July 15-18
2025

2025 Biotec

XIX Congress of the Spanish Society of Biotechnology

Faculty of Biosciences. Universitat Autònoma de Barcelona

Abstracts of communications

ASE BIO Asociación Española de Bioempresas

ADM Biopolis Tailor-made Biotechnology

sebiot Sociedad Española de Biotecnología

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

LETTER FROM THE PRESIDENT

WELCOME TO BIOTEC 2025

The Organizing and Scientific Committee are pleased to welcome you to the **XIX Congress of the Spanish Society of Biotechnology, BIOTEC 2025**. As you know, this congress will take place in Barcelona, at the Faculty of Biosciences of the Universitat Autònoma de Barcelona, from July 15 to 18, 2025. For the Spanish Society of Biotechnology, it is a great pleasure to hold this new edition of BIOTEC in person.

The National Biotechnology Congresses (BIOTEC), organized by SEBiot since 1986, represent a great opportunity for researchers and professionals in the biotechnology sector to establish connections and collaborations with other colleagues, as well as with other stakeholders in this field, from private companies to public administrations and other national organizations.

The scientific program of the congress is aimed at providing updates on a wide range of topics of interest by leading experts, as well as offering opportunities for young researchers to share their progress with the scientific community.

It is both important and rewarding to see the discussions that arise in the poster area between PhD students, master's students, and senior researchers as they share their results and seek solutions to scientific problems. But it won't all be work; we also wanted to include some fun moments that will allow us to continue discussing biotechnology and generate new collaborations. For this, we have planned a welcome cocktail, a gala dinner, and some other surprises.

Finally, we would like to thank you for your attendance and participation in BIOTEC 2025. We hope you enjoy the meetings, presentations, debates, and, ultimately, the Congress.

María José Hernáiz
Spanish Biotechnology Society President

sebiot
Sociedad Española de Biotecnología

continue –>

July 15-18
2025

Biotec 2025

XIX Congress of the Spanish Society of Biotechnology

Faculty of Biosciences.
Universitat Autònoma de Barcelona

COMMITTEES

HONOURARY COMMITTEE

H.E. Minister of Science, Innovation and Universities **Diana Morant**

Honourable Minister of Research and Universities **Núria Montserrat**

Honourable Rector of the UAB **Javier Lafuente**

Director General Biocat/Bioregion **Robert Fabregat**

Ilm. Degà Biosciences **Isidre Gibert**, Director of the School of Engineering **Ian Blanes** and Ilm. Degà Sciences **Juan José Donaire**

EXECUTIVE COMMITTEE

Dra. María José Hernáiz Gómez
Degano, Facultad de Farmacia, Universidad Complutense de Madrid

Dra. Auxiliadora Prieto Jiménez
Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC)

Dra. María Francisca Vicente Pérez
Asesora Científica

Dra. Asunción Longo González
Escola d'Enxeñaría Industrial. Universida de Vigo

Dr. Francisco Valero Barranco
Escola d'Enginyeria. Universitat Autònoma de Barcelona

LOCAL COMMITTEE

D. Francisco Valero

Dr. Francesc Gòdia

Dra. Montserrat Sarrá

Dra. Marina Guillén

Dr. Óscar Enrique Romero

Dr. Xavier García-Ortega

Dr. Arnau Gasset

Departament d'Enginyeria Química, Biològica i Ambiental. Escola d'Enginyeria. Universitat Autònoma de Barcelona

continue –>

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

COMMITTEES

SCIENTIFIC COMMITTEE

Dr. Baltasar Mayo. Instituto de Productos Lácteos de Asturias (IPLA-CSIC)

Dr. Enrique Samper. Cheaf Businees and transformation officer Life Length

Dra. Fátima Al-Shahrour. Centro Nacional de Investigaciones Oncológicas (CNIO)

Dra. Mar Castellano. Centro de Biotecnología y Genómica de Plantas (UPM-INIA-CSIC)

Dr. Pedro Lozano. Facultad de Química. Universidad de Murcia

Dra. Eva María Martín del Valle. Facultad de Ciencias Química. Universidad de Salamanca

Dr. Juan Manuel Bolívar. Departamento de Ingeniería Química y materiales (UCM)

Dr. Xavier García-Ortega. Departament d'Enginyeria Química, Biològica i Ambiental (UAB)

Dra. Silvia Osuna. Departamento de Química (Universitat de Girona)

Dr. Fernando López Gallego. Heterologous biocatalysis laboratory (CIC biomaGUNE)

Dra. Susana Velasco. Instituto de síntesis química y catálisis heterogéne (Universidad de Zaragoza - CSIC)

Dr. Marcos Egea. Departamento de Ingeniería Agronómica (UPCT)

Dra. Marta Pérez. Centro de Biología y genómica de plantas (UPM)

Dra. Carmen Michán. Departamento de Bioquímica y Biología Molecular (U. Córdoba)

Dra. Isabel Pardo. Biotecnología de polímeros (CIB-CSIC)

Dr. Enrique Martínez Force. Departamento de Bioquímica y Biología molecular de productos vegetales. (I. Grasa-CSIC)

Dra.Eva García Ruíz. Grupo de Biología y Biocatálisis (ICP-CSIC)

Dra. Natalia Hernández Herreros. Biotecnología de polímeros (CIB-CSIC)

Dr. Ricardo Macarrón. VP Myeloid Diseases-Biology at Incyte)

Dra. Celia Nieto Jiménez. Departamento de Ingeniería Química y textil (Universidad de Salamanca)

Dr. Víctor González Menéndez. Fundación Medina

Dr. Carlos Barreiro Méndez. Departamento de Biología Molecular (Universidad de León)

Dr. Josu López Fernández. Consultor FI Group

continue –>

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

SPONSORS

Protective Partners

DIAMOND

PLATINUM

GOLD

continue –>

July 15-18
2025

2025 Biotec

XIX Congress of the Spanish Society of Biotechnology

Faculty of Biosciences.
Universitat Autònoma de Barcelona

INDEX

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

BIOCATALYSIS FOR SUSTAINABLE BIOSURFACTANTS: ENZYMATIC INNOVATIONS IN RHAMNOLIPID SYNTHESIS

María José Hernáiz Gómez-Dégano
Complutense University

Abstract

Rhamnolipids (RLs) are glycolipid biosurfactants of increasing industrial interest due to their excellent surface activity, biodegradability, and bioactive properties, including antibacterial, antibiofilm, and anti-quorum sensing effects. However, large-scale production is still constrained by high costs and low yields.

Enzymatic synthesis has emerged as a sustainable alternative to microbial fermentation, offering advantages such as high regioselectivity, reduced environmental impact, and greater process flexibility. This presentation highlights recent developments in the enzymatic synthesis of rhamnolipids and related glycolipids using Pseudomonas stutzeri lipase, with a focus on improving efficiency, selectivity, sustainability, and scalability.

Innovative approaches include the application of green solvents, alternative energy sources such as mechanochemistry, microwave irradiation, and ultrasound, all of which significantly accelerate reaction rates while preserving high conversion and regioselectivity. Enzyme immobilization on various supports further enhances catalytic activity, stability, and reusability. Additionally, molecular modeling techniques, such as docking and molecular dynamics, offer valuable insights into substrate binding and enzyme selectivity, facilitating process optimization.

Beyond synthesis, the biological activity of the resulting glycolipids is also explored. The synthesized rhamnose esters demonstrate strong antibacterial, antibiofilm, and anti-quorum sensing activity against both Gram-positive and Gram-negative pathogens. These findings underscore the potential of biocatalytic strategies for the eco-friendly and cost-effective production of functional biosurfactants, with promising applications in pharmaceutical, cosmetic, and environmental fields.

Financial support and acknowledgments

TED2021-130430B-C21, PDC2022-133817-I00 and PID2023-150195OB-I00

Keywords

Rhamnolipids • Lipases • Green Solvents • Sustainable Energy Sources • Immobilization of Enzymes • Molecular Modelling Studies • Biological Evaluation

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

IMMOBILIZE TO GO FURTHER: FROM THE DESIGN TO THE INTEGRATION OF IMMOBILIZED BIOCATALYSTS

David Roura Padrosa
inSEIT AG

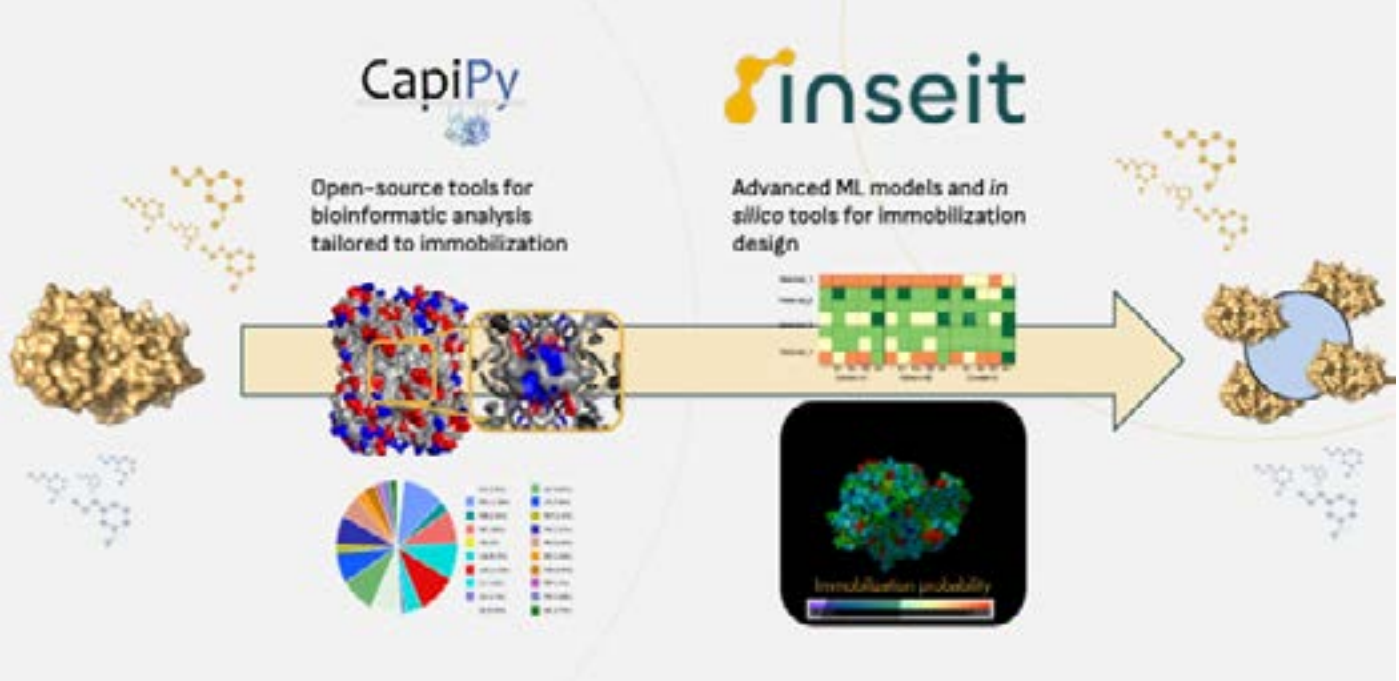
Abstract

Industrial biocatalysis continues to evolve with the integration of computational biology and in silico methods, aiding in the discovery and optimization of biocatalysts. From storing and deciphering vast amounts of genetic data to designing and studying macromolecules such as proteins, all fields have benefited from this revolution. In the field of structural biology and enzyme engineering, for example, there is a continuous development of novel tools and better algorithms for the evolution and design of enzymes, however, the application of such tools to enzyme immobilisation is very limited.

To bridge this gap, in 2020, we developed CapiPy, the first iteration of a series of bioinformatic tools that allow experienced scientists to perform simple analyses of the protein of interest, to better plan and understand the results of its immobilization. CapiPy has been used both a priori and a posteriori to design improved immobilized enzymes. Furthermore, as a natural evolution of CapiPy, we at inSEIT have expanded the software's capabilities. The novel iteration is capable of performing deeper analyses, and we have integrated machine learning technologies into the design process. Through this approach, we have been able to enhance previous immobilized biocatalysts and create novel ones that have been successfully applied for the synthesis of a variety of synthons.

Keywords

enzyme immobilization • bioinformatics • biocatalysis



July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

KEYNOTE COMMUNICATIONS

Biocatalysis

DE LA NATURALEZA A LA INTELIGENCIA ARTIFICIAL: DISEÑANDO LA BIOCATÁLISIS DEL FUTURO

Manuel Ferrer[1]; Paula Vidal[1]; Laura Fernández-López[1]; Ana Robles-Martín[2]; Rubén Muñoz-Tafalla[2]; Joan Giménez-Dejoz[2]; Rafael Bargiela[1]; Víctor Guallar[2]

1. Instituto de Catalisis y Petroleoquimica (ICP), CSIC, Instituto de Catalisis y Petroleoquimica (ICP), CSIC , Instituto de Catalisis y Petroleoquimica (ICP), CSIC; 2. Barcelona Supercomputing Center (BSC)

Abstract

En un mundo que avanza hacia un desarrollo sostenible, la biocatálisis se posiciona como una herramienta estratégica para abordar muchos de los retos recogidos en los Objetivos de Desarrollo Sostenible (ODS) de las Naciones Unidas. A través del uso de enzimas y sistemas biológicos, ya sean naturales o diseñados, es posible transformar residuos, reducir el consumo energético y minimizar el uso de sustancias peligrosas, todo ello con un impacto medioambiental significativamente menor que los métodos químicos convencionales. La catálisis enzimática permite operar a temperaturas y presiones más suaves, con una notable eficiencia incluso en presencia de disolventes orgánicos o líquidos iónicos. Sus aplicaciones abarcan desde la producción de biocombustibles y bioplásticos, hasta la captura de carbono, la gestión de residuos urbanos o la degradación de plásticos persistentes, contribuyendo directamente a los ODS. Sin embargo, para maximizar su impacto global, es necesario diseñar sistemas biocatalíticos más potentes, flexibles y personalizados. Sin embargo, esta investigación se enfrenta a varios desafíos. Por un lado, la identificación de nuevas enzimas y organismos robustos sigue siendo limitada por la complejidad y coste del cribado experimental. Por otro, los sistemas biocatalíticos existentes, aunque funcionales, necesitan mejoras significativas para operar con eficacia en contextos industriales reales. En este sentido, la convergencia entre la biología y la inteligencia artificial está abriendo nuevas vías para acelerar el descubrimiento, diseño y optimización de enzimas y microorganismos mediante técnicas como la predicción estructural, el modelado funcional y la edición del genoma. Esta charla abordará el camino que va desde la exploración de la biodiversidad natural hasta la construcción de sistemas biocatalíticos sintéticos. Presentaré cómo la integración de herramientas ómicas, la bioinformática y los avances en inteligencia artificial, como AlphaFold2, que permite identificar y generar miles de estructuras tridimensionales precisas, junto con técnicas de deep learning, simulaciones computacionales y algoritmos basados en IA, combinadas con innovadoras herramientas de biología sintética, nos permite avanzar hacia una biocatálisis del futuro: más eficiente, más adaptable y alineada con los Objetivos de Desarrollo Sostenible. En definitiva, propondré una visión de la biotecnología moderna como una disciplina que, al combinar el conocimiento biológico con las tecnologías digitales, puede liderar la transición hacia una sociedad verdaderamente sostenible. Las publicaciones a resumen algunas de nuestras herramientas y aplicaciones más relevantes en el campo.

Financial support and acknowledgments

Los autores agradecen la financiación de la Unión Europea (H2020, proyecto 101000327-FuturEnzyme; Horizonte Europa, proyecto 101060625-Nymphe; HORIZON-MSCA-2022-PF-01, proyecto 101104264-BIO DEGRADE), del Ministerio de Ciencia, Innovación y Universidades y de la Agencia Estatal de Investigación (MICIU/AEI/10.13039/501100011033), así como de los fondos FEDER, la Unión Europea y el instrumento NextGenerationEU/PRTR. También se agradece el apoyo del Ministerio de Ciencia e Innovación mediante la ayuda PRE2020-091825, y del Programa de Atracción de Talento Investigador “César Nombela” (ayuda 2024-T1/ECO-31227), de la Consejería de Educación, Ciencia y Universidades de la Comunidad de Madrid.

ENGINEERING ARTIFICIAL METALLOENZYMES: PROTEIN-METAL HYBRIDS FOR TUNABLE BIOCATALYSIS

Aitziber L. Cortajarena
CIC biomaGUNE

Abstract

Proteins, with their exceptional structural versatility, biocompatibility, and biodegradability, are emerging as powerful tools in both biomedicine and technology. We focus on engineered repeat proteins, a class of proteins known for their stability, robustness, and ability to be tailored for specific functions and supramolecular assembly properties. This work focuses on engineered protein assemblies and protein–nanomaterial hybrids for applications in sustainable catalysis.

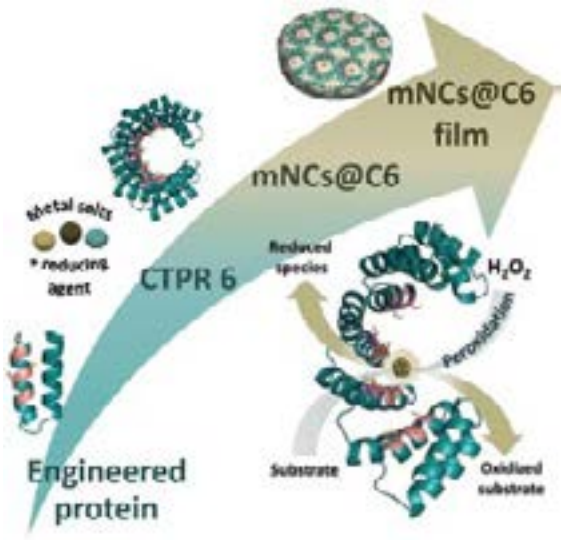
We have developed strategies to create ordered protein-based biomaterials by re-engineering protein-protein interactions. Leveraging these self-assembly properties, we have created spatially organized multi-enzyme systems by programming spatial organization into protein scaffolds. These assemblies enhance substrate channeling and cofactor recycling, resulting in significantly higher catalytic efficiency. Furthermore, by incorporating metal-binding residues such as histidines and cysteines, we can drive metal coordination, leading to the formation of tailored protein-nanomaterial composites, particularly nanozymes and artificial metalloenzymes, exhibiting a diverse range of catalytic activities and processable into solid films for increased reusability. Additionally, we are exploring engineered artificial metalloenzymes (ArMs) with abiotic metal cofactors, to catalyze new-to-nature reactions. Tailored immobilization on solid supports enables their use as heterogeneous biocatalysts. In addition, the catalytic properties of these protein-nanomaterial hybrids are being exploited in applications beyond catalysis, such as the development of advanced sensors. Altogether, these strategies highlight the power of engineered protein assemblies and protein–nanomaterial hybrids to act as versatile catalytic platforms.

Financial support and acknowledgments

PDC2021-120957-I00 PID2022-137977OB-I00

Keywords

Protein engineering • Artificial metalloenzymes • Nanozymes|Biocatalysts • Multi enzymatic pathways • Protein scaffolds • Catalytic biomaterials



TOWARD MORE BALANCED WINES: MICROBIAL CONTROL IN TIMES OF CLIMATE CHANGE

Mª Jesús Torija Martínez
Department of Biochemistry and Biotechnology, Faculty of Oenology. Universitat Rovira i Virgili. Tarragona, Spain

Abstract

In recent decades, climate change has posed new challenges in winemaking, primarily related to the increase of sugar concentration in grapes, which leads to wines with higher alcohol content and, in some cases, diminished quality. As a result, reducing ethanol levels in wine has become a key objective in most wine-producing regions worldwide. To address this issue, researchers have explored various strategies, with microbiological approaches standing out due to the metabolic diversity of wine yeasts. One promising strategy involves the use of selected non-Saccharomyces yeasts as fermentation starters, either through co-inoculation or sequential inoculation with Saccharomyces cerevisiae. These mixed fermentations not only have the potential to lower ethanol content but also to enhance the wine's aromatic complexity. Many combinations of S. cerevisiae and non-Saccharomyces strains have been tested to optimize these effects. However, to achieve consistent results, a better understanding of how these yeasts behave and interact during fermentation is essential. These microbial interactions can be either synergistic or antagonistic, with negative effects often arising from competition for key nutrients (like nitrogen, vitamins, or metal ions) or from the production of compounds that suppress or inhibit competing yeasts. In parallel, the bioactive compound melatonin has emerged as a molecule of interest in wine microbiology. Known for its antioxidant properties, melatonin has been detected in several fermented beverages, including wine. Yeasts can synthesize melatonin during alcoholic fermentation; however, its intracellular role and biosynthetic pathway remain poorly understood. Recent studies have shown that melatonin interacts with proteins involved in the glycolytic pathway, but only in Saccharomyces and certain non-Saccharomyces species with high fermentative capacity. In contrast, such interactions are absent in less fermentative yeasts, and no melatonin-protein binding is observed during respiratory metabolism, even in strains that accumulate intracellular melatonin. Notably, when cells switch from respiration to fermentation, melatonin-protein interactions reappear rapidly. This supports the hypothesis that melatonin plays a regulatory role specifically associated with fermentative metabolism and sugar catabolism.

Further investigation into yeast-yeast interactions, species-specific carbon metabolism, and the functional role of melatonin is essential for optimizing microbial management strategies and the production of more balanced wines in the context of climate change.

Financial support and acknowledgments

The work was supported by the projects PID2019-108722RB-C33 and PID2022-137807OB-C21 (MCIN/AEI/FEDER, UE/ https://doi.org/10.13039/501100011033).

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

PROS AND CONS OF ADAPTIVE EVOLUTION FOR IMPROVEMENT AND DIVERSIFICATION OF DAIRY STARTERS

Beatriz Martínez
IPLA-CSIC

Abstract

Lactococcus lactis and Lactococcus cremoris are key components of mesophilic starter cultures widely used in the dairy industry, primarily for cheese production. As dairy starters, their main role is to efficiently metabolize lactose—the predominant sugar in milk—and produce lactic acid, thereby lowering the pH to enable milk clotting and inhibit the growth of spoilage microorganisms and food-borne pathogens. These bacteria also contribute to the development of organoleptic properties that, together with manufacturing practices, impart a distinctive signature to the final product. In addition, certain strains are being explored as alternative cell factories for the production of high-value compounds.In both fermentation and biotechnological applications, the viability and metabolic performance of these starters are critical and there is growing demand for robust strains capable of withstanding stress, while also offering novel traits to enhance competitiveness and productivity. Adaptive evolution has emerged as a promising strategy to meet these objectives.In this talk, I will present examples of adaptive evolution that illustrate its potential from both academic and industrial perspectives. This approach has shed light on the domestication process of L. lactis, tracing its transition from plant-associated environments to dairy niches. Moreover, applying adaptive evolution under specific stress conditions has led to the selection of strains with improved phenotypes. In particular, adaptive evolution under cell envelope stress (AE-CES)—using a cell wall-active bacteriocin as the selective pressure—has been applied to both wild isolates from dairy environments and industrial starters. The results highlight the remarkable plasticity of L. lactis in deploying diverse strategies to mitigate cell envelope damage. Notably, AE-CES generated evolved clones that retained key technological traits, while acquiring additional beneficial phenotypes.However, this approach also revealed limitations, especially when applied to strains carrying critical plasmid-encoded functions. In some cases, traits such as lactose fermentation and phage resistance were lost. Despite these drawbacks, the phenotypic diversity obtained through AE-CES demonstrates that it is a viable method for introducing new characteristics into industrial L. lactis dairy starters.

Financial support and acknowledgments

Grant PID2023-149852OB-I00 funded by MCIU /AEI /10.13039/501100011033 / FEDER, UEGrant IDE/2024/000721 funded by AGENCIA SEKUENS (Gobierno de Asturias) and FEDER, EU.

Keywords

Dairy starters • strain improvement • adaptive evolution

ENZYMATIC MODIFICATION OF POLYPHENOLS TO OPTIMIZE THEIR PHYSICOCHEMICAL AND BIOACTIVE PROPERTIES

Jose Luis González-Alfonso[1]; David Rodríguez-García[1]; Irene Paz-Mouriño[1]; Mercedes Moreno[1]; Carlos Uceda[1]; Laura Barahona[2]; Cristina Alonso[3]; Luisa Coderch[3]; Tom Desmet[4]; María Fernández-Lobato[2]; Francisco J. Plou Gasca[1]

1. ICP-CSIC; 2. CBMSO-CSIC; 3. IQAC-CSIC; 4. Ghent University

Abstract

Polyphenols are a group of compounds found in plants, which have health benefits for humans. They can act as antioxidants, reduce inflammation, and may even help protect against cancer or support brain health. These qualities make them very interesting to people studying nutrition and wellness. Polyphenols are common in foods like fruits, vegetables, tea, coffee, and wine. They are also present in the byproducts from farming and food processing. For example, olive pomace, which is the material left after making olive oil, contains polyphenols such as hydroxytyrosol, oleuropein, and tyrosol. These could be used in health foods, supplements, cosmetics or medicines. Similarly, grape pomace, the residue from winemaking, has polyphenols like resveratrol, catechins, and quercetin. These could be turned into valuable products. Using these byproducts not only creates useful items but also reduces waste. In this context, our group participates in a European project called LIFE CYCLOPS (http://life-cyclops.eu), which explores the extraction of polyphenols from the waste of olive oil and wine industries. Another example is apple pomace, left after making apple juice. It contains phloretin, a polyphenol that has antioxidant, anti-inflammatory, antidiabetic and anti-cancer effects. Phloretin is also studied for use in skincare products.The main problem with polyphenols in foods is that the body does not absorb them very well. Only a small amount of what people eat actually gets into the bloodstream to provide benefits. Changing the structure of polyphenols can be a way to improve this. For example, a glucosyl moiety can be added to a polyphenol, a process called glucosylation. This makes the polyphenol dissolve better in water and stay stable, so it is easier to use and absorb. Another method, called acylation, adds an acyl group to the polyphenol. This makes it better at mixing with fatty substances, which can help it pass through the body's barriers more easily. In this study, we share results from using enzymes to modify different polyphenols through glucosylation and acylation. The enzymatic reactions worked with high yield. These methods are also practical for use in large-scale production. Additionally, we studied the effect of the modifications on physicochemical and bioactive properties.

Financial support and acknowledgments

This work was supported by the projects (1) PDC2022-133134-C21/C22 "ACYLGLUFLAV_APP" MCIN/AEI/ 10.13039/501100011033 from "European Union NextGenerationEU/PRTR". (2) Grant 101074544 "LIFE Program" (LIFE21-ENV-ES-CYCLOPS) funded by European Union. (3) PID2022-136367OB-C31/-C32 "GLYCOENZ-GREEN" from MCIN/AEI/10.13039/501100011033 and FEDER, a Way of Making Europe.

Keywords

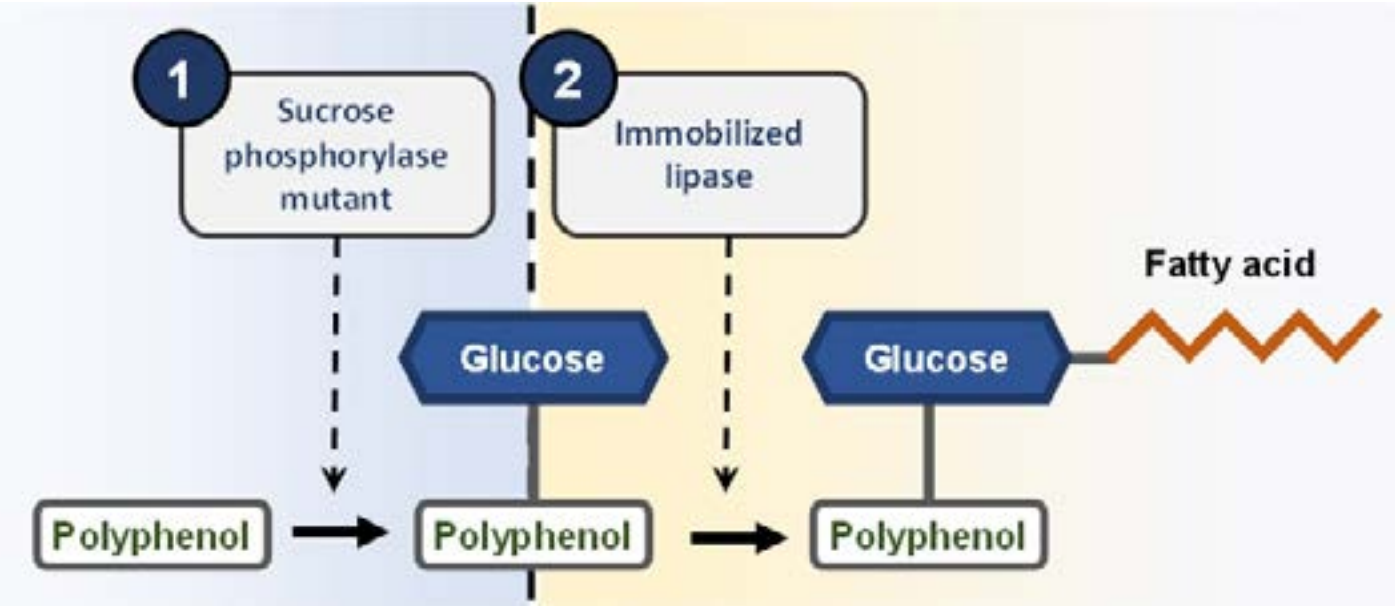
Polyphenols • Glycosidases • Glucosylation • Acylation • Lipases • Food Industry • Byproducts

continue –>

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

KEYNOTE COMMUNICATIONS

Food Biotechnology



July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

APPLIED BIOTECHNOLOGY: TECHNOLOGY TRANSFER IN HIGH-VALUE CULTURES

Veronica Romero Gil
OLEICA

Abstract

Applied biotechnology plays a key role in improving high-value starter crops, not only in terms of productivity but also in sustainability and resilience to climate change. This talk will address current strategies and practical cases of technology transfer from research centers to the productive sector, with an emphasis on starter crops with high economic and nutritional value. It will analyze public-private collaboration models, regulatory challenges, and biotechnological tools such as selection, imposition, and application across different sectors. Additionally, specific experiences will be presented that have enabled the effective adoption of these technologies in local contexts, promoting innovation and advancement in the food sector. The presentation aims to open a space for reflection on how to accelerate technology transfer to positively impact strategic value chains.

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

mABXIENCE: HIGH QUALITY, ACCESSIBLE AND AFFORDABLE MEDICINES FOR THE WORLD

Ricardo Vicente Ullán
mAbxience. Manufacturing Supervisor

Abstract

mAbxience Biotechnology Company mAbxience is a leading vertically integrated biopharmaceutical company, committed to improving patient access to treatments worldwide. Our in-house, core R&D competencies set us apart in the development of biosimilars and innovator biologics.

We provide end-to-end services in the B2B biopharmaceutical space, ensuring everything we deliver worldwide is world-class. From R&D expertise in biologics, specialized in biosimilars. We have over a decade of experience in the development and commercialization of biopharmaceuticals, supporting access to high-quality medicines. We incorporate cutting edge technology in our development and manufacturing platform across three Biologic Drug Substance (BDS) facilities and our partner filling sites. Our facilities are spread across two continents (Europe and America), with single-use bioreactors offering a combined capacity of 50,000L and great flexibility, from R&D batches to commercial batches.

mAbxience Holding - mAbxience Research SL (Madrid, Spain).- mAbxience SA (Lugano, Switzerland).- Three GMP multi-product plants: Genhelix (Spain). Up to 24.000L Cell Culture Capacity (1st line – 6 x 2000L and 2nd line – 3 x 4000L). Garín (Argentina). Up to 24.000L Cell Culture Capacity (1st line – 6 x 2000L and 2nd line – 6 x 2000L). Munro (Argentina). Installed Capacity: 2 x 1000L mAbxience PipelinemAbxience developed and commercialized two biosimilar medicines for oncology, available to thousands of patients across the globe via our B2B partnerships in worldwide markets: MB01 rituximab biosimilar and MB02 bevacizumab biosimilar, since 2014 and 2016 respectively. Now we have a highly attractive pipeline of biosimilar and innovative biopharmaceuticals targeting areas of oncology, hematology, osteoporosis and pediatrics. Our development of biopharmaceuticals adheres to a strict “quality by design” standard using the latest innovations in upstream, downstream and cutting edge-analytics. We guarantee all biosimilars are equivalent in terms of quality, safety and efficacy to the reference products, by subjecting the drugs to exhaustive comparability testing and clinical trials, regulated by the competent authorities. Nowadays mAbxience has six biosimilars under development.mAbxience CDMO (Contract Development and Manufacturing Organization)

Thanks to our independent CDMO platform, we provide integrated services adapted to your requirements: from research batches to commercial batches, big or small. We tailor solutions to meet our partners’ needs.

mAbxience ValuesmAbxience was born from a desire to bring hope to as many patients as possible. We are also guided by a quality philosophy that permeates everything we do. We are committed to producing world-class biopharmaceutical products through optimized processes and by working closely with our partners.

Keywords

mAbxience • Biopharmaceutical company • Biosimilars • Single-Use Bioreactors • CDMO

HOW FUNGI WILL SAVE US FROM THE CLIMATE CHANGE APOCALYPSE

Pablo Cruz-Morales
Technical Univeristy of Denmark, NNF Center for Biosustainability

Abstract

For about 150 years industrial chemistry has revolved around petroleum. Our transportation, food, and health are dangerously linked to this non-renewable resource that will be depleted in about 50 years. The world urgently needs new chemical processes that can support us sustainably.

Fungi are extraordinary chemists; they make molecules for competition, attack, defense, communication, deception, energy-storage, and structure-building. We study fungal chemistry to learn how to use it to make polymers, drugs, agrochemicals, fuels for aviation, rocketry, and shipping.

Our work involves collecting and cultivating fungi, usually complex species with limited knowledge available. We look at the natural chemicals they produce, and decode the chemical recipes stored in their DNA. We then transfer the DNA-written recipes to simpler, laboratory-friendly fungi who can then make the chemicals. We modify the recipes to create variants of the natural chemicals, iterating this process until we get molecules suitable for human needs.I this talk I will present our fungal chemical discovery and engineering platform and some of their potential applications.

Financial support and acknowledgments

Work at my lab is supported by the Novo Nordisk Foundation

Keywords

Fungi • sustainability • biosynthesis • Biomanufacturing

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

EXTRACYTOPLASMIC FUNCTION (ECF) SIGMA FACTORS IN THE RESPONSE OF PSEUDOMONAS AERUGINOSA TO ENVIRONMENTAL AND HOST STIMULI

Marian Llamas
Estación Experimental del Zaidín-Consejo Superior de Investigaciones Científicas

Abstract

Extracytoplasmic function sigma (sECF) factors are signal response proteins that control bacterial gene expression at the level of transcription initiation by modifying the affinity of RNA polymerase (RNAP) for DNA. Due to its abundance, diversity and importance, sECF-signalling represents one of the major signal transduction mechanisms in bacteria. These proteins are abundantly present in bacteria with complex lifestyles able to survive and colonize many different environments, like Pseudomonas aeruginosa. This environmental bacterium can be found in water, soil and the rhizosphere of plants. However, its relevance is related with being an opportunistic pathogen of humans where it causes a wide array of life-threatening acute and chronic infections. Among other problems, P. aeruginosa is a leading cause of hospital-acquired infections and the main reason for lung function deterioration and mortality in cystic fibrosis patients. P. aeruginosa infections are often life-threatening and difficult to treat because this bacterium is intrinsically resistant to multiple antibiotics and can easily acquire new resistances. Due to its global spread and increasing number of antimicrobial resistances (AMR), this bacterium is one of the WHO priority pathogens requiring research and development of new eradication strategies. Signalling through sECF factors is extensively present in P. aeruginosa and represents a main adaptation strategy for this pathogen in the host by regulating processes as important during infection as iron acquisition, cell wall stress responses, and the production of virulence factors. Because the ability of the pathogen to sense and adapt to the host is critical during infection, interference with signalling mechanisms involved in this process represents a promising strategy to fight bacterial pathogens. We have identified three proteases required for activation of sECF factors signalling cascades in P. aeruginosa. Because proteases are known to be druggable proteins, regulatory proteases involved in modulation of the activity of signal transduction systems represent excellent drug targets, a concept that we are looking to exploit.

Financial support and acknowledgments

This work has been funded by MCIN/AEI/10.13039/501100011033 Spanish agency withprojects PID2020-115682GB-I00 and PID2023-149253NB-I00.

Keywords

Pseudomonas aeruginosa • sigma factor • gene regulation • signalling|iron acquisition • virulence • proteolysis • antimicrobial resistance • therapeutic target

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

MICROBIAL COMMUNITIES AND CONTAMINANTS: CHALLENGES OF FUNCTIONAL ANALYSIS

Balbina Nogales; Theo Obrador-Viel; Alberto Contreras-Moll; Justine M. Bitalac; Rocío D.I. Molina; M. Mar Aguiló-Ferretjans; Rafael Bosch; Joseph A. Christie-Oleza
Universitat de les Illes Balears

Abstract

Despite significant advances in our understanding of how microorganisms degrade environmental pollutants, inferring catabolic functions and identifying key degraders from metagenomic data remains a complex task. This approach faces several challenges. For instance, in the case of aromatic compounds, it is difficult to distinguish genes involved in pollutant degradation from those related to the metabolism of naturally occurring aromatic compounds. Moreover, some enzymes—such as reductases, decarboxylases, and others—may play roles in pollutant degradation but are often overlooked in sequence homology-based analyses because they have not been associated with characterized hydrocarbon degradation pathways. The challenge is even greater when investigating plastic degradation. The identification of plastic-degrading organisms and the functional annotation of relevant genes are hindered by the limited availability of well-characterized degradation pathways and the difficulty in reliably assessing degradation potential. These limitations underscore the need to isolate relevant environmental strains. Such isolates are crucial not only for elucidating the metabolic pathways underlying plastic degradation but also for providing valuable insights into the microorganisms and mechanisms responsible for plastic breakdown in natural environments.

Financial support and acknowledgments

PID2022-139042NB-I00 funded by MCIN/AEI /10.13039/501100011033TED2021-129739B-I00 funded by MICIU/AEI /10.13039/501100011033 and the EU NextGenerationEU/ PRTRPDC2022-133849-I00 financiado por MICIU/AEI /10.13039/501100011033 y por la Unión Europea Next GenerationEU/ PRTR

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

NANOSTRUCTURED DRUG DELIVERY SYSTEMS FOR TARGETED ANTIMICROBIAL THERAPY

Manuel Arruebo
1. Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, Zaragoza, Spain; 2. Department of Chemical Engineering. University of Zaragoza, Campus Río Ebro-Edificio I+D, Zaragoza, Spain

Abstract
Despite all the awareness campaigns and the continuous recommended reduction in antibiotic prescriptions and use, antimicrobial resistance (AMR) remains in the top ten global public threats for human health, food security, and development. The most recent global analysis of AMR funded by the Wellcome Trust Foundation and the UK Department of Health and Social Care predicts that 39 million people will die from AMR between 2025 and 2050. This is equivalent to three deaths every minute [1].Nanotechnology has contributed significantly to the development of therapeutic and diagnostic systems used in the reduction of AMR infections. As alternatives to antibiotics, several nanoparticulated systems show intrinsic antiseptic effects that combine multiple mechanisms of antimicrobial action reducing the chances for bacteria to develop resistance. The combination of antibiotics or the combination of other antimicrobials (e.g., lytic enzymes) interfering with different targets in bacteria or the use of immunonanoparticles directed against specific receptors in bacteria are also different approaches to reduce AMR. Having some of those nanoparticulated antimicrobial systems growth-independent and non-specific multiple bactericidal modes of action, mainly by membrane disruption and oxidative stress generation, the chances for bacteria to develop AMR are minimized. We have evaluated the antimicrobial action of several of those systems against pathogenic resistant bacteria in their planktonic, sessile, and also when infecting intracellularly (e.g., small colony variants of Staphylococcus aureus) eukaryotic cells. Some of those systems are also benchmarked against the standard care using an excisional wound-splinting model in SKH1 hairless mice to evaluate their effectiveness when treating infected topical wounds.

Financial support and acknowledgments
The author acknowledges the Spanish Ministry of Science and Innovation (grant numbers PID2020-113987RB-I00 and PID2023-146091OB-I00) for funding, and for the Severo Ochoa Programme for Centers of Excellence in R&D (CEX2023-001286-S MICIU/AEI/10.13039/501100011033). I also thank the financial support from Instituto de Salud Carlos III (Spain; Fortalece Call, grant number FORT23/00028).

Keywords
Antimicrobial resistance • Nanoparticles • Nanomedicine • Drug delivery • Bacteria

CHALLENGES ADDRESSED IN THE DEVELOPMENT OF IMMUNOBIOGRAM: A NOVEL IVD PLATFORM OF CELLULAR TECHNOLOGY THAT APPLIES THE PARADIGM OF PRECISION MEDICINE TO THE PERSONALIZATION OF IMMUNOSUPPRESSIVE DRUGS

Isabel Portero
CEO & Founder, BIOHOPE SL

Abstract

The clinical management of immunosuppression in immunology remains largely empirical, lacking tools to predict individual pharmacodynamic responses to immunosuppressive agents. The Immunobiogram is a novel in vitro diagnostic (IVD) platform based on functional immune cell profiling, designed to personalize immunosuppressive therapy by integrating principles of Precision Medicine. The development and validation of this technology involved addressing a series of technical, regulatory, and translational challenges.

From a developmental perspective, a critical first step was the standardization of peripheral blood mononuclear cell (PBMC) isolation protocols to ensure cell viability and reproducibility across patient samples. Optimization of ex vivo PBMC culture conditions and the selection of an appropriate immune activation pathway (favoring T-cell–mediated responses) were essential for assay consistency. A proprietary support matrix was engineered to generate stable and reproducible concentration gradients of multiple immunosuppressive drugs within a single culture system. The assay readout was based on a fluorescence-based metabolic viability assay, selected for its sensitivity, dynamic range, and compatibility with high-throughput formats. A key challenge was the transformation of raw fluorescence intensity data into a clinically interpretable pharmacodynamic profile capable of guiding therapeutic decisions.Regulatory challenges included the analytical validation of assay precision, reproducibility, and linearity, as well as the establishment of clinical performance benchmarks in the absence of a universally accepted gold standard. Clinical implementation challenges centered on the integration of pharmacodynamic data into existing transplant care workflows. This required the development of clinician-facing decision-support algorithms, strategies to mitigate institutional risk aversion, and evidence generation to support cost-effectiveness and reimbursement.

In summary, the Immunobiogram addresses a critical gap in transplant medicine by providing a mechanistically informed, patient-specific tool for immunosuppressive drug selection. Its development exemplifies the complex interplay of assay engineering, clinical relevance, and regulatory compliance required for next-generation Precision Medicine diagnostics.

Keywords

Immunology • Precision Medicine • Transplant Medicine • Immunosuppresant Drugs • Personalized Medicine

continue –>

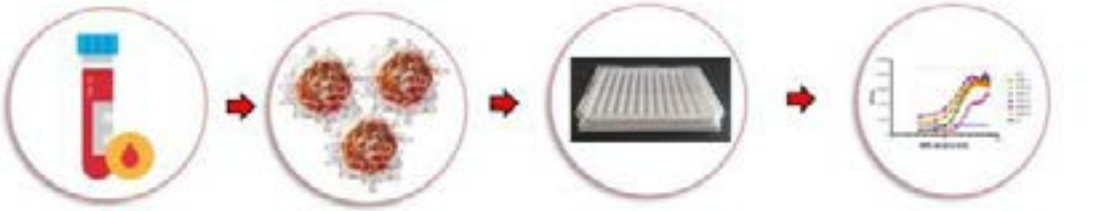
July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

KEYNOTE COMMUNICATIONS

Biotechnology and Health

The Immunobiogram: biotechnology component

The **Immunobiogram®** simulates the immune response of the patient's activated cells to different immunosuppressants to identify the most appropriate treatment for each patient.



The peripheral blood mononuclear cells (PBMCs) are isolated

4-day live cell culture of activated immune cells.

PBMC Exposure to individual immunosuppressants and measurement of activity.

Dose-response curves for each immunosuppressant

A functional assay made accessible

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

KEYNOTE COMMUNICATIONS
Environmental Biotechnology

20 YEARS OF MICROBIAL ELECTROCHEMICAL TECHNOLOGIES: FROM LAB TO FULL SCALE

Abraham Esteve-Núñez
Universidad de Alcalá

Abstract

Two decades after Geobacter was found to be the key actor for electric current generation in a sediment, real applications for removing environmental pollutants (electrobioremediation) have been developed after intense multidisciplinary research (Tucci et al 2021). Wastewater (ww) has been certainly the most tested matrix for hosting Microbial Electrochemical Technologies (MET) due to the potential conversion of the chemical energy contained in organic pollutants into electrical power. However, we needed roughly ten years to realize Microbial Fuel Cells could not compete with commercial solutions based on renewables energies. Despite this bottleneck researchers in the field have developed a plethora of devices and applications with electromicrobiology as key actor. In a wastewater context, the largest electrobioremediation strategy so far corresponds to a solution so-called METland®, where electrochemical concepts are integrated in already existing wastewater treatment solution: the constructed wetlands (Peñacoba-Antona et al. 2022). The hybrid solution is indeed a biofilter made of electroconductive sustainable materials to enhance microbial oxidation of pollutants and reduce the footprint of this nature-based solution as low as 50 m^2 for treating ww from 1000 pe. The system has evolved to modular construction so it can be used as plug and play solutions for treating also industrial water from oil&gas, food&beverage, automotive and pharma sectors to name a few. Solid electrodes (eg. rods, plates, granules, and felts) are typically used as electroconductive materials to support biofilm growth in conventional microbial electrochemistry, diffusion and migration processes could limit the performance for optimal biodegradation rates. To overcome such limitation, we developed a game changer: the microbial electrochemical fluidized bed reactor (ME-FBR). Core element is a fluid-like electrode to minimize mass transfer and energy limitations while simultaneously enhancing the activity of both electroactive planktonic and electroactive biofilms in the bioreactor. Indeed, a fluid-like anode has been shown to be efficient for removing organic pollutants and nitrogen from industrial brewery wastewater. Moreover, ME-FBR allow to develop a nutrient recovery strategy by culturing purple phototrophic bacteria to transform brewery wastewater into protein source and bioplastics (Muniesa et al., 2025), or even fixed CO2 to bioelectrosynthesized acetate (Llorente et al. 2024).

Finally, electrobioremediation generate “useful electrons” that may drive desalination if performed devices calles Microbial Desalination Cells. So, MDC represents a hybrid concept in which energy from an organic pollutants can be directly used in a passive device to produce fresh water (i.e. indirect use of energy). MDC technology has been extensively studied in order to increase the desalinated water production while maintaining low energy requirements resulting in the first full-scale demonstration of MDC technology (Ramirez et al. 2022).

Keywords

metland • electromicrobiology • MET

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

BIOENGINEERING SYNTHETIC CELLULAR ORGANELLES TO ENHANCE SUSTAINABLE BIOCOMPUND PRODUCTION

Henning Kirst
Universidad de Córdoba

Abstract

The enormous complexity of metabolic pathways, their regulation and crosstalk create major obstacles for metabolic engineering, because small changes made to the system, by introducing a new metabolic pathway for example, can often have unpredictable consequences. Thus, effective production strains need to go through many rounds of time-consuming optimization.

For metabolic engineering to become most effective, ideally an autonomous operating metabolic module is introduced decoupled from the cell's regulatory and metabolic networks. Self-assembling, easy-to-modify and interspecies transferable Bacterial microcompartments (BMCs) are promising scaffolds for the next generation of metabolic engineering addressing the current challenges of containing toxic intermediates, minimizing metabolic crosstalk and creating a favorable microenvironment within compartment.

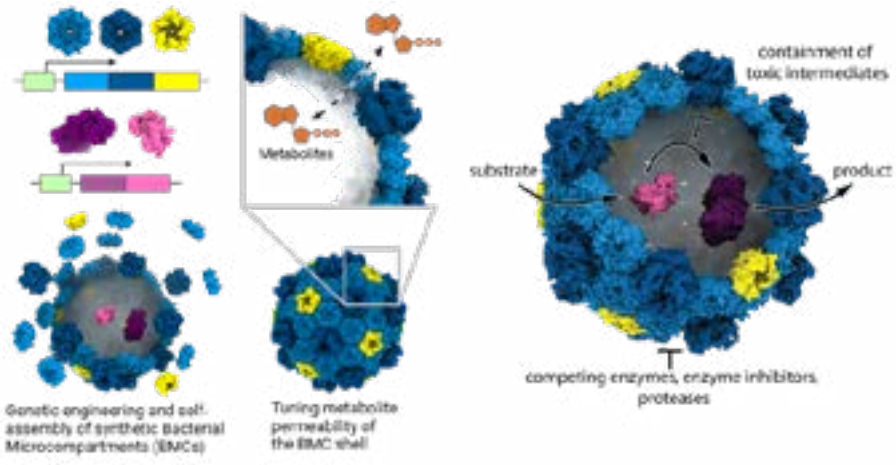
We have developed a comprehensive tool kit to produce synthetic BMC shells in E. coli, load these in vivo with multiple heterologous cargo enzymes and measure the BMC shell molecular permeability. These enabling tools allowed us to construct a synthetic cellular organelle capable of producing pyruvate from format and acetate. Application of BMC based synthetic cellular organelles enabling metabolic routes that wouldn't be feasible without compartmentalization will be discussed. As an example, we will highlight New-to-Nature CO_2 fixation routes, promising to outperform Rubisco mediated CO_2 fixation.

Financial support and acknowledgments

This work is currently supported by CIENCIA - Ministerio de Ciencia, Innovación yUniversidades; Reference: PID2023-153001OB-I00 and RYC2021-034890. It was also supported by the National Science Foundation Award MCB 1733552 and the US Department of Energy, Basic Energy Sciences, Contract DE-FG02-91ER20021 as well as National Institutes of Health, National Institute of Allergy and Infectious Diseases (NIAID), grant 5R01AI114975-08. The work at the Molecular Foundry was supported by the Office of Science, Office of Basic Energy Sciences, of the US Department of Energy under Contract DE-AC02-05CH11231.

Keywords

Synthetic Biology • Metabolic Engineering • Bacterial Microcompartments



July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

EXTRACYTOPLASMIC FUNCTION (ECF) SIGMA FACTORS IN THE RESPONSE OF PSEUDOMONAS AERUGINOSA TO ENVIRONMENTAL AND HOST STIMULI

Marian Llamas
Estación Experimental del Zaidín-Consejo Superior de Investigaciones Científicas

Abstract

Extracytoplasmic function sigma (sECF) factors are signal response proteins that control bacterial gene expression at the level of transcription initiation by modifying the affinity of RNA polymerase (RNAP) for DNA. Due to its abundance, diversity and importance, sECF-signalling represents one of the major signal transduction mechanisms in bacteria. These proteins are abundantly present in bacteria with complex lifestyles able to survive and colonize many different environments, like Pseudomonas aeruginosa. This environmental bacterium can be found in water, soil and the rhizosphere of plants. However, its relevance is related with being an opportunistic pathogen of humans where it causes a wide array of life-threatening acute and chronic infections. Among other problems, P. aeruginosa is a leading cause of hospital-acquired infections and the main reason for lung function deterioration and mortality in cystic fibrosis patients. P. aeruginosa infections are often life-threatening and difficult to treat because this bacterium is intrinsically resistant to multiple antibiotics and can easily acquire new resistances. Due to its global spread and increasing number of antimicrobial resistances (AMR), this bacterium is one of the WHO priority pathogens requiring research and development of new eradication strategies. Signalling through sECF factors is extensively present in P. aeruginosa and represents a main adaptation strategy for this pathogen in the host by regulating processes as important during infection as iron acquisition, cell wall stress responses, and the production of virulence factors. Because the ability of the pathogen to sense and adapt to the host is critical during infection, interference with signalling mechanisms involved in this process represents a promising strategy to fight bacterial pathogens. We have identified three proteases required for activation of sECF factors signalling cascades in P. aeruginosa. Because proteases are known to be druggable proteins, regulatory proteases involved in modulation of the activity of signal transduction systems represent excellent drug targets, a concept that we are looking to exploit.

Financial support and acknowledgments

This work has been funded by MCIN/AEI/10.13039/501100011033 Spanish agency withprojects PID2020-115682GB-I00 and PID2023-149253NB-I00.

Keywords

Pseudomonas aeruginosa • sigma factor • gene regulation • signalling • iron acquisition • virulence • proteolysis • antimicrobial resistance • therapeutic target

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

MICROBIAL COMMUNITIES AND CONTAMINANTS: CHALLENGES OF FUNCTIONAL ANALYSIS

Balbina Nogales; Theo Obrador-Viel; Alberto Contreras-Moll; Justine M. Bitalac; Rocío D.I. Molina; M. Mar Aguiló-Ferretjans; Rafael Bosch; Joseph A; Christie-Oleza
Universitat de les Illes Balear

Abstract

Despite significant advances in our understanding of how microorganisms degrade environmental pollutants, inferring catabolic functions and identifying key degraders from metagenomic data remains a complex task. This approach faces several challenges. For instance, in the case of aromatic compounds, it is difficult to distinguish genes involved in pollutant degradation from those related to the metabolism of naturally occurring aromatic compounds. Moreover, some enzymes—such as reductases, decarboxylases, and others—may play roles in pollutant degradation but are often overlooked in sequence homology-based analyses because they have not been associated with characterized hydrocarbon degradation pathways. The challenge is even greater when investigating plastic degradation. The identification of plastic-degrading organisms and the functional annotation of relevant genes are hindered by the limited availability of well-characterized degradation pathways and the difficulty in reliably assessing degradation potential. These limitations underscore the need to isolate relevant environmental strains. Such isolates are crucial not only for elucidating the metabolic pathways underlying plastic degradation but also for providing valuable insights into the microorganisms and mechanisms responsible for plastic breakdown in natural environments.

Financial support and acknowledgments

PID2022-139042NB-I00 funded by MCIN/AEI /10.13039/501100011033TED2021-129739B-I00 funded by MICIU/AEI /10.13039/501100011033 and the EU NextGenerationEU/ PRTRPDC2022-133849-I00 financiado por MICIU/AEI /10.13039/501100011033 y por la Unión Europea Next GenerationEU/ PRTR

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

KEYNOTE COMMUNICATIONS
Industrial Biotechnology

ENZYMES AS KEY CATALYSTS FOR THE SUSTAINABLE SYNTHESIS OF BIOPLASTICS: FROM BIOMASS TO 2,5-FURANDICARBOXYLIC ACID CATALYZED BY HMFO

Kírian Bonet-Ragel; Darly Concha Núñez; Garazi Ortiz-Orruño; Oscar Romero; Marina Guillén Montalbán
Universitat Autònoma de Barcelona (UAB)

Abstract

Polyethylene furanoate bioplastics (PEF) have emerged as promising environmentally friendly materials that could replace the widely used polyethylene terephthalate (PET). PEFs are synthesized from renewable resources and produced through the polymerization of the building block 2,5-furandicarboxylic acid (FDCA). The enzymatic synthesis of FDCA plays a central role in this process, starting from 5-hydroxymethylfurfural (HMF), a molecule obtained from lignocellulosic feedstocks rather than petroleum. Through the action of a hydroxymethylfurfural oxidase (HMFO), HMF undergoes oxidation to yield FDCA (Figure 1), providing a biocatalytic route that aligns with green chemistry principles. One of the main hurdles to widespread enzyme use in industry is the economic burden tied to their production and processing. To manage this, innovative approaches of immobilization aim to simplify recovery and enable enzyme recycling, while also aligning with continuous manufacturing setups. A particularly interesting approach involves the use of substrate-binding domains like cellulose-binding modules (CBMs). When enzymes are genetically engineered to include these modules, they can directly and selectively attach to cellulose-rich surfaces, eliminating the need for additional chemical treatments. This method enhances immobilization metrics and could significantly broaden the practical implementation of enzymes in various biotechnological contexts. Thus, the conversion of HMF into FDCA through biocatalysis represents a key step in developing sustainable monomers for bio-based plastics. In this work, an octuple-mutated variant of HMF oxidase (8BxHMFO) was functionally enhanced via genetic fusion with the cellulose-binding module CBM3 and subsequently cross-linked with glutaraldehyde to enhance the stability of the biocatalyst. This configuration enabled robust catalytic performance under intensified conditions, including the increase of initial HMF concentration (up to 10-fold). Space–time yield and product titres were markedly improved under these settings. Oxygen availability, acting as a co-substrate, was also tested to evaluate its influence on bioconversion efficiency. Moreover, a sequential substrate feeding approach was implemented to mitigate the inhibitory effects associated with intermediate accumulation, leading to further optimisation. Altogether, the methodology demonstrates a scalable and eco-efficient route for FDCA synthesis, reinforcing the role of immobilised enzyme systems in green chemical manufacturing.

Financial support and acknowledgments

Projects FURENPOL PLEC2021-007690 funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR, ROBUSTOO project funded by European Union's Horizon Europe research and innovation program under Grant Agreement 101135119 and SGR 00143 (2017) funded by AGAUR (Generalitat de Catalunya).

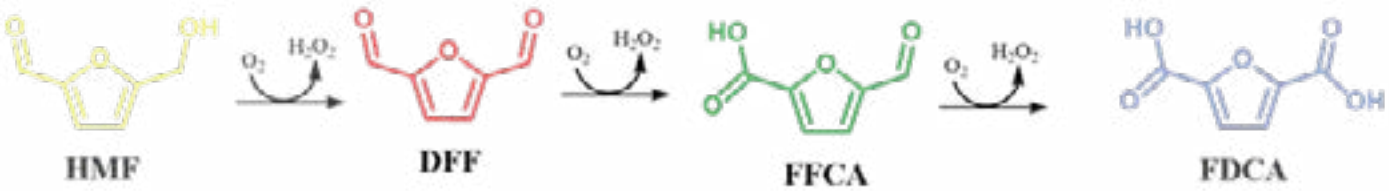


Figure 1: Oxidation pathway from HMF to FDCA.

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

SUSTAINABLE BIOTECH? PROVE IT, SHOW ME THE METRICS!

Pablo Domínguez de María
Sustainable Momentum, SL

Abstract

Enzymes may offer eco-friendly options to traditional organic synthesis. Yet, like any other chemical process, biocatalysis require water, solvents and energy, what inevitably generates waste. Developing reliable metrics to evaluate the environmental impact of enzymatic reactions, particularly at early stages, results crucial[1]. The traditional E-Factor (kg waste · kg product^{-1})[2] is intuitive, albeit it is often used to assess wastes that are fully treated in the chemical plant (wastewater, solvents, etc), and do not remain in the planet[3]. When such wastes are treated, CO_2 is the ultimate residue, formed upon wastewater treatment, from solvent incineration, and from the energy used in the (bio)catalytic reaction[3,4]. That CO_2 remains in the planet and should be therefore the key aspect to be environmentally considered, to put forth processes that may balance sustainability with commercial viability. This talk discusses a rapid tool to estimate the "Global Warming Potential (GWP)" – expressed as kg CO_2 · kg product^{-1} – of (bio)catalytic reactions, deducing equations based on reaction parameters such as "conversion", "substrate loading", "reaction time", and "temperature"[5]. The method compares reaction conditions and identifies key CO_2-producing hotspots, pinpointing greener biomanufacturing options while preserving the efficiency of biocatalytic systems.

Keywords

Environmental metrics • Biocatalysis

1. P. Domínguez de María. "Biocatalysis, sustainability and industrial applications: Show me the metrics". *Curr. Op. Green Sust. Chem.* **2021**, 31, 100514.
2. P. Domínguez de María. "On the need for gate-to-gate environmental metrics in biocatalysis: fatty acid hydration catalyzed by oleate hydratases as a case study". *Green Chem.* **2022**, 24, 9620.
3. P. Domínguez de María. "Primary and secondary waste in (bio)catalysis: What matters is not what is produced, but what permanently remains!". *Curr. Op. Green Sust. Chem.* **2025**, 52, 101003.
4. P. Domínguez de María, S. Kara, F. Gallou. "Biocatalysis in water or in non-conventional media? Adding the CO_2 production for the debate". *Molecules* **2023**, 28, 6452.
5. P. Domínguez de María. "General equations to estimate the CO2 production of (bio)catalytic reactions in early developments stages". *RSC Sustainability* **2024**, 2, 3817.

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

ADVANCING BIOPROCESS ENGINEERING FOR A SUSTAINABLE AND EFFICIENT PRODUCTION OF BACTERIAL POLYMERS

Alberto Rodríguez Martín
Centro de Investigaciones Biológicas Margarita Salas - Consejo Superior de Investigaciones Científicas (CIB-CSIC)

Abstract

Microbial bioplastics have emerged as promising alternatives to conventional polymers due to their thermal and mechanical properties, as well as the global push toward a circular economy and sustainable development. However, large-scale production remains limited by the high cost of raw materials, bioreactor operation and downstream processes. A viable strategy to overcome these challenges is the use of liquid and gaseous waste streams, which can significantly reduce costs and improve process sustainability. In this context, our research group has developed laboratory-scale bioprocesses for converting such residue into polyhydroxyalkanoates (PHAs). Three show cases using two types of waste streams will be presented. In the first bioprocess, we worked with Rhodospirillum rubrum, a purple non-sulphur bacteria (PNSB) with a versatile metabolism able to produce PHA and hydrogen simultaneously from synthesis gas, which represents the main product of the pyrolysis of municipal waste in landfills. In this biosystem, we developed and optimized syngas dark fermentation, studying the operational pressure as a key design variable to enhance yield and bioprocess performance. Using a dynamic CO-dose strategy, we were able to reduce the operational time in 10 days and doubling biohydrogen productivity to 27 mmol H2·(L·h)-1 respect other previous studies with PNSB. The second example consists on the use of oil streams as feedstocks, using two model PHA-producing strains: Pseudomonas putida and Cupriavidus necator in aerobic fermentations. In those bioprocesses, optimization of carbon, nitrogen, phosphorous and oxygen availability are fundamental to reach high-cell density cultures and maximize PHA accumulation. Working with P. putida, we have optimized liquid feeding strategies in continuous fed-batch fermentations. Moreover, we have studied different mixtures of enriched air to removing oxygen limiting conditions. Our optimal configuration reached 42.6 g PHA/L and 1.2 g PHA·(L·h)-1 in just 36 h. In the case of C. necator, we investigated the valorization of used cooking olive oil in continuous fed-batch fermentations. Specifically, we studied the influence of oxygen transfer rate (OTR) in bioprocess performance. Our observations indicated that an OTR higher than 0.06 mmol O2·(L·h)-1 boosted PHA accumulation, while lower values of this variable directed the carbon flux to cell growth. Our optimal conditions achieved 95.3 g PHA/L and 1.8 g PHA·(L·h)-1 in 54 h, with a 99% conversion of the carbon source.These results offer new strategies for fast, cost-effective, and sustainable PHA production in high-cell density cultures, providing valuable insights for efficient scale-up to larger bioreactor systems.

Financial support and acknowledgments

This work has been supported by the Spanish Ministry of Science and Innovation under research grants BIOCIR (PID2020 112766RB C21) and OPENVIRO (PID2023-146557OB-C21), funded by AEI/10.13039/501100011033.

Keywords

Bacterial polymers • Polyhydroxyalkanoates • Bioprocess optimization • Bioreactor engineering

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

DOWNSTREAM INTENSIFICATION BY INTEGRATING TANGENTIAL FLOW FILTRATION TECHNOLOGIES

Ruth Ordóñez Sanz
Bioprocess Specialist (Bionet)

Abstract
Traditionally, the upstream of bioprocessing has received greater attention, technological development and investment, resulting in high productivities and yields. In contrast, downstream processing has lagged behind, becoming a bottleneck due to the inherent challenges in modeling, automation and scale-up.The versatility of tangential flow filtration (TFF) makes it a valuable technology for use at multiple stages of downstream processing. This technology serves as the foundation for the intensification strategy presented in this work.Bioprocess intensification aims to meet the key needs of downstream operations: achieving high yields while maintaining product quality. This balance is attained through various design and operational strategies.One such strategy is continuous bioprocessing, and an application example within the pharmaceutical industry will be presented to illustrate its implementation with TFF.

Keywords
TFF • Tangential Flow Filtration • Membranes • Downstream • Intensification

LIGHTING UP PLANT BIOTECHNOLOGY: AUTOBIOLUMINESCENCE AS A TOOL FOR PROTOTYPING SYNTHETIC GENE CIRCUITS AND MONITORING PLANT VIRAL INFECTIONS

Marta Vazquez-Vilar[1]; Camilo Calvache[1]; Marta Rodriguez-Rodriguez[1]; Victor Vazquez-Vilriales[1]; Elena Garcia-Perez[1]; Aubin Fleiss[2]; Mustafa Ezzedin[3]; Karen S. Sarkisyan[2]; Diego Orzaez[1]
1. IBMCP-CSIC; 2. Imperial College London; 3. Universidad de Valencia

Abstract

Plant Synthetic Biology is transforming how we engineer plants, enabling the construction of synthetic gene circuits (SGCs) that expand plant functionalities for sensing, signaling, and bioproduction. A key challenge in advancing this field is the need for fast, affordable, and quantitative tools to test and optimize these circuits directly in planta. To address this, we developed an autobioluminescent reporter system based on the Neonothopanus nambi fungal bioluminescent pathway (FBP), offering a powerful, substrate-free platform for both circuit prototyping and real-time monitoring of viral infections.

By transiently expressing all four genes of the FBP in Nicotiana benthamiana leaf discs, we created a fully self-sustained bioluminescent reporter system that eliminates the need for exogenous luciferin and enables continuous, non-invasive tracking of gene expression. A key design feature was using HispS, the rate-limiting enzyme in the pathway, as the transcriptional entry point, which significantly enhanced dynamic range. Coupling this with enhanced GFP as a ratiometric normalizer resulted in the NeoLuc/eGFP system, which achieves performance comparable to standard luciferase assays.

This autobioluminescent system has been validated across a wide range of SGCs, including those responsive to agrochemicals, hormones, and light. It allows real-time monitoring of expression dynamics, identification of regulatory bottlenecks, and fine-tuning of induction strategies such as copper- and red-light-controlled switches. Notably, it has also proven valuable for optimizing high-expression systems based on Geminivirus (BeYDV) and TMV replicons—widely used platforms in plant molecular farming. In parallel, we leveraged the FBP as a novel biosensor for detecting plant viral infections. By constitutively expressing three of the FBP enzymes (H3H, Luz, and CPH) and delivering HispS substitutes via viral vectors, we enabled autonomous luminescence specifically in infected tissues, allowing visual tracking of TMV and PVX spread in planta. Building on this concept, we developed sentinel plants that activate luminescence in response to specific viral proteins. Together, these developments position autobioluminescence as a transformative tool in plant biotechnology. It enables dynamic, real-time feedback for circuit optimization, provides insights at the macroscopic level of the performance of high-expression systems, and opens new avenues for substrate-free, field-deployable pathogen detection. This work illuminates the way forward for building smarter biosensors and next-generation biofactory plants.

Financial support and acknowledgments

PID2022-141438OB-I00MCIN/AEI/10.13039/501100011033/ FEDER, UE

Keywords

plant synthetic biology • autobioluminescence • sentinel plants • reporter

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

A PRICKLY ISSUE

Pietro Gramazio[1]; James W. Satterlee[2]; David Alonso[1]; Andrea Arrones[1]; Gloria Villanueva[1]; Mariola Plazas[1]; Sandra Knapp[3]; Joyce Van Eck[4]; Jaime Prohens[1]; Santiago Vilanova[1]; Zachary B. Lippman[2]
1. Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain; 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA; 3. Natural History Museum, London, UK; 4. Boyce Thompson Institute, Ithaca, NY, USA

Abstract

Prickles are sharp projections of plant epidermis that serve as a defence mechanism against herbivory, but present significant challenges in agricultural contexts, complicating cultivation, harvesting, and postharvest processes. To identify genetic regulators of prickle development, we generated interspecific mapping populations of eggplant (Solanum melongena) with introgressions of prickly wild relatives. Through fine mapping, we found that prickle loss is caused by a mutation in a duplicated member of the LONELY GUY (LOG) classical cytokinin hormone biosynthetic gene family. High-quality genome assemblies and sequencing of diverse Solanum accessions revealed 16 independent LOG mutations associated with prickle loss in cultivated and wild species, highlighting recurrent co-option of LOG paralogs during evolution. Beyond Solanum, mutations in LOG genes were identified in distant prickly taxa, such as roses (Rosa sp.), jujube (Ziziphus jujuba) and the giant spider-flower (Tarenaya hassleriana). LOG family genes are also involved in the prickly barbs of the awns of rice (Oryza sativa) and barley (Hordeum vulgare), underscoring a convergent evolution among species spanning unrelated and highly diverged lineages. Genome editing with CRISPR-Cas9, was utilized to knock-out the LOG gene to eliminate prickles in the scarlet eggplant (S. aethiopicum), in the foraged Australian desert raisin (S. cleistogamum) and in the forest nightshade (S. prinophyllum) without pleiotropic effects, demonstrating the practical application of our findings in crop improvement. These insights pave the way for breeding prickleless varieties in economically significant plants, improving safety and efficiency in cultivation and harvesting, as well as for domesticating wild prickly foraged plants. Our work also deepens the understanding of convergent evolution of plant innovations, such as the development of prickles.

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

VIVALDI: INNOVATIVE BIO-BASED CHAINS FOR CO_2 VALORISATION AS ADDED-VALUE ORGANIC ACIDS

Albert Guisasola Canudas
Universitat Autònoma de Barcelona

Abstract

VIVALDI proposes an innovative integrated biotechnological solution for the conversion of off-gas emissions into CO2-based chemicals. A multidisciplinary consortium of 16 partners from 9 EU countries representing technology developers, bio-based industries (BIs), end-users and knowledge hubs is working together to present BIs with an opportunity to shift towards circular economy by turning their CO_2 emissions into added-value products, which can be again utilised e.g. as raw materials in the production of biomaterials. VIVALDI uses real off-gas emissions from four key BI sectors (Pulp & Paper, Food & Drinks, Bioethanol and Biochemicals) to produce 4 industrially relevant organic acids with different applications and market penetration: lactic acid (LA), succinic acid (SA), itaconic acid (IA) and 3-hydroxypropionic acid (3-HP). After capturing the CO_2 from industrial off-gas streams, it will be electrochemically converted to methanol (MeOH) and formic acid (FA), which can serve as feedstocks for the bioproduction of the targeted acids. The acid production is carried out by a microbial fermentation process with specific engineered yeast strains of Pichia pastoris. Nutrients required for the bioproduction are recovered from the wastewaters using microbial electrochemical technologies, in which microorganisms are catalyzing electrochemical reactions. The individual technologies have been first optimized and the electrochemical reduction of CO_2 to methanol and formic acid have been integrated to the bioproduction step making it possible to produce added-value compounds from CO_2 in the same environment. Finally, after custom-made downstream processing, the organic acids have been industrially benchmarked to ensure that they comply with current industrial standards. The benefits of VIVALDI's implementation have been quantified with a comprehensive sustainability and circularity assessment (technical, environmental and socio-economic). To alleviate the adoption of VIVALDI's solutions, market opportunities and regulation bottlenecks for the early adoption have been identified.

Financial support and acknowledgments

The VIVALDI project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000441

Keywords

CO_2 electroreduction • bioelectrochemistry • fermentation • downstream

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

NEXT-GENERATION USP DEVELOPMENT: DRIVING INNOVATION AND ROBUSTNESS IN CHO-BASED BIOPROCESSING

Evan Gilman-Burke
Leanbio SL

Abstract

The upstream process (USP) is the cornerstone of biopharmaceutical manufacturing, determining both the productivity and quality of recombinant protein therapeutics. As industry pressure intensifies to deliver complex biologics faster and more cost-effectively, next-generation USP development has emerged as a critical driver of innovation. This evolution is particularly evident in CHO (Chinese Hamster Ovary) cell-based bioprocessing, which remains the dominant platform for therapeutic protein production due to its capacity for human-like post-translational modifications and regulatory acceptance.

Next-generation USP strategies aim to overcome the limitations of traditional process development by integrating advanced tools and approaches that enhance scalability, robustness, and product consistency. Key enablers include high-throughput miniaturized systems, intensified seed trains, and perfusion-based production platforms. These technologies allow for deeper process understanding, faster iteration cycles, and more predictive scale-up, thereby reducing development timelines and increasing process resilience.

Design of experiments (DoE), combined with multivariate data analysis, plays a pivotal role in defining critical process parameters, their interactions and ultimately their impact on the quality of the protein therapeutic. Meanwhile, the use of chemically defined media and feeds, tailored to the specific metabolic and productivity profiles of optimized CHO clones, ensures consistency and reduces variability. Perfusion strategies, such as N-1 intensification and high-density inoculum, are also transforming USP paradigms. These methodologies enable higher volumetric productivity, reduced footprint, and better facility utilization, especially in multi-product facilities or continuous manufacturing schemes. Moreover, the integration of PAT (Process Analytical Technology) and automation tools supports adaptive process control, moving the industry closer to real-time release and closed-loop manufacturing.

This abstract highlights how next-generation USP development is not merely about incremental improvements but represents a shift toward more intelligent, data-driven, and agile process design. By embracing these innovations, CHO-based bioprocessing can achieve higher levels of robustness, scalability, and product quality — essential attributes in the rapidly evolving landscape of biologics manufacturing.

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

RATIONAL DESIGN OF EFFICIENT ENZYMES

Sílvia Osuna
ICREA & Universitat de Girona

Abstract

Enzymes exist as an ensemble of conformational states, whose populations can be altered through substrate binding, allosteric interactions, post-translational modifications, and even by introducing mutations into their sequence. In many enzymatic systems, full catalytic potential is only retained when in the presence of binding partners, either from additional proteins to generate heterocomplexes or from the formation of homo-oligomers. Despite progresses and recent advances in generative models for protein design, designing efficient enzymes and predicting oligomeric assemblies presenting a tight communication for enhancing a specific catalytic function remains challenging. Such tightly intertwined enzymatic assemblies modulate the conformational heterogeneity of the systems and allow the adoption of the multiple catalytically relevant conformational states needed for catalysis.

In this talk, we will explore our developed computational pipelines that integrate AlphaFold2 with machine learned interatomic potentials to guide enzyme design and foster protein assembly, thereby enabling the cooperative interactions and conformational transitions crucial for enhanced catalytic performance. We will also discuss how our approach, which combines coevolutionary analysis and correlation-mediated allosteric networks, can be leveraged to redesign enzymes and modify protein–protein interfaces for improved catalytic activity. Over the years, our work with diverse enzyme systems has generated strong evidence that rational design strategies can effectively yield active enzyme variants. Moreover, we demonstrate that the current challenge of predicting distal active sites to enhance functionality in computational enzyme design can ultimately be met.

Keywords

molecular dynamics • AlphaFold2 • rational enzyme design

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

PREDICTIVE CONTROL IN BIOLOGIC DRUG MANUFACTURING: LEVERAGING AI TO PREVENT PROCESS DEVIATIONS

Jordi Gibert
Klinea Biotech & Pharma Engineering

Abstract

Biologic drug manufacturing involves complex, highly sensitive processes where even minor deviations can compromise product quality and yield. Predictive control, powered by artificial intelligence, offers a proactive approach to maintaining process stability by identifying and correcting deviations before they escalate.

This presentation will explore how predictive models, built on historical and real-time process data, can forecast critical parameter shifts—such as changes in cell culture conditions or purification dynamics—and trigger timely adjustments. By integrating these models with process control systems and digital twins, manufacturers can achieve greater consistency, reduce variability, and enhance overall process robustness.

The session will also address key considerations for implementing predictive control in GMP environments, including data infrastructure, model lifecycle management, and alignment with regulatory expectations. Attendees will gain a clear understanding of how predictive control can be strategically applied to improve reliability and efficiency in biologic drug production.

CHITIN DEACETYLASES FOR THE PRODUCTION OF SEQUENCE-DEFINED CHITOSAN OLIGOSACCARIDES. UNDERSTANDING AND ENGINEERING SPECIFICITY

Antoni Planas; Mònica Texidó; Xevi Biarnés
Institut Químic de Sarrià, University Ramon Llull

Abstract

Chitin is a linear polysaccharide of β-1,4-linked N-acetylglucosamine (GlcNAc) monomers, whereas chitosans are highly or fully deacetylated chitin polymers. Depolymerization of chitin results in chitin oligosaccharides (COS) and de-N-acetylation of chitin and COS yield chitosans and partially acetylated chito-oligosaccharides (paCOS), respectively. COS and paCOS have gained recent interest due to their biological activities as antifungals in agriculture for crop protection against fungal pathogens, stimulants of plant growth or improving plant abiotic stress tolerance, and as immunostimulants in animals, being target compounds for sustainable and environmentally friendly applications. Their biological activities not only depend on the degree of polymerization (DP) and degree of acetylation (DA) but also on their pattern of acetylation (PA) that defines the distribution of N-acetylglucosamine (GlcNAc) and glucosamine (GlcNH2) units along the oligosaccharide chain. Current chemical production of COS and paCOS (by depolymerization and deacetylation of chitin under harsh acidic and basic conditions) render complex mixtures with random deacetylation patterns. Most of the bioassays are performed with mixtures of products with inconsistent results due to batch to batch variations.We are developing a bottom-up approach in which first COS are produced by a cell factory approach; engineered E. coli strains expressing rhyzobial chitin synthases produce COS with defined DP from DP4 to DP7 depending on the enzyme and engineered active site mutants. Next, enzymatic deacetylation of COS with chitin deacetylases (CDAs) has become an attractive alternative to access sequence-defined paCOS and a number of CDAs with different regio-selectivities have been identified and characterized in the last decade.

Chitin deacetylases (CDA) are members of family 4 carbohydrate esterases (CE4) which operate by metal-assisted general acid/base catalysis. We are interested in understanding the structural bases of substrate specificity by CE4 enzymes, their mechanism of action and biological functions, as well as the use of engineered variants as biocatalysts for the production of sequence-defined paCOS. Here we will report on recent developments to expand the toolbox of CDAs as biocatalyst to access a panel of tailored paCOS: Vibrio cholera CDA and engineered variants for single regio-specific deacetylation on which the subsite capping model was first proposed, Pochonia chlamydosporia CDA and Pestalotiopsis sp. CDA with a distributive mode of action, and more recently the extension to some peptidoglycan deacetylases that are also active on COS.

Financial support and acknowledgments

Work supported by grants GLYCOENGIN (PID2022-138252OB-I00) and paCOSVALOR (PDC2022-133580-I00) to A.P. from MICIU/AEI, Spain M.T. acknowledges a predoctoral fellowship from MICIU/AEI.

Keywords

chitin deacetylases • specificity • chitosan oligosaccharides • protein engineering • biocatalysis|enzyme catalysis

BIOCATALYSIS AND IONIC LIQUIDS AS ENABLING TOOLS FOR THE SUSTAINABLE CO2 TRANSFORMATION INTO BIS(CYCLIC CARBONATE) ESTERS

Rocio Villa; Francisco Velasco; Susana Nieto; Eduardo Garcia-Verdugo; Pedro Lozano
1. Department of Biochemistry and Molecular Biology B and Immunology, Faculty of Chemistry, University of Murcia, Spain; 2. Department of Organic and Inorganic Chemistry, Faculty of Chemistry, University Jaime I, Castellón, Spain

Abstract

The planet inevitably undergoes oxidative processes, resulting in the generation of CO_2 as universal waste product. The emission of CO_2 into the atmosphere, along with the industrial production of recalcitrant plastic materials that are disposed of in landfilled or incinerated, remain among the most impactful sources of environmental damage.

Both the Ionic liquid technologies and the inherent advantages of enzymes as natural catalysts have emerged as powerful tools for carrying out the transformation of CO_2 into valuable products, such as cyclic carbonate esters.

In this work, a green approach for CO_2 capture and chemo-enzymatic transformation into bis(cyclic carbonate) esters from CO_2, glycidol, and anhydrides under solvent-free conditions is presented (Figure 1).

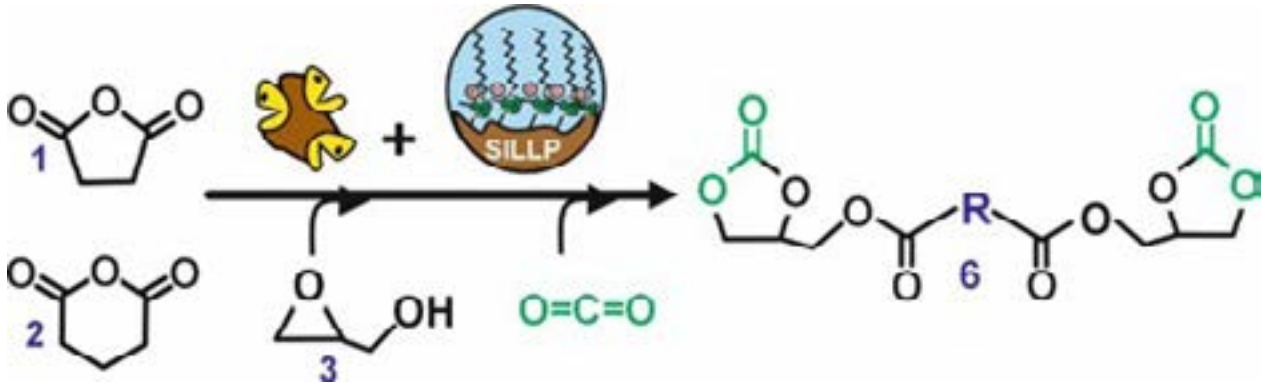
The chemo-enzymatic process is based on two catalytic steps, which can be carried out in separated operations or within a one-pot system, taking advantage of the synergic effects observed when combining ILs and Novozym 435 as biocatalyst. In the first reaction, lipase-catalyzed (trans)esterification reactions of different diacyl donors (e.g., glutaric anhydride, succinic anhydride, etc.) with glycidol in solvent-free under mild reaction conditions (70 °C, 6 h) to produce the diglycidyl ester derivatives in up to 41% yield. By a second step, the synthesis of bis (cyclic carbonate) esters was carried out as a result of the cycloaddition reaction of CO_2 on these diglycidyl esters, catalyzed by the covalently attached 1-decyl-2-methylimidazolium IL (supported ionic liquid-like phase, SILLP), achieving up to 65% yield after 8 h at 45 °C and 1 MPa CO_2 pressure. Finally, both key elements of the reaction system (biocatalyst and SILLP) were fully recovered and reused for at least 5 operational cycles. Additionally, different metrics have been applied to assess the greenness of the solvent-free chemo-enzymatic synthesis of bis(cyclic carbonate) esters.

Financial support and acknowledgments

This work was partially supported by MICINN-FEDER-AEI 10.13039/501100011033 (PID2021- 124695OB-C21/C22 and CPP2023-010883), and SENECA (21884/PI/22, 22518/PDC/24).

Keywords

Biocatalysis • Ionic Liquids • CO_2 transformation • Sustainable Chemistry



SELF-SUFFICIENT BIOCATALYST-DRIVEN CO_2 CONVERSION: UPCYCLING INDUSTRIAL FEEDSTOCKS INTO HIGH-VALUE PRODUCTS

Sady Roberto Rodríguez; Marina Guillén, Oscar Romero
Department of Chemical, Biological and Environmental Engineering. Engineering School. Universitat Autónoma de Barcelona. Barcelona, Spain

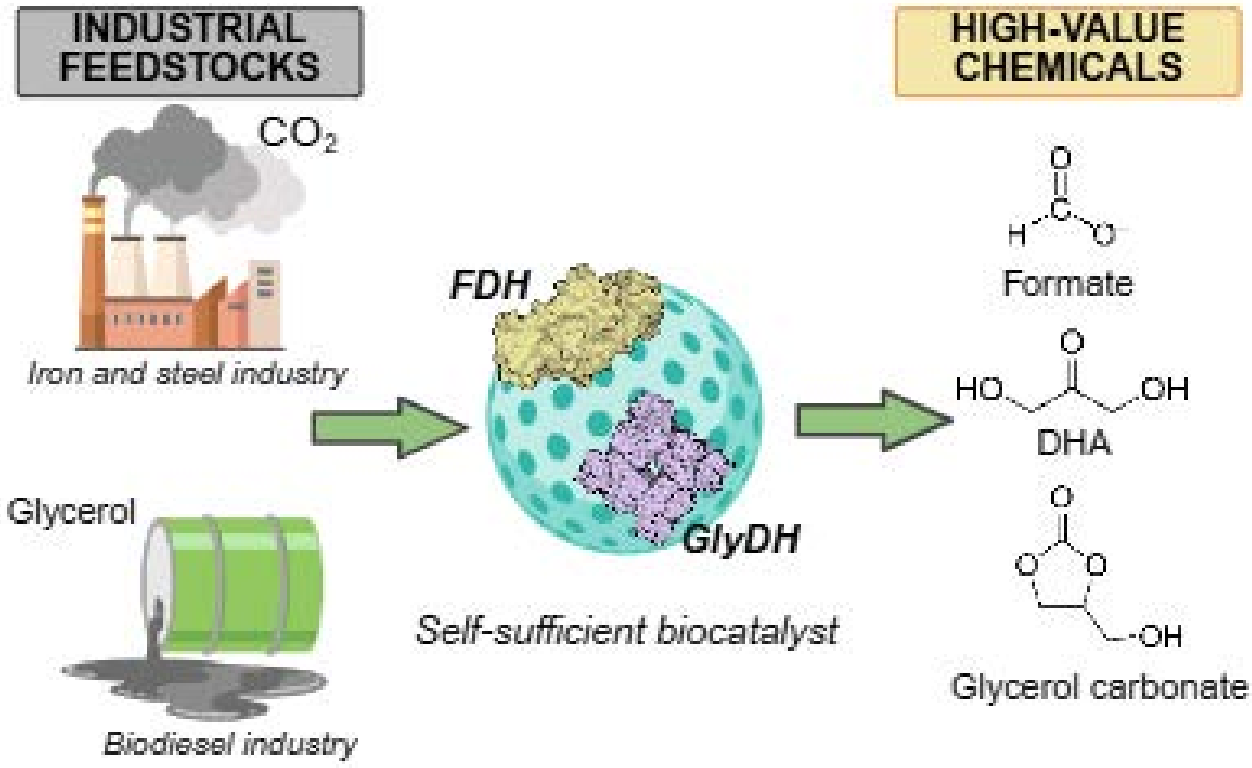
Abstract
Decarbonizing industry requires a transition from energy-intensive fossil carbon processes to more sustainable green technologies that valorize CO_2 as a valuable waste. Biocatalysis offers a promising approach for integrating Carbon Capture and Utilization (CCU) technologies with the conversion of industrial waste into value-added chemicals. To meet the demanding conditions of industrial environments, enzyme immobilization plays a key role—enhancing biocatalyst stability, reusability, and overall reaction efficiency, thus enabling scalable and robust CCU applications. The present work explores an industrially relevant multi-enzyme CCU platform to valorise CO2 and glycerol by integrating formate dehydrogenase (FDH) and glycerol dehydrogenase (GlyDH) enzymes for the co-production of formate and dihydroxyacetone (DHA), with in situ cofactor regeneration. First, a self-sufficient biocatalyst —prepared on Ni+2-ReliZyme as the carrier— was optimized in terms of activity and stability through a sequential one-step purification/co-immobilization strategy using cell lysates. To intensify the reaction and reduce overall gas consumption, the CO_2 transfer rate was optimized, resulting in one of the highest formate concentrations reported via enzymatic synthesis, 66.1 ± 1.4 mM (3 g / L), achieved with a low volumetric input flow rate (0.1vvm). To bridge the gap between industrial environments and bench-scale studies, the biocatalysis performance was assessed under industrially relevant conditions, using a crude gas mixture that mimics emissions from the iron and steel industry, as well as crude glycerol derived from biodiesel production. The results demonstrate the feasibility of this system for sustainable CO_2 conversion into formate, 43.3 ± 1.3 mM (2 g / L). Likewise, the valorization of crude glycerol into DHA was achieved, along with glycerol carbonate as a byproduct. The remarkable biocatalyst enabled reaction intensification with significant yields for all products, improved stability and reusability over five reaction cycles, and reduced DHA inhibition. Finally, downstream processing for the separation of formate and DHA was successfully implemented through the selective adsorption of DHA onto an anion exchange resin (89.4 ± 0.4%) and the sustainable isolation of formate via liquid–liquid extraction using 2-methyltetrahydrofuran (85.9 ± 0.1%). Therefore, the outstanding co-production of three high-value molecules in a one-pot system was achieved through a CCU approach aimed at the valorization of industrial waste.

Financial support and acknowledgments
S.R.R. acknowledges the support of Generalitat de Catalunya AGAUR for his Joan Oró predoctoral fellowship (2022FI-B00955). Authors acknowledge the support of the Generalitat de Catalunya, the 2021 SGR 00143 grant, and the project MEPLAB-CO2 (TED2021-129732A-I00), funded by MCIN/AEI/10.13039/501100011033 and co-funded by the European Union through the “NextGenerationEU”/PRTR initiative.

Keywords
CO_2 reduction • Carbon Capture and Utilization • Multi-enzymatic system • Waste valorization • Co-inmobilization • Downstream purification

continue –>

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona



July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

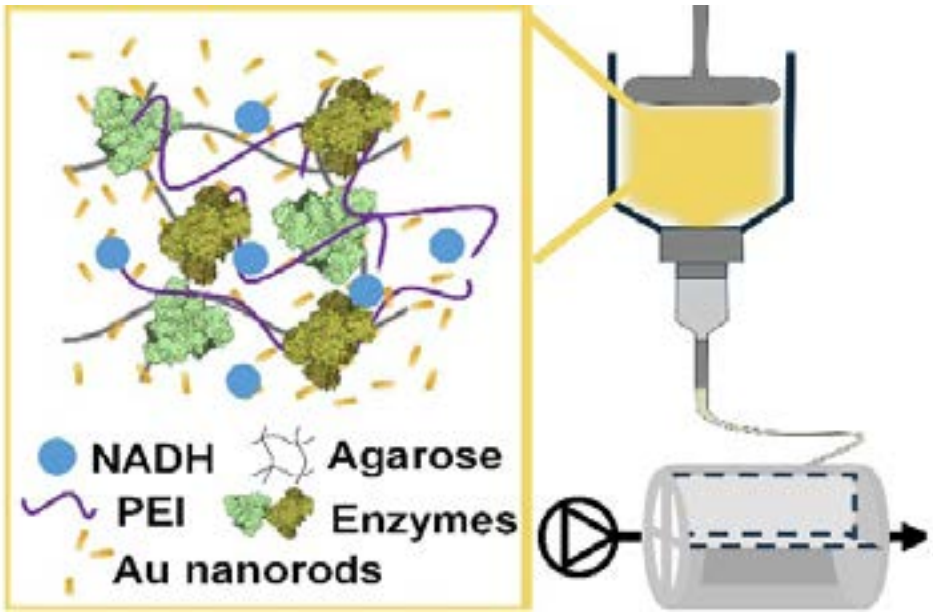
SELF-SUFFICIENT AND AUTOSENSING BIOCATALYTIC INKS FOR 3D-PRINTED BIOREACTORS

Daniel Andrés Sanz; Fernando López Gallego; Dorleta Jiménez de Aberasturi; Uxue Aizarna-Lopetegui; Clara García-Astrain
Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Donostia San Sebastián, Spain

Abstract
Additive manufacturing (3D printing) offers an accessible and precise platform to transform digital designs into functional materials. By integrating this technology with enzymatic catalysis, we aim to expand the design space for continuous flow bioreactors using cofactor-dependent enzymes. In this work, we developed a biocatalytic ink capable of co-immobilizing enzymes and cofactors within a printable hydrogel matrix. The ink, based on agarose and polyethyleneimine, is printed as porous monoliths that retain enzymes and absorb cofactors via ionic interactions. Incorporation of gold nanorods enables in operando sensing of substrates and cofactors—such as NAD^+ and isopropanol—through Surface Enhanced Raman Scattering (SERS), complementing fluorescence microscopy techniques for reactor characterization. Using this optimized formulation, we fabricated 3D-printed reactors with varied geometries and evaluated their performance in the continuous reduction of ethyl acetoacetate by NADH-dependent dehydrogenases. Notably, cross-shaped reactors maintained stable product yields over 24 hours operation while minimizing enzyme and cofactor leaching. Our results demonstrate a self-sufficient biocatalytic platform that sustains enzymatic activity in continuous flow without the addition of exogenous cofactors, paving the way for on-demand shaped and self-monitoring enzymatic reactors based on cofactor-dependent enzymes.

Financial support and acknowledgments
Basque Government's Predoctoral Programme (PRE_2023_2_0165) and ERC-Co (#METACELL-818089)

Keywords
Flow biocatalysis • 3D printing • NAD(P)H recycling



OSMAC AND METABOLOMIC ENGINEERING STRATEGIES TO IMPROVE THE CHEMICAL INFORMATION OF STANJEMONIUM SPECTABILE AND ENHANCE THE PRODUCTION OF A NOVEL COMPOUND

Gioele Pecin[1]; Victor González Menéndez[1]; Frederick Annang[1]; Marina Sánchez-Hidalgo[1]; Jesús Martín[1]; Mercedes Pérez Bonilla[1]; Adrian Enrique Gadar-Lopez[2]; Pablo Cruz-Morales[2]; Fernando Reyes[1]; Olga Genilloud[1]
1. Fundación MEDINA; 2. DTU Biosustai

Abstract

Fungi are an incredible source of novel natural products, and one of the main sources for new drugs and therapeutics. Unfortunately, their metabolic potential is not fully exploited due to silent or poorly expressed biosynthetic gene clusters (BGCs), whose products remain often undetected in standard laboratory conditions. In this study, the “One Strain Many Compound” (OSMAC) approach was applied to Stanjemonium spectabile strain CF-278320, isolated as epiphyte on the endemic plant Bupleurum gibraltaricum, and its holotype strain CBS 340.70 to stimulate the production of novel compounds. The OSMAC experiment consisted of 78 fermentation conditions in total, including three cultivation times (7, 14 and 21 days), 15 different media, and 11 modifications of the original media.

The chemical dereplication of the extracts performed with LC/HRMS data against our internal database, combined with a molecular networking analysis of the MS/MS data, revealed the presence of several trichothecenes and Trichothecium fingerprint toxins. These data, along with a phylogenetic analysis based on Maximum Likelihood and Bayesian models, suggested a relocation of the strains as a monophyletic group within the Trichothecium clade. Furthermore, the analysis of the chemical space of the extracts unveiled the presence of a novel fusaristatin-like compound with strong cytotoxic activity produced by the strains. The production of the novel compound was enhanced by testing the best cultivation condition that resulted from the OMSAC experiment in 10 different cultivation times and 2 formats. Finally, the compound was purified and its structure elucidated using HRMS and NMR. The sequencing of the whole genome of strain CF-278320 allowed us to identify the putative BGC responsible for the production of the novel compound through the bioinformatic tool antiSMASH. In an attempt to demonstrate the validity of our hypothesis, we used an engineered strain of the black yeast Exophiala viscosa JF 03-4F as heterologous host to express the putative cluster. The heterologous construction consisted in the parallel insertion of the two genes encoding for a Type-1 PKS and a 3-aminoacidic domain NRPS in the Exophiala genome using Cas9 sgRNA plasmids. Two transformant strains were successfully obtained, each one producing a different structural analogue of the original compound.

Financial support and acknowledgments

This project has received funding from the European Union’s Horizon Europe programme underthe Marie Skłodowska-Curie grant agreement No 101072485

Keywords

OSMAC • Metabolomic • Heterologous Expression • Stanjemonium spectabile • Exophiala limosa

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

FROM SECRETOME TO INNOVATION: A NOVEL FUNGAL HYALURONATE LYASE EXPANDING THE GLYCOENZYME TOOLBOX

Marina Minguet Lobato[1]; Fadia V. Cervantes[1]; David Fernández Polo[1]; María Fernández Lobato[2]; Francisco J. Plou[1]
1. ICP-CSIC, Madrid, Spain; 2. CBM-UAM-CSIC, Madrid, Spain

Abstract

Filamentous fungi rely on complex and dynamic secretomes, ensembles of membrane-associated and secreted proteins, to degrade extracellular biomasses, adapt to nutrient availability, and interact with their environments. These secreted proteins, many of them carbohydrate-active enzymes, play key roles in fungal survival and have positioned fungi as valuable enzyme providers for industrial biotechnology. However, the current enzymatic repertoires remain limited, and unlocking new fungal diversity is crucial for developing more sustainable and customised bioprocesses.Here, we present the characterisation of a novel hyaluronate lyase secreted by Purpureocillium sp. This enzyme was identified as part of the fungal extracellular machinery and shows no prior functional annotation. Heterologous expression in Pichia pastoris allowed production at 1.0 g/L, and the enzyme demonstrated remarkable thermal stability and high activity on high-molecular-weight hyaluronic acid.

This new enzyme operates via an endolytic mechanism, releasing a unique profile of hyaluronan oligosaccharides, including both unsaturated even-numbered and rare, odd-numbered products. This enzymatic pattern is uncommon among known hyaluronidases and broadens the spectrum of structurally diverse oligosaccharides that can be obtained. Additionally, we established a novel methodology to produce oligosaccharide mixtures with antioxidant properties, underscoring its value for biomedical and cosmetic applications.

Altogether, this work exemplifies how unexplored fungal secretomes can lead to the discovery of novel biocatalysts with distinctive activities and product specificities that enrich the current glycoenzyme toolkit and opens new avenues for sustainable bioprocesses, particularly in fields where bio-based customization of oligosaccharides is of growing interest.

Financial support and acknowledgments

This work was supported by:
(1) Project GLYCOENZ-GREEN (PID2022-136367OB-C31/C32), funded by MCIN/AEI/10.13039/501100011033 and FEDER, a Way of Making Europe;
(2) Project SUST_CHIT_BIOPR (TED2021-129288B-C21/22) funded by MCIN/AEI/10.13039/501100011033 and by the European Union Next Generation EU/PRTR.

Keywords

hyaluronate lyase • fungal secretome • unsaturated hyaluronan oligosaccharides • hyaluronic acid • heterologous expression

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

ALKALINE PH-REGULATABLE PROMOTERS FOR PROTEIN EXPRESSION IN K. PHAFFII: THE CONTRIBUTION OF THE TRANSCRIPTION FACTORS CRZ1 AND RIM101

Joaquin Arino[1]**;** Marcel Albacar[1]; Antonio Casamayor[1]; Abdelghani Zekhnini[1]; Alex Schmidt[2]; Asier González[1]
1. Institut de Biotecnologia i Biomedicina & Dept. Bioquímica i Biol. Molecular, UAB, Cerdanyola del Vallès, Spain; 2. Biozentrum, University of Basel, Basel, Switzerland

Abstract
The yeast Komagataella phaffii (formerly Pichia pastoris) is widely used for heterologous protein production. As in other fungi, alkalinization of the medium (shift to pH 7.8-8.2) results in fast and extensive transcriptional remodeling [1]. Alkaline-sensitive promoters such as pPHO89 and pHSP12, have been recently proposed as novel methanol-free, regulatable protein expression systems and used to drive the expression of industrially relevant proteins [2]. However, the molecular bases of the alkaline response in K. phaffii are fully unknown. Crz1 and Rim101 are transcription factors (TFs) that mediate alkaline pH responses in various fungi. Here, we identify the K. phaffii CRZ1 and RIM101 genes, and we create single and double mutant strains using CRISPR/Cas9 techniques. Phenotypic characterization shows that crz1 cells are strongly sensitive to Ca2+ ions (a landmark for this mutation in fungi), mildly sensitive to alkaline pH and hypertolerant to the cell wall damaging agent sodium dodecyl sulfate (SDS). In contrast, rim101 cells are markedly hypersensitive to alkaline pH and to Na+ and Li+ ions, and SDS, but not to calcium. RNA-seq transcriptomic analysis reveals that mutation of RIM101 affects 55% of genes whose expression is altered at pH 8.0, whereas lack of Crz1 alters only 11% of these. Thirty-eight genes are co-regulated by both Crz1 and Rim101. The PMC1 vacuolar Ca2+-ATPase is regulated by Crz1, whereas the ENA2 Na+-ATPase, both PHO84 phosphate transporters, and diverse genes involved in iron homeostasis are Rim101-dependent. PHO89 was also Rim101 dependent, but HSP12 was not regulated by any of the TFs. These effects were confirmed by proteomic analyses after 3 h of alkalinization. While Rim101 appears as a major player in the transcriptional response to alkalinization, nearly 40% of these responses are controlled by still unknown pathways that are independent of Rim101 and Crz1. We aim to characterize these processes in future studies.

Financial support and acknowledgments
Supported by grants PID2020-113319RB-I00 and PID2023-150535OB-I00 (Ministerio de Ciencia, Innovación y Universidades & Agencia Estatal de Investigación) to JA and AC, and grant 2023 PROD 00006 (AGAUR, Generalitat de Catalunya) to JA.

Keywords
alkaline pH-regulatable promoters • transcription factors • phenotypic mutant analysis • transcriptomics • proteomics • Komagataella phaffii • heterologous protein expression

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

OPTIMIZING POLYHYDROXYALKANOATE RECOVERY THROUGH BDELLOVIBRIO-INDUCED CELL LYSIS AND PREDATOR-PREY DYNAMICS INTEGRATION

Natalia Hernández-Herreros; Sergio Salgado; Maria Auxiliadora Prieto
Polymer Biotechnology Group. Microbial & Plant Biotechnology Department. Biological Research Center Margarita Salas (CIB-CSIC), Madrid, Spain

Abstract

Bdellovibrio bacteriovorus HD100 is an obligate predatory bacterium that has attracted growing interest as a sustainable biotechnological tool, particularly for applications in the medical and industrial sectors. This unique organism invades the periplasmic space of Gram-negative prey, utilising their intracellular contents to fuel its growth. One promising application of *B. bacteriovorus* is in the eco-friendly extraction of polyhydroxyalkanoates (PHAs), which are biodegradable polymers that are gaining prominence in the bioplastics industry[1]. Compared to conventional chemical extraction techniques, biological lysis using these predatory bacteria offers a greener and potentially more cost-effective approach. In addition, using mixed microbial cultures (MMC) for PHA production under non-sterile conditions further improves the economic viability of the process. In combination with the treatment of PHA-rich biomass to inhibit polymer hydrolysis, enabling separate processing from biomass production, this constitutes a holistic strategy[2].

In our study, we explored the integration of *B. bacteriovorus* into MMC-based workflows as a biological strategy for PHA recovery. To address key challenges, such as understanding prey specificity, predation dynamics, and the effects of biomass composition, we developed a high-throughput, real-time analytical method using flow cytometry microscopy, enabling single-cell resolution tracking of predatory activity. The impact of different biomass pretreatments on predation efficiency was also investigated, with a particular focus on the role of prey membrane integrity. Furthermore, we analyzed prey preference patterns in both pure and mixed cultures and examined the impact of predator-to-prey ratios on biomass reduction. Our results demonstrate that *B. bacteriovorus* can selectively target certain prey, even among closely related strains, and that optimizing the predator-prey ratio can lead to up to an 80% decrease in prey biomass.

These findings provide valuable insights into the ecological parameters influencing predation and support the use of B. bacteriovorus as an effective, sustainable agent for PHA recovery and bioplastic production.

Financial support and acknowledgments

This work has been funded by the project Agriloop (Grant agreement No. 101081776) from the European Union's Horizon Europe research and innovation programme and the UK Research and Innovation.

Keywords

PHA recovery • Predatory bacteria • Population dynamics • Circular economy

References

1. Martínez, V., Herencias, C., Jurkevitch, E., Auxiliadora Prieto, & M., 2016. Engineering a predatory bacterium as a proficient killer agent for intracellular bio-products recovery: The case of the polyhydroxyalkanoates. Nature Publishing Group.
2. Tayou Nguemna, L., Marzulli, F., Scopetti, F., Lorini, L., Lauri, R., Pietrangeli, B., Crognale, S., Rossetti, S., Majone, M., Villano, M., 2023. Recirculation factor as a key parameter in continuous-flow biomass selection for polyhydroxyalkanoates production. Chemical Engineering Journal 455, 140208.

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

CHARACTERIZATION OF A NOVEL FUNGAL A-GLUCOSIDASE AND ITS BIOTECHNOLOGICAL POTENTIAL FOR PRODUCING BIOACTIVE COMPOUNDS

Eglè Narmontaitè[1]; José Luis González-Alfonso[2]; Francisco J. Plou[2]; María Fernández-Lobato[1]
1. Department of Molecular Biology-UAM. Center of Molecular Biology (CSIC-UAM). Madrid, Spain; 2. Institute of Catalysis and Petrochemistry, CSIC. Madrid, Spain

Abstract

Increasing interest in healthier diets is driving demand for functional foods, which offer both nutritional value and health benefits, making them a key area of growth and innovation in the food industry. Non-digestible oligosaccharides form an important component of functional foods, with their production for prebiotic and dietary applications typically involving polymer hydrolysis or monomer glycosylation. These include fructooligosaccharides (FOS), isomaltooligosaccharides (IMOS), β-galactooligosaccharides (GOS), among others. IMOS accounts for 60% of the food and beverage market, with a projected 7.1% compound annual growth rate (CAGR) by 2033, and) stand out among other oligosaccharides due to their excellent stability in food products, cost-effectiveness, and easy availability. IMOS are glucose-based oligosaccharides composed of D-glucose units linked by α(1→6), α(1→2), α(1→3), and α(1→4) glycosidic bonds, forming branched and sometimes cyclic structures with a degree of polymerization ranging from 2 to 10. IMOS possess prebiotic properties, a low glycemic index, and promote mineral absorption, bowel function, and bifidogenic response. Additionally, they exhibit immunomodulatory effects and help regulate cholesterol levels and mineral absorption. IMOS can be produced by α-glucosidases from Glycoside Hydrolase families GH13 and GH31, which are characterized by a common (β/α)8-barrel fold. These enzymes hydrolyze oligosaccharides containing α(1→4) links releasing glucose units, and under saturating substrate conditions, they can also exhibit transglycosylating activity, forming IMOS. Some α-glucosidases can utilize alternative glucosyl-acceptors, such as other carbohydrates, vitamins, and phenolic compounds, leading to the production of new bioactive molecules.

In this study, a novel fungal α-glucosidase was heterologously expressed purified, and biochemically characterized. The enzyme exhibited broad substrate specificity, hydrolyzing substrates with α-(1→4) linkages and disaccharides containing α-(1→3), α-(1→2) and α-(1→6) linkages. The transglycosylation activity of this enzyme was evaluated using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). A diverse array of MOS and IMOS was detected in the reaction mixture, with kojibiose, maltotriose, isomaltotretraose, and panose identified as the main products. Maximum IMOS/MOS production was obtained when 93% of the initial maltose was hydrolyzed, and at this point of the reaction, a total of 70.34 g/L of IMOS and 25.62 g/L of MOS was produced, which corresponds to ~ 43 % (w/w) of the total carbohydrates in the reaction mixture. The enzyme also demonstrated the ability to utilize some polyphenols as alternative acceptors, successfully glycosylating piceid, hydroxytyrosol, and resveratrol. The broad acceptor specificity and efficient transglycosylation activity of this new biocatalyst make it a promising candidate for further exploration in enzyme-based production and modification of bioactive compounds.

Financial support and acknowledgments

PID2019-105838RB-C31/-C32, PID2022-136367OB-C31/C32 and PDC2022-133134C21/22. Comunidad de Madrid contratos predoctorales con Ref. PIPF-2022/BIO-25376.

Keywords

α-Glucosidase • isomaltooligosacharides • maltooligosacharidos • piceid • hydroxytyrosol

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

EXPLORING THE DIVERSITY AND METABOLIC POTENTIAL OF PREUSSIA FROM XEROPHYTIC AND MARINE HABITATS IN THE MEDITERRANEAN BASIN

Daniel Guerra-Mateo[1]; Víctor González-Menéndez[2]; José F. Cano-Lira[1]; Olga Genilloud[2]; Josepa Gené[1]
1. Universitat Rovira i Virgili; 2. Fundación Medina

Abstract
The genus Preussia represents one of the most diverse and metabolically active group of ascomycetes in the family Sporormiaceae (Pleosporales). It is primarily associated with soil, plant material, and dung, substrates rich in microbial life that demand an active metabolism to compete for nutrient sources. To date, more than 20 secondary metabolites have been identified among Preussia species, many of which exhibit antifungal and antibacterial properties. The discovery of such compounds is essential in an era of rapidly increasing drug-resistant pathogens. As a result, there is an increasing interest in exploring novel metabolites, particularly from fungi inhabiting extreme or poorly studied environments. In this context, the Mediterranean Basin stands out as a biodiversity hot-spot, where fungi from xerophytic and marine habitats remain largely underexplored. This study aimed to assess the diversity of Preussia in these habitats and characterize their secondary metabolites. Samples were collected from endemic xerophytic plants and lichens, gypsum soil and marine sediments. Preussia strains were identified using a polyphasic approach, combining morphology and phylogenetic analysis. Phylogenetic relationships were used to select representative strains from different clades with taxonomic and metabolic interest, including seven known species of Preussia and ten distinct phylogenetic lineages that may represent novel species. All those diverse taxonomic strains were cultivated in three liquid and two solid different media to promote metabolite production. The microbial extracts obtained were analysed by liquid chromatography coupled to high-resolution mass spectrometry to characterize their secondary metabolite profiles. The metabolic profiles of these lineages revealed 24 different molecules, including antimicrobials with antibacterial, antifungal or antiprotozoal activities (e.g., Deoxypreussomerin B, Monocerin, Palmarumycin, Preussin and Ravenelin), cytotoxic compounds (e.g., Leptosin), plant growth inhibitors (e.g., Resorcylide), protein transport inhibitors (e.g., Brefeldin), as well as 16 additional potential unknown metabolites produced by Preussia spp. that need further chemical and biological characterization. These results highlight the relevance of diversity surveys in underexplored areas and demonstrate the value of phylogenetic analysis as a cost-effective strategy in the discovery of new microbial bioactive compounds.

Keywords
Preussia • Phylogeny • Metabolites • Antimicrobials

SMART PEPTIDE DESIGN: AI-POWERED SYNTHETIC BIOLOGY TOOLS FOR NEXT-GENERATION ANTIBIOTIC DESIGN

Marc Torrent Burgas
Universitat Autònoma de Barcelona

Abstract

Antimicrobial peptides (AMPs) are among the most promising next-generation therapeutics, but their application has been hindered by challenges related to stability, toxicity, and inefficient delivery. Here, we explore whether integrating synthetic biology with computational frameworks can accelerate the discovery and development of new AMPs with improved pharmacokinetics and target selectivity. Addressing this question is critical, as conventional AMP design strategies often yield candidates with high bactericidal activity but poor pharmacological properties, leaving a gap between scientific innovation in the laboratory and clinically viable drugs.To overcome these limitations, we developed a modular, in vivo-mimetic computational platform capable of designing diverse peptide libraries with tunable physicochemical properties. Complementing this, we assembled a toolkit of AI-driven algorithms to predict and optimize peptide sequences for enhanced bacterial membrane penetration, targeted antibiotic delivery, and reduced host cytotoxicity. By iteratively combining wet-lab synthesis with in silico screening, we refined millions of putative sequences down to a small number of high-performing candidates within weeks—dramatically reducing the timescale of rational design.Preliminary results show that these AI-designed peptides are effective against both gram-negative and gram-positive bacteria, even at nanomolar concentrations, while maintaining serum half-lives exceeding six hours. Furthermore, toxicity assays reveal a therapeutic index improvement of over 30% compared to conventional AMPs. In murine infection models, these peptides reduced bacterial load by more than 2–3 log units, indicating strong in vivo efficacy. These findings demonstrate that our approach not only enhances AMP discovery but also yields functional delivery agents with translational potential.By transforming antibiotic design into a data-driven, high-throughput process, this work establishes a foundation for modular, user-defined peptides that can be rapidly adapted to address emerging resistance threats. Future efforts will focus on comprehensive in vivo efficacy testing, immunogenicity profiling, and integration with microfluidic screening platforms to enable on-demand synthesis of targeted antimicrobial therapeutics.

Financial support and acknowledgments

Financial support from the Spanish Ministry of Economy and Innovation (PDC2021-121544-I00 funded by MCIN/AEI /10.13039/501100011033 and European Union Next GenerationEU/ PRTR; project PID2020-114627RB-I00 funded by MCIN/AEI /10.13039/501100011033) and the Departament de Recerca i Universitats de la Generalitat de Catalunya (2023PROD00021). This work has been co-financed by the Spanish Ministry of Science and Innovation with funds from the European Union NextGenerationEU, from the Recovery, Transformation and Resilience Plan (PRTR-C17.I1) and from the Autonomous Community of Catalonia within the framework of the Biotechnology Plan Applied to Health.

Keywords

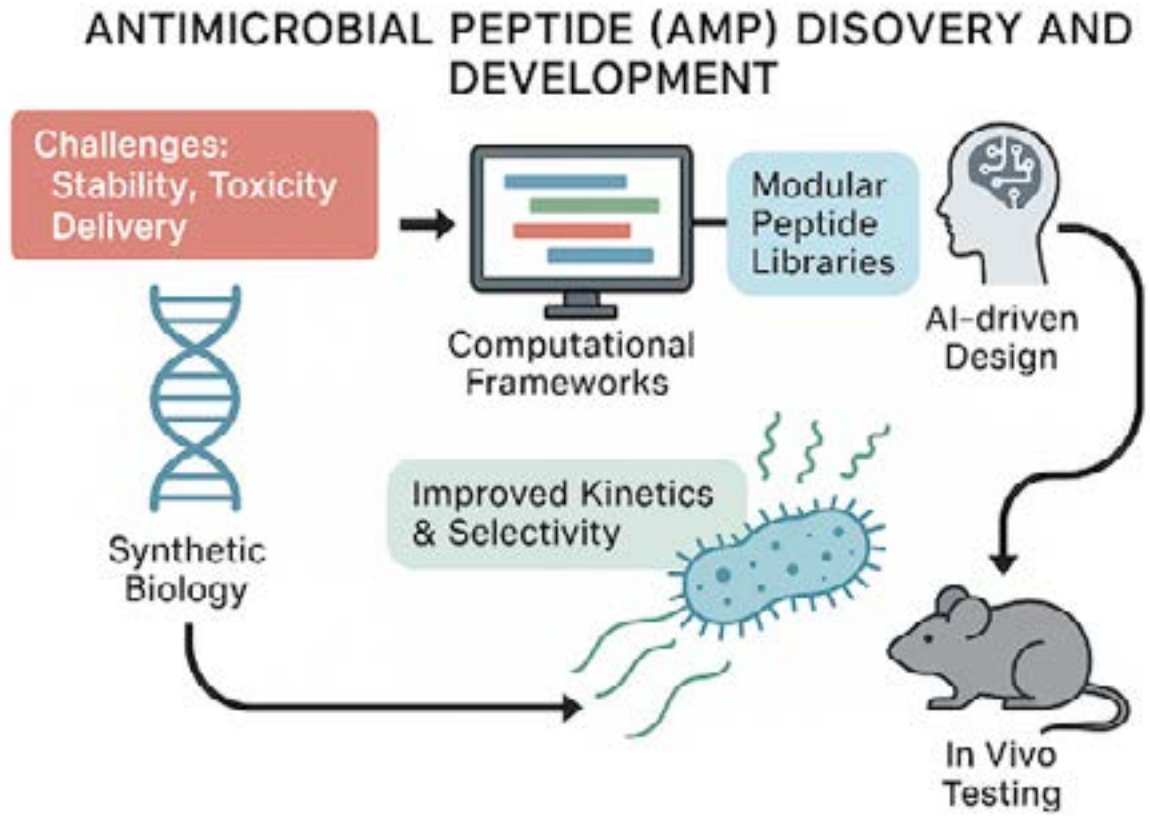
Antimicrobial peptide • Artificial intelligence • Protein and peptide design • Antibiotic discovery

continue –>

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

ORAL COMMUNICATIONS

Biotechnology and Health



July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

ORAL COMMUNICATIONS
Biotechnology and Health

LIPIDOMIC AND TRANSCRIPTOMIC PROFILING OF HEK293-DERIVED EXTRACELLULAR VESICLES AND HIV-1 GAG VIRUS-LIKE PARTICLES REVEALS FUNCTIONAL AND SAFETY IMPLICATIONS

Marc García-Trujillo[1]; Jesús Lavado-García[2]; Pol Pérez-Rubio[1]; Laura Cervera[1]; Francesc Gòdia[1]
1. Universitat Autònoma de Barcelona; 2. Technical University of Denmark

Abstract
Virus-like particles (VLPs) and extracellular vesicles (EVs) are promising platforms for drug delivery and vaccine development. Despite their shared vesicular nature, limited comparative analyses exist regarding their RNA and lipid cargo. This study presents a comprehensive multi-omic analysis of VLPs derived from HIV-1 Gag and EVs produced in HEK293 cells, aiming to identify compositional differences that may influence their functionality and safety.

Transcriptomic profiling revealed broader and more pronounced RNA cargo changes in VLPs compared to EVs, with significant enrichment of mRNAs related to protein degradation, RNA regulation, and translation. In contrast, EVs derived from cells grown to confluence showed upregulation of histone-encoding mRNAs, suggesting transcriptomic shifts linked to cell density.mRNA transcripts from adenoviral genes E1A, E1B, and IX, integrated into the HEK293 genome from its original transformation with adenovirus type 5 DNA, were detected in all vesicle populations, with a marked enrichment in VLPs. This raises biosafety concerns, as such transcripts could also be present in other HEK293-derived products, including viral vectors intended for clinical use.EVs and VLPs also contained microRNAs (miRNAs) with known tumor suppressor or oncogenic roles. The presence of tumor suppressor miRNAs could be harnessed to support therapeutic strategies in oncology, particularly by modulating gene expression in favor of antitumor responses. However, the co-packaging of oncogenic miRNAs raises important safety concerns, as their unintended delivery may promote proliferation, immune evasion, or metastatic behavior in recipient cells. These findings underscore the need for rigorous evaluation and selective cargo control in vesicle-based therapeutic strategies.

Motif enrichment analysis of mRNAs and lncRNAs revealed distinct RNA signatures across vesicle types, including motifs associated with RNA-binding proteins and viral packaging elements. This suggests opportunities to engineer vesicles with customized RNA cargo by exploiting motif-mediated selection mechanisms.Lipidomic analysis showed reduced total lipid content in VLPs compared to EVs. However, VLPs were enriched in lipids linked to membrane stability, such as cholesterol and sphingomyelin, consistent with their assembly through lipid raft domains. Overall, these results reveal critical differences in RNA and lipid composition between VLPs and EVs and underscore the importance of detailed molecular characterization in the design of vesicle-based therapeutic platforms. This work provides a valuable resource for guiding the rational engineering of VLPs and EVs tailored to specific clinical applications.

Keywords
Transcriptomics • Lipidomics • HEK293 cells • Virus-like particles • Extracellular vesicles

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

RESILIENCE AND MICROBIAL ADAPTATION OF A LACTATE-DRIVEN DARK FERMENTATION PROCESS UNDER OPERATIONAL DISTURBANCES

Ysis E. Lanzoni-Vargas[1]; Issac A. Gómez-Gutiérrez[2]; Hugo Méndez-Acosta[2]; Alma Toledo-Cervantes[2]; Raúl Muñoz[1]; Sara Cantera[1]; Cristina Martínez-Fraile[1]; Octavio García-Depraect[1]
1. Institute of Sustainable Processes, University of Valladolid, Valladolid, Spain; 2. Department of Chemical Engineering, CUCEI-Universidad de Guadalajara, Guadalajara, Jalisco, México

Abstract
In recent years, there has been a growing interest in biological hydrogen production as a sustainable alternative for clean energy. Within this context, lactate-driven dark fermentation (LD-DF) has emerged as a promising biotechnology due to its favorable metabolic features and compatibility with organic waste valorization (Regueira-Marcos et al., 2024; Leroy-Freitas et al., 2024). However, relatively little research has been carried out on the operational robustness of LD-DF, especially under disturbance conditions that commonly occur in real-world applications. This study set out to investigate the resilience of an LD-DF process operated under mesophilic conditions when exposed to two types of relevant disturbances: substrate interruption (feast/famine cycles) and temperature shocks. The investigation not only focused on hydrogen production performance but also examined microbial community dynamics in response to these perturbations. Experiments were conducted using a two-stage continuous stirred tank reactor (CSTR) system, operated for 57 days at 37 °C and fed with a simulated household food waste substrate using a mixed microbial inoculum. Process stability, volumetric hydrogen production rates (VHPR), and energy outputs were monitored across multiple perturbation–recovery cycles. The results demonstrated that the system exhibited a high degree of resilience under both perturbation types. Following the feast/famine perturbations, which were applied in two independent cycles, hydrogen production initially dropped but was successfully restored during the recovery phases, with volumetric hydrogen production rates (VHPR) consistently exceeding 1.2 L H2 L^{-1} d^{-1} in the post-recovery steady states. In the case of temperature shocks, also applied in duplicate, the process exhibited a more pronounced inhibition, with VHPR decreasing to as low as 0.09 L H2 L^{-1} d^{-1} at 20 °C. Nevertheless, under restored mesophilic conditions, the system gradually recovered, ultimately achieving the highest VHPR recorded (1.97 L H2 L^{-1} d^{-1}) in the final post-perturbation phase. These findings highlight the ability of LD-DF systems to adapt and maintain functional performance following operational stress. 16S rRNA gene amplicon sequencing revealed a functional restructuring of the microbial community throughout the study. Initially dominated by homofermentative lactic acid bacteria (*Lactobacillus, Lactococcus*), the community progressively shifted toward hydrogen-producing genera, such as *Clostridium sensu stricto* and *Ruminococcaceae*, particularly during recovery and stabilization phases. These shifts were closely associated with improved hydrogen production. Overall, this study provides new insights into the adaptive capacity of LD-DF systems, contributing to the understanding of how microbial consortia respond to stress and guiding the development of resilient biohydrogen production technologies.

Financial support and acknowledgments
This research was funded by the Spanish Ministry of Science and Innovation (PID2022-139110OA-I00) project CIRCULAR-BIOMED. The financial support from the Regional Government of Castilla y León (UIC393) are also gratefully acknowledged.

Keywords
Environmental Biotechnology • Bioenergy • Circular economy • Biohydrogen • Waste management

BIOCONVERSION OF LIGNIN WASTE INTO NEW CHEMICAL BUILDING BLOCKS FOR THE SUSTAINABLE PRODUCTION OF BIO-BASED PLASTICS

Carlos del Cerro Sánchez; Helena Gómez Álvarez; Pablo Espada Núñez; Gonzalo Durante Rodríguez; Eduