diana Gomes	CICS-UBI – Health Sciences Research Centre, University of Beira Interior (UCIBIO – Applied Molecular Biosciences Unit/i4HB – Institute for Health and Bioeconomy)
Fani Sousa	CICS-UBI – Health Sciences Research Centre, Universidade da Beira Interior
Gabriela Paiva	Department of Bioprocess Engineering and Biotechnology, School of Pharmaceutical Sciences, São Paulo State University (UNESP)
Jose Palomar	Departamento de Ingeniería Química, Universidad Autónoma de Madrid
Luís Passarinha	UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, Nova School of Science and Technology, Universidade Nova de Lisboa, Caparica, Lisboa (i4HB – Institute for Health and Bioeconomy/CICS-UBI – Health Sciences Research Centre/Laboratório de Fármaco-Toxicologia – UBIMEDICAL)
Mariana Oliveira	Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro
Rita Carapito	CICS-UBI – Health Sciences Research Centre, Universidade da Beira Interior
Sandra Bernardo	CICS-UBI – Health Sciences Research Centre, Universidade da Beira Interior
Valéria C. Santos-Ebinuma	Department of Bioprocess Engineering and Biotechnology, School of Pharmaceutical Sciences, São Paulo State University (UNESP)

Critical criteria senger RNA-based vaccines

Chantal Pichon

Center Molecular Biophysics, CNRS, Orléans-FRANCE

Abstract

Messenger RNA (mRNA) has emerged as a promising biopharmaceutical for a vast array of therapeutic applications, the main application being the vaccination. The recent development of mRNA-based vaccines for COVID-19 has proved the potentiality of this vaccine modality. It is a versatile platform to express any type of antigens. mRNA offers a strong safety compared to DNA because it cannot be integrated in host genome. The translation machinery being in the cytosol, the nuclear import is not required which is of benefit for hard to transfect cells. To be protected against nucleases and internalized efficiently inside cells, mRNA must be formulated.
This talk will summarize the the molecular design of mRNA vaccines and lipid-based mRNA formulations. Purification and stabilization strategies are highly important throughout the vaccine production process. The regulatory guidelines related to characterization and storage stability of RNA-based biopharmaceuticals will be presented. Last, I will discuss on challenges that still have to be overcome for a cost-effective and worldwide distribution of mRNA vaccines.

References

Baptista B, et al., 2021. Pharmaceutics. 13(12):2090.
Vervaeke P, et al., Adv Drug Deliv Rev. 2022 Mar 26;184:114236.

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Shape-versatile biomaterials prepared at all-aqueous interfaces

Mariana B. Oliveira[1], Raquel C. Gonçalves[1], Sara Vilabril[1], Catarina M. S. S. Neves[1], Mara G. Freire[1], João A. P. Coutinho[1], João F. Mano[1]

[1] Department of Chemistry, CICECO – Aveiro Institute of Materials. University of Aveiro. 3810-193 Aveiro, Portugal

Abstract

Cell encapsulation in biomaterial microcompartments is a useful tool to deliver protected cell cargo into defective tissues. However, the processing of micrometric structures is often dependent on emulsion agents including oils and organic solvents, often related with poor cell viability, or requiring complicated washing procedures. All-aqueous processing methodologies arose as promising strategies to process biomaterials. Aqueous two-phase systems have been used as surrogates of classical water/oil-based emulsions to enable the formation of, for example, microparticles. Experimental approaches explored for classical emulsion systems for biomaterial fabrication have led mostly to the formation spherical structures. For example, the interfacial complexation of polyelectrolytes at the interface of millimetric and micrometric droplets has enabled the cytocompatible encapsulation of stem cells [1]. However, the preparation of compartments with other shapes has proven challenging. Although previous attempts were made to fix the shape of objects at the interface of aqueous emulsions, structures that can be handled outside those interfaces could not be obtained. We here report an advance on the all-aqueous fabrication of self-standing biomaterial tubes.

References

[1] Vilabril, S., Nadine, S., Neves, C. M. S. S., Correia, C. R., Freire, M. G., Coutinho, J. A. P., Oliveira, M. B., Mano, J. F., One-Step All-Aqueous Interfacial Assembly of Robust Membranes for Long-Term Encapsulation and Culture of Adherent Stem/Stromal Cells. Adv. Healthcare Mater. 2021, 10, 2100266.

Acknowledgements

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Computer-aided identification of potential antiviral peptides from *Hancornia speciosa* Gomes leaf protein

Matheus M. Pereira[1]

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Abstract

Nowadays, the discovery of antiviral drugs against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still required. After the development strategy of antiviral therapy using vaccines, the identification of novel and effective SARS-CoV-2 antiviral drugs is the next goal for several pharmaceutical companies. Generally, plants are used to obtain phytochemicals, on the other hand, the valorization of plant proteins as source of bioactive peptides is neglected. In this work, a computational strategy using molecular docking and MM/GBSA analysis was applied to identify antiviral peptides in Ribulose 1,5-bisphosphate carboxylase (Rubisco) from *Hancornia speciosa* Gomes. Firstly, the amino acid sequence of Rubisco was obtained from UniProt and the hydrolysis prediction was performed. The 90 peptides obtained were evaluated regarding their instability index and allergenicity. After the screening, 7 peptides were selected and their secondary structure predicted. The peptides structures predicted were applied to molecular docking analysis against spike glycoprotein of SARS-CoV-2. The model with lowest RMSD and higher bind affinity of each peptide-protein complex were selected and applied to MM/GBSA analysis. According to the results obtained, the most promising peptide (Pep_45) display competitive behavior and could inhibit the bind of spike glycoprotein of SARS-CoV-2 with angiotensin-converting enzyme 2 (ACE2) human receptor, preventing the virus contact with human cells.

Acknowledgements

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Application of ionic liquids as adjuvants in polymer-based aqueous biphasic systems to improve their purification performance

Leonor S. Castro[1], Patrícia Pereira[2], Luís A. Passarinha[3,4,5], Augusto Q. Pedro[1], Mara G. Freire[1]

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[5] Laboratory of Pharmacology and Toxicology – UBIMedical, University of Beira Interior, Covilhã, Portugal

Abstract

Protein biopharmaceuticals, among which interferon alpha-2b (IFNα-2b) that can be used in the treatment of chronic hepatitis C, have become an indispensable product of current medicine [1]. However, their current high costs derived from the lack of cost-effective downstream strategies still limits their widespread use. In this sense, aqueous biphasic systems (ABS) are a viable alternative for the clarification and purification of biopharmaceuticals. This work investigates the use of ionic liquids (ILs) as adjuvants in ABS constituted by polyethylene glycol (PEG, 600 g/mol) and polypropylene glycol (PPG, 400 g/mol) to purify recombinant IFNα-2b from *Escherichia coli* lysates. IFNα-2b was found to be preferentially partitioned to the PEG-rich phase, being also the phase enriched in IL. In comparison with the ABS without adjuvant, systems comprising ILs composed by aromatic cations and anions with high hydrogen-bond basicities as adjuvants led to enhancements in the purification factors from 2.28 ± 0.06 to 6.77 ± 0.49 [2]. Overall, these results demonstrate the ability of ILs to tune the characteristics of the ABS coexisting phases towards improved purification process, also being able to preserve the secondary structure of IFNα-2b.

References

[1] L.S. Castro, G.S. Lobo, P. Pereira, M.G. Freire, M.C. Neves, A.Q. Pedro, *Vaccines* 9 (4): 328. 2021.

[2] L.S. Castro, P. Pereira, L.A. Passarinha, M.G. Freire, A.Q. Pedro, *Sep Purif Technol* 248: 117051. 2020.

Acknowledgements

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Gellable aqueous biphasic systems as a platform for biopharmaceuticals encapsulation

Bojan Kopilovic, João A.P. Coutinho, Mara G. Freire

Department of Chemistry, CICECO - Aveiro Institute of Materials, University of Aveiro, 3810-193 Aveiro, Portugal

Abstract

Biopharmaceuticals produced using recombinant or hybridoma technologies offer the potential to treat a wide range of diseases, however they are not without drawbacks. Many biopharmaceuticals lose their stability easily in commercial formulations because they are proteins. As a result, protein stability is becoming increasingly crucial as the supply of protein products grows in almost every sector, particularly in the pharmaceutical industry. In addition, one of the key challenges of the more desired oral delivery of protein therapeutics is protein instability. Proteins must be properly stabilized and kept at a high quality for an extended period if they are to be employed as therapeutics. If they are not stabilized, their therapeutic efficacy decreases, requiring the development of suitable formulations and administration techniques.

Aiming to preserve biopharmaceutical stability, this work describes a unique approach using an already known platform. Aqueous biphasic systems (ABS) with phase-forming components with gelling ability were used for biopharmaceuticals encapsulation. Gellable ABS formed from biopolymers for protein encapsulation has been investigated. An emulsification method for microsphere formation has been developed. Protein encapsulation, release profile in simulated gastric fluids and stability have been determined.

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The Successful Partnership Between Deep Eutectic Solvent Formulations and Biopolymers in the Transdermal Delivery of NSAIDs

Sónia N. Pedro[1], Maria S. M. Mendes[1], Bruno M. Neves[2], Isabel Filipa Almeida[3,4], Paulo Costa[3,4], Inês Correia-Sá[5], Carla Vilela[1], Mara G. Freire[1], Armando J. D. Silvestre[1] and Carmen S. R. Freire[1]

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) are amongst the most commonly prescribed medications; however, their oral administration is associated with moderate side effects [1]. Therefore, transdermal delivery can be a desirable alternative to provide their therapeutic effect with safety and efficacy [2]. In an attempt to replace organic solvents, used in commercial formulations [3] we have considered the use of deep eutectic solvents (DES). To this purpose, we have studied aqueous solutions of bio-based DES to enhance the solubility of ibuprofen, a widely used NSAID. These solutions allowed to increase the drug solubility up to 7917-fold when compared to its water solubility. It is possible to incorporate these formulations in alginate-based systems, without toxicity associated to macrophages and without affecting the drug's therapeutic action. Upon application in human skin, the alginate-based hydrogels containing DES aqueous solutions with ibuprofen were capable to improve the drug permeation across human skin by 8.5-fold in comparison with the hydrogel counterpart containing only ibuprofen. The results herein highlight the advantages of combining DES formulations with biopolymers to enhance the transdermal administration of pharmaceuticals.

References

[1] Harirforoosh, S.; Asghar, W.; Jamali, F. *J. Pharm. Pharm. Sci.* 2013, 16, 821–847.
[2] Ong, C.K.S.; Lirk, P.; Tan, C.H.; Seymour, R.A *Clin. Med. Res.* 2007, 5, 19–34.
[3] Hadgraft, J.; Whitefield, M.; Rosher, P.H. *Skin Pharmacol. Appl. Skin Physiol*. 2003, 16, 137–142.

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Amino acids as a powerful tool for DNA and RNA purification

Fani Sousa[1]

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Abstract

Nucleic acid-based biopharmaceuticals are attracting great interest due to their potential to create breakthrough treatments for incurable diseases. Gene-based therapeutics, such as DNA, iRNA or mRNA, are thus, novel and complex products that bring unique challenges in product development. The recombinant production of biopharmaceuticals has already been established, however the envisioned therapeutic application of pDNA, mcDNA, or RNA requires their recovery as highly pure products. So, the challenges related to the purification of biopharmaceuticals motivated the exploitation of high-binding capacity supports combined with selective ligands in affinity techniques. Thus, amino acid-based affinity chromatography was implemented as a chromatographic approach to first purify pDNA, and more recently, for the purification of mcDNA and RNA, using amino acids as specific ligands. In general, a high selectivity towards the target nucleic acids was achieved, allowing their purification from complex lysates. This selectivity mainly results from the establishment of multiple non-covalent interactions, leading to the biorecognition of the biologically active biomolecules. The use of monolithic supports enabled the improvement of the global performance of the purification process, by increasing the binding capacity and decreasing the chromatographic run-time, which contributes to the recovery of biopharmaceuticals with the required stability and biological activity.

Acknowledgements

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Biopharmaceuticals preparative chromatography – Biophysical online and in situ characterization of the adsorption process

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Abstract

In recent years, the major driving force for pharmaceutical industry growth has been the production of biopharmaceuticals. Still, their purification continues to be considered the bottleneck of the manufacturing process. Also, the request for mechanistic understanding of processes has completely changed the paradigm of process development and process validation in biopharmaceutical manufacturing. This is also true for preparative chromatography, the dominant technique used in biomolecules purification. The retention and separation mechanisms involved, are still not fully understood. To overcome this limitation and to seed light onto the mechanisms of interaction between biomolecules and the chromatographic resins is important a biophysical characterization of the adsorption process. This presentation will provide the state-of-the-art in experimental approaches to monitor biomolecule – surface interactions. Focus will be put on flow microcalorimetry (FMC) and small-angle X-ray scattering (SAX), as two non-labeling techniques capable of simulating a dynamic chromatography system allowing online and in situ monitoring of the adsorptive process. Recent applications of these in situ monitoring techniques to better understand separation of biomolecules with pharmaceutical interest will be presented.

References

- G.F. L. Silva *et al.*, Biotechnol. J. 2019, 14, 1800632. Doi:10.1002/biot.201800632, 2019
- G.L. Silva *et al.*, Biotechnology and Bioengineering 2019, 116, 76–86. Doi: 10.1002/bit.26843
- S.A.S.L. Rosa *et al.*, J. Chrom. A 2018, 1569, 118–12. Doi:10.1016/j.chroma.2018.07.050
- S. Rauwolf *et al.*, J. Phys. Chem. Lett. 2021, 12, 9384−9390. Doi:10.1021/acs.jpclett.1c02398

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Bio-based excipients to enhance the stability of Avian Immunoglobulin Y (IgY) antibodies

Catarina Almeida[1], Luís C. V. Silva[1], Maria Carolina Silva[1], Márcia C. Neves[1], Mara G. Freire[1]

[1] CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

Abstract

Avian immunoglobulin Y (IgY), present in chicken's egg yolk, is a promising antibody to be used as biopharmaceutical [1]. IgY displays high binding avidity and immunogenicity, being non-invasibly recovered [2]. By being a polyclonal antibody, identifies numerous epitopes on an antigen, being employed for instance in the treatment of diseases [1-3]. Since IgY comes from a complex source their recovery at high purity and yields and preservation are difficult, constraining their use as biopharmaceuticals [1]. IgY antibodies were isolated from commercial chicken egg´s yolk and purified. Their stability was evaluated by Circular Dichroism Spectroscopy under 1-3 weeks of storage at -20 ºC, with and without sucrose, trehalose, sorbitol, and xylitol. The IgY purity and percentage of aggregates formed during storage were revealed by Size Exclusion- High Performance Liquid Chromatography, and protein profile unveil by dodecyl sulphate polyacrylamide gel electrophoresis. All compounds exhibit promising results, since a reduction in the percentage of aggregates was found in IgY formulations during storage. β-sheets in IgY secondary structure were verified, and no substantial evidence of its degradation occur during storage with the stabilizers, implying that its secondary structure was maintained. Novel bio-based compounds have been recognized as promising stabilizers to enhance the stability of IgY antibodies, envisaging their use as excipients in IgY therapeutic formulations.

References

[1] Kovacs-Nolan, Jennifer and Mine, Yoshinori. Avian and Poultry Biology Reviews. 2004, 15, 25–46.
[2] Pereira, E. P.V., M. F. van Tilburg, E. O.P.T. Florean, and M. I.F. Guedes. International Immunopharmacology. 2019, 73, 293–303.
[3] Kovacs-Nolan, Jennifer, and Yoshinori Mine. Annual Review of Food Science and Technology. 2012, 3, 163–82.

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This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). This work has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 899921). M.C. Neves acknowledges FCT, I.P. for the research contract CEECIND/00383/2017 under the CEEC Individual 2017.

Ionic liquid-based chromatographic supports for RNA purification

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Abstract

Therapies based on non-coding RNAs arise as recent strategies for the treatment of several diseases, especially those caused by gene defects. Notwithstanding, purification technology that can assure RNA purity, integrity, and stability remains a great obstacle that needs to be surpassed. Therefore, there is a high necessity for the development of highly selective and profitable downstream processes for RNA purification. In this work, an alternative method using Ionic Liquids(ILs)-based chromatographic supports is proposed. ILs are a group of organic salts with great features for purification and stabilization of nucleic acids, allowing a variety of combinations of cation/anion chemical structures aiming the design of task-specific ILs. Silica-confined ILs (SILs) have been recently studied in chromatography for biomolecules purification [1]. Thus, the application of SILs in RNA purification is highly innovative and can lead to the establishment of alternative and advantageous downstream methods. Herein, novel SILs such as SilPrMImCl, SilPrMImPrACl, SilPrNM$_2$ButCl and SilPrNM$_2$EtACl were synthetized, and screened regarding binding and elution conditions under hydrophobic and ionic conditions. SilPrMImPrACl displayed a better performance, achieving the separation between RNA and genomic DNA. This imidazolium-based support showed great ability for RNA purification from a lysate sample, in a simple and rapid step using mild conditions.

References

1. Neves, M., et al., Improved ionic-liquid-functionalized macroporous supports able to purify nucleic acids in one step. Materials Today Bio, 2020. 8: p. 100086.

Acknowledgements

This work was supported by the Portuguese Foundation for Science and Technology (FCT), through the project UIDB/00709/2020, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, by the project PTDC/BII-BBF/29496/2017 funded by FEDER, through COMPETE2020-POCI, and by national funds, through FCT/MCTES. Authors also acknowledge Project Yscript (101047214) from European Union Programme Pathfinder EIC. R. Carapito and M.C. Neves acknowledge FCT for the PhD fellowship (REF:2020.07980.BD) and the research contract CEECIND/00383/2017, respectively.

Enzyme purification using silica-based supported ionic liquid-like phase materials

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Abstract

Enzymes are widely studied biomolecules with relevant applications in several industries. Specifically, L-asparaginase (ASNase) is an aminohydrolase enzyme widely applied as a biopharmaceutical, as an acrylamide reduction agent, and as an enzyme-based biosensor for L-asparagine quantification[1]. While widely distributed in nature, e.g., plants, animals, and microorganisms, most commercial ASNase are from recombinant microorganisms, whose production can be performed through fermentation[1]. However, downstream processing of ASNase accounts for up to 80% of total production cost[2]. Thus, a novel cost-effective downstream process is of emerging concern to allow its widespread use.

This work aims the development of a cost-effective purification process for ASNase from recombinant *Bacillus subtilis* cell lysates obtained through ultrasound sonication. Silica-based supported ionic liquid-like phase materials were studied as cost-effective ASNase purification supports by a simple adsorption method. The optimization of experimental conditions, e.g., medium pH and material/cell lysate ratio was performed regarding ASNase purity. Through this approach, process costs, energy consumption, and waste produced, may be significantly reduced, leading to ASNase price decrease, thereby allowing its widespread application.

References

[1] Nunes, J. C. F. et al. L-Asparaginase-based biosensors. Encyclopedia 1, 848–858 (2021).
[2] Lopes, A. M. et al. Therapeutic l-asparaginase: upstream, downstream and beyond. Crit. Rev. Biotechnol. 37, 82–99 (2017).

Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). This work was also financially supported by the project POCI-01-0145-FEDER-031268-funded by FEDER through COMPETE2020—Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE) through FCT/MCTES, and by FAPESP (2018/06908-8). Ana P. M. Tavares and Márcia C. Neves acknowledge FCT for the research contract CEECIND/2020/01867 and CEECIND/00383/2017, respectively. Valéria C. Santos-Ebinuma acknowledges FAPESP for financial support. João C. F. Nunes acknowledges SPQ and FCT for the PhD fellowship (SFRH/BD/150671/2020).

Novel purification platform for pre-miRNAs by customized oligo-affinity chromatography

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Abstract

RNA technology boom has just begun, and most techniques and related processes are still in development[*1*]. Small non-coding RNAs (such as miRNAs) control gene expression and their dysregulation can impact on biological mechanisms of several diseases[*2*]. Current trials based on RNA therapeutics rely mostly on the use of synthetic RNAs[*1*]. Still, issues associated with the presence of contaminants restrains the rapid translation of these candidates onto real clinical applications. Although techniques such as *in vitro* transcription and recombinant expression, can provide access to relatively high amounts of stable RNA, further purification is still required [*3*, *4*]. Thus, we propose the development of new oligo-affinity chromatography purification platform for specific pre-miRNAs. These miRNAs produced by recombinant technology are intended to be purified using specifically designed oligonucleotides that are complementary towards different pre-miRNAs. This envisions a superior and cost-effective manner to obtain RNA-based therapeutics. An initial screening of oligo-based affinity ligands, by surface plasmon resonance (SPR), has been performed and the oligos are currently being immobilized onto a solid support for their study in different purification strategies.

References

1. B. Baptista, M. Riscado, J. A. Queiroz, C. Pichon, F. Sousa, *Biochem. Pharmacol.*, 114469 (2021).
2. A. Fire *et al.*, *Nature*. **391**, 806–811 (1998).
3. P. Pereira *et al.*, *Appl Microbiol Biotechnol*. **100**, 3723–3734 (2016).
4. P. Pereira, J. A. Queiroz, A. Figueiras, F. Sousa, *J. Chromatogr. B*. **1021**, 45–56 (2016).

Acknowledgements

This work was supported by the Portuguese Foundation for Science and Technology (FCT), through the project UIDB/00709/2020 and UIDP/00709/2020, and by the project PTDC/BII-BBF/29496/2017 funded by FEDER, through COMPETE2020-POCI, and by national funds, through FCT/MCTES. R. Carapito acknowledges FCT for the PhD fellowship (2020.07980.BD).

Pegylation as a strategy to improve biological drugs

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Abstract

The covalent attachment of polyethylene glycol (PEG) to proteins is an important route to develop improved biomolecules for biomedical, biotech and pharmaceutical applications. PEG conjugation can shield the protein antigenic epitopes, reduce degradation by proteolytic enzymes, enhance long-term stability and maintain or even improve pharmacokinetic and pharmacodynamics characteristics of the protein drug. Nonetheless, knowledge of the PEGylation process from reaction to downstream processing is of paramount importance for the industrial application and processing scale-up. In this presentation we will discuss the main steps in protein PEGylation, mainly PEGylation reaction, separation of the products and final characterization of structure and activity of the resulting species.

References

Santos, J.H.P.M.; Torres, K.M.; Meneguetti, G.P.; Amaro, B.P.; Rangel-Yagui, C.O. Protein PEGylation for the Design of Biobetters: From Reaction to Purification Processes. Braz. J Pharm. Sci., v.54 no.spe , 2018.

Acknowledgements

The authors acknowledge the National Council for Scientific and Technological Development (CNPq – Brazil), the Coordination for the Improvement of Higher Education Personnel (CAPES-Brazil), the São Paulo Research Foundation (FAPESP – Brazil, grant # 2016/22065-5) and the Portuguese Foundation for Science and Technology (FCT) for the doctoral grant SFRH/ BD/102915/2014 to J. H. P. M. Santos.

Extraction of recombinant membrane proteins using alternative solvents

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Abstract

Recombinant proteins have contributed significantly to medical biotechnology, especially as biopharmaceuticals in the treatment of various diseases. Recombinant protein manufacturing has become a requirement and vital component of most current small drug development efforts, with membrane proteins (MemP) playing a crucial role [1]. The hydrophobic structure of MemP makes it challenging to produce pure, stable, and physiologically active membrane proteins, and so we must rely on effective purification processes to do so. Aqueous biphasic systems (ABS) have been recently proposed as a key methodology for integrating several downstream units (e.g., cell lysis and capture) and reducing the costs while maintaining the stability of the extracted biomolecule. Thus, as a first step, this study aims to study the ability of various surfactants and ionic liquids to solubilize the cell wall and plasma membrane of *Komagataella pastoris* in order to recover intracellularly produced human cyclooxygenase 2 (COX-2) as a model recombinant MemP. The ultimate goal is the combination this step with the development of an ABS capable of extracting and purifying the MemP in a single step, in order to develop an integrated process. Overall, the approach described here will be a step forward in the development of efficient processes to meet the critical demand for high-quality MemP, while the integration of two downstream units may potentially reinforce the process's sustainability by reducing cost and its environmental footprint.

References

[1] Pham, P.V. Chapter 19 - Medical Biotechnology: Techniques and Applications. In Omics Technologies and Bio-Engineering, Barh, D., Azevedo, V., Eds.; Academic Press: 2018; pp. 449-469.

Acknowledgements

This research was funded by FEDER through COMPETE 2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES from the project IL2BioPro-PTDC/BII-BBF/30840/2017. This work was developed within the scope of the projects CICECO-Aveiro Institute of materials, UIDB/50011/2020 & UIDP/00285/2020, financed by national funds through the Portuguese Foundation for Science and Technology (FCT). Augusto Q. Pedro acknowledge FCT for the research contract CEECIND/02599/2020 under the Scientific Stimulus - Individual Call. Leonor S. Castro acknowledges FCT for her PhD grant 2020/05090/BD.

Recovery and purification of antibodies using three-phase partitioning systems based on aqueous biphasic systems

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Abstract

Antibodies, such as immunoglobulin G (IgG), are relevant for several therapeutic applications [1]. Despite the technological improvements achieved, the purification processes of antibodies are still based on several and expensive steps [2]. Here, we propose the use of three-phase partitioning (TPP) systems based on aqueous biphasic systems (ABS) as a simpler and cost-effective alternative. Systems composed of polyethylene glycol (PEG) and citrate buffer at pH 7 were studied, with and without the use of ionic liquids (ILs) as adjuvants, to manipulate the selectivity and recovery efficiency of the process. The process was optimized in terms of PEG molecular weight as well as of IL structure and concentration using human polyclonal IgG from serum. Regarding the recovery yield and purity level, the best performance was obtained for the TPP with PEG 1000 $g \cdot mol^{-1}$, with the use of ILs as adjuvants leading to improvements. The optimized TPP were then successfully applied for the recovery and purification of monoclonal antibodies from cell culture supernants, showing technological flexibility. Overall, TPP systems based on ABS can be used as alternative platforms to recover and purify IgG from complex biological matrices.

References

1. Katz, U.; Achiron, A.; Sherer, Y.; Shoenfeld, Y. Safety of intravenous immunoglobulin (IVIG) therapy. *Autoimmun. Rev.* **2007**, *6*, 257–259.
2. Yang, O.; Qadan, M.; Ierapetritou, M. Economic analysis of batch and continuous biopharmaceutical antibody production: A review. *J. Pharm. Innov.* **2020**, *15*, 182–200.

Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). F.A. e Silva acknowledges FCT for the researcher contract CEECIND/03076/2018 under the Scientific Employment Stimulus – Individual Call 2018. E.V. Capela and A.F.C.S. Rufino acknowledge FCT for the PhD grants SFRH/BD/126202/2016 and SFRH/BD/138997/2018, respectively.

Three-phase partitioning systems for the separation of DNA and DNase I

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Abstract

New therapeutic areas have been emerging, thus, the development of downstream processes capable of extracting, purifying, and preserving deoxyribonucleic acid (DNA) integrity by reducing the degradation by endonucleases is still needed to allow the large-scale production of DNA [1,2]. Aqueous biphasic systems (ABS) are constituted of two water-soluble compounds and above given conditions lead to the formation of two phases. ABS composed of polymers and salts, have been reported as alternative extraction and pre-purification approaches for plasmid DNA (pDNA) [3]. However, other compounds such as ionic liquids (ILs) can be used in ABS. ILs are molten salts known as designer solvents allowing to customize their properties. Though, no attempt has been made with DNA, particularly in the development of IL-based ABS capable of separating contaminating endonucleases from nucleic acids. In this work, three-phase partitioning systems (TPP) formed by ABS composed of biocompatible cholinium-based ILs are used for the separation of double-stranded DNA (dsDNA) from deoxyribonuclease I (DNase I). With the designed ILs, dsDNA can be completely extracted to the IL-rich phase, while DNase I is precipitated at the ABS interface. Moreover, cholinium glycolate ([N111(2OH)][Gly]) allows the dsDNA simultaneous extraction, purification, and preservation at long-term, representing a relevant system to be applied in bioprocessing when envisioning DNA-based therapy products.

References

[1] J. Stadler, R. Lemmens and T. Nyhammar, *J. Gene Med*., 2004, **6**, S54-S66.
[2] D. R. Gill, I. A. Pringle and S. C. Hyde, *Gene Ther.*, 2009, **16**, 165-171.
[3] S. C. Ribeiro, G. A. Monteiro, J. M. S. Cabral and D. M. F. Prazeres, *Biotechnol. Bioeng.*, 2002, **78**, 376-384.

Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC) and CICS-UBI projects UIDB/00709/2020 & UIDP/00709/2020 financed by national funds through the FCT/MCTES. Ana P.M. Tavares acknowledges the FCT for the research contract CEECIND/2020/01867 and Ana I. Valente acknowledges the FCT PhD grant (SFRH/BD/08352/2021).

Can egg yolk antibodies (IgY) be used to avoid or fight bacterial infections in aquaculture systems?

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Abstract

Aquaculture has received remarkable attention as an alternative to traditional fishing activities limited by fishing quotas, hence new techniques have been developed aiming to increase its production and profit. However, over-exploitation, pollution, infectious diseases, and antimicrobial resistance have emerged as consequences [1]. *Vibrio anguillarum* is a Gram-negative bacterium causing fish infections in aquaculture systems, leading to significant economic losses[2]. This infection is treated with antibiotics; however, bacteria resistance to such drugs urges the development of alternative therapeutic strategies. The use of antibodies, namely avian Immunoglobulin Y (IgY) purified from hen egg yolks, come as a promising approach for the control such infections [3]. In this work, production, purification, and characterization of chicken IgY antibodies against *V. anguillarum* whole-cell extracts were performed. IgY antibodies extracted from hyperimmune chicken eggs were purified (>95% purity) and its antimicrobial potential against *V. anguillarum* evaluated. The purified IgY revealed a promising bacteriostatic effect that promoted a 50% bacterial growth inhibition when supplemented to microbial cultures. These results support the use of specific IgY antibodies as alternative antimicrobial agents to prevent and fight infections by *V. anguillarum* in aquaculture systems and set up the basis for further downstream refinement.

References

[1] Food and Agriculture Organization of the United Nations. The State of World Fisheries and Aquaculture. 2016.
[2] Novriadi R. Vibriosis in Aquaculture. OmniAkuatika. 2016;12(1):1–12.
[3] Baloch, A. R., Zhang, X. Y., & Schade, R. (2015). IgY Technology in aquaculture - a review. Reviews in Aquaculture, 7(3), 153–160

Acknowledgements

This work was developed in the scope of the projects Centro de Neurociências e Biologia Celular POCI-01-0145-FEDER-007440 (Ref. FCT UID/NEU/04539/2013) and CICECO-Aveiro Institute of Materials POCI-01-0145-FEDER-007679 (Ref. FCT UID/CTM/50011/2013), financed by national funds through the FCT –Fundação para a Ciência e a Tecnologia /MEC. M.E.Rosa and R. S. Vieira-Pires also acknowledge HBT - Saúde e Biotecnologia Lda as industry partner and FCT for the doctoral grant SFRH/BD/136995/2018 and FCT Investigator IF/00123/2013. M. G. Freire acknowledges European Research Council (ERC) for the Starting Grant ERC-2013-StG-3377.

Microbial Cell Factories for High Quality Biopharmaceuticals Production

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Abstract

Biopharmaceuticals, encompassing vaccines and protein therapeutics, comprise the fastest growing class of drug compounds on the pharmaceutical market. This growth has spurred innovation in manufacturing to improve product titers and reduce the upstream operation costs. There is now a proliferation of outstanding stories in the biotechnology industry where recombinant protein titers have been improved up to and beyond the 5 g/L level. Although further improvements in product titers are still achievable, a broader goal for host cell and protein engineering that encompasses product quality and consistency, in addition to high yield, has now an uncontrollable reality. Furthermore, it is likely that future cell culture engineering efforts will place greater emphasis on controlling structural and biological quality of the target recombinant product. So, this communication intends to reinforce the attention on the ability to understand, predict, and control biopharmaceuticals quality during the biosynthesis process and depict the recent developments and ongoing challenges to engineer cells (namely in bacteria, yeasts and mammalian cells) for high-quality membrane proteins production [1, 2].

References

1- Duarte *et al.*, Impact of glycerol feeding profiles on STEAP1 biosynthesis by *Komagataella pastoris* using a methanol-inducible promoter, *Applied Microbiology and Biotechnology* 105 (2021): 4635-4648.

2- Pedro *et al.*, Smoothing membrane protein structure determination by initial upstream stage improvements, *Applied Microbiology and Biotechnology* 103 (2019): 5483-5500.

Acknowledgements

This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. This work was also supported by UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences-UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy-i4HB.

Aliivibrio fischeri L -Asparaginase production in *Bacillus subtillis: from orbital shaker to stirred tank bioreactor*

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Abstract

L-Asparaginase (L-ASNase) is an enzyme widely used in the treatment of lymphoid malignancies and similar diseases, acting to lower the concentration of L-asparagine in the blood plasma by catalyzing the hydrolysis of L-asparagine, preventing the proliferation of cancer cells. Commercial L-ASNase used as biopharmaceutical are produced exclusively as recombinant enzymes from *Escherichia coli* and *Dickeya dadantii*. Although those formulations are efficient as oncological agents, they have some limitations that prevent a broader use, such as hyper sensibility, side effects and low yields triggering high costs. These issues have driven our research group to evaluate *Bacillus subtilis* as an expression platform for the recombinant *Aliivibrio fischeri* L-ASNase production. The enzyme heterologous production was studied in orbital shaker and Stirred tank bioreactor. The results showed that the enzyme was successful cloned and produced by engineered *B. subtilis*. Moreover, the results showed elevated L-ASNase activity produced on cultivation in orbital shaker and stirred tank biorreactor, indicating the potential of yield improvement under bioreactor cultivation. In this way, a new recombinant L-ASNase bioprocess is presented.

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Development of L-Asparaginase protein releasing systems for recombinant *Bacillus subtilis*

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Abstract

The DNA recombinant technique made it possible to produce new biomolecules and generate high production yields. Nevertheless, the purification of these molecules is still a challenge, especially if they remain in the intracellular environment, as is the case for most industrial enzymes. The L-Asparaginase is an important enzyme for the food and pharmaceutical industry. To facilitate the L-Asparaginase purification and reduce the process' costs, we aimed to develop and test two protein releasing systems in *Bacillus subtilis*. Our first construction was the L-Asparaginase secretion through the Sec system. To reach it, we created a signal peptide library. Testing the library showed us that the NucB peptide is the most efficient signal peptide for the protein secretion in *B. subtilis*. The second system we will develop is the programable cell lysis. This will be grounded on the Carbon Catabolite system. As the carbon source ceases in the final stage of the cultivation, the bacterium will produce an enzyme that lysis the cell releasing the intracellular content. Therefore, we will be able to determine the most effective L-Asparaginase protein releasing system for L-Asparaginase produced in recombinant *B. subtilis*.

References

TERWILLIGER, T.; et al. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. **Blood Cancer J**, v. 7, n. 6, 2017.

RAY, M.; et al. Isolation and Characterization of Microbial Asparaginase to Mitigate Acrylamide Formation in Food. **Advances in Plant & Microbial Biotechnology**, p. 95-100, 2019.

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Recombinant E6 protein biosynthesis and purification from *Escherichia coli* cells: a target for cervical cancer

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Abstract

Cervical cancer is the 4th leading cause of cancer death in women worldwide, arising from lesions caused by human papillomaviruses (HPVs), which have an oncogenic role that is linked to two oncoproteins, E6 and E7 [1]. High-risk HPV E6 interacts with E6AP, causing p53 degradation via the proteasome pathway. As a result, E6 has been used as a target in the development of anti-HPV drugs [2]. Nonetheless, large amounts of protein with high purity are required to conduct biointeraction studies with the selected drugs. Therefore, our goal was to recombinantly overexpress E6 and investigate different extraction/purification methods to isolate the protein in its native folding. E6 protein biosynthesis was developed using *Escherichia coli* in LB medium supplemented with different glucose concentrations and its induction was achieved by the addition of IPTG and zinc sulfate. To isolate the protein, it was explored different lysis methods. The results showed that E6 was successfully produced with 2 fusion tags MBP and His_6 and the best lysis method consisted of sonication/ice cycles. Finally, affinity chromatography was applied as the main capture step for the purification.

References

[1] Yeo-Teh NSL, et al. High-Risk Human Papillomaviral Oncogenes E6 and E7 Target Key Cellular Pathways to Achieve Oncogenesis. *Int J Mol Sci.* 19(2018)1706.
[2] Almeida AM, et al. Cervical cancer and HPV infection: ongoing therapeutic research to counteract the action of E6 and E7 oncoproteins. *Drug Discov Today*. 24(2019)2044.

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Recombinant laccase production by *Komagataella pastoris*

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Abstract

Laccases (EC 1.10.3.2) are multicopper enzymes that use molecular oxygen to catalyse the oxidation of several substrates, such as phenolic compounds and aromatic amines [1]. Laccases have multiple applications in the industry and biotechnology, such as bioremediation of wastewaters, paper pulp bleaching, wine clarification, organic synthesis, and polymerization reactions.

The most common natural source of laccase is fungi, in particular the white-rot fungus *Trametes versicolor*. However, the bioprocess using these microorganisms has several problems and limitations, such as the low amount of enzyme produced, and does not meet the actual industrial demand [2]. Genetically modified microorganisms have been used to produce a recombinant laccase. *Komagataella pastoris* is very promising due to its ease of genetic manipulations, rapid growth and ability to secrete recombinant proteins [3].

This work describes the production of recombinant laccase from *T. versicolor* using *K. pastoris* as a heterologous host under the control of the alcohol oxidase (AOX1) promoter. The expression in *K. pastoris* was successful and an extracellular recombinant laccase was produced. The fermentation conditions were optimized for factors influencing laccase expressions such as methanol concentration and copper concentration. The protein was characterized by SDS-PAGE and biological activity.

References

[1] P.J. Strong, H. Claus, Laccase: A Review of Its Past and Its Future in Bioremediation, Crit. Rev. Environ. Sci. Technol. 41 (2011) 373–434. https://doi.org/10.1080/10643380902945706.

[2] Q. Li, J. Pei, L. Zhao, J. Xie, F. Cao, G. Wang, Overexpression and characterization of laccase from Trametes versicolor in Pichia pastoris, Appl. Biochem. Microbiol. 50 (2014) 140–147. https://doi.org/10.1134/S0003683814020124.

[3] B.G. Ergün, P. Çalık, Lignocellulose degrading extremozymes produced by Pichia pastoris: current status and future prospects., Bioprocess Biosyst. Eng. 39 (2016) 1–36. https://doi.org/10.1007/s00449-015-1476-6.

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Bioprocess integration of Interferon alpha 2b using Pluronics®

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Abstract

Bioprocess integration is essential to design competitive and more sustainable manufacturing processes than those used nowadays. This can be achieved using aqueous biphasic systems (ABS), namely by combining the biopharmaceuticals upstream and downstream steps (*e.g.* clarification and concentration) [1]. Using the GRAS organism *Komagataella pastoris* for the extracellular expression of interferon α-2b (IFNα-2b), this work aims to develop an integrated bioprocess using ABS composed by Pluronics®. Preliminary results indicate that the supplementation of the culture medium with Pluronics® highly enhances the extracellular expression of monomeric IFNα-2b, outperforming the effect of polysorbate-20, currently applied to this end. Then, the partition of IFNα-2b from *K. pastoris* broth was evaluated in ABS composed by Pluronics® and salts or ionic liquids, being found that it was preferentially partitioned toward the bottom phase in Pluronic® L31-cholinium chloride-based ABS. Ongoing work is focusing on evaluating the partitioning of biomass in the selected systems and evaluate the effect of ABS phase-forming components on cell viability, both deemed crucial to achieve the ultimate goal of creating an integrated bioprocess.

References

[1] F. Magalhães, A.P.M. Tavares, M.G. Freire, Advances in aqueous biphasic systems for biotechnology applications, *Curr Opin Green Sustain Chem* 27: 100417. 2021.

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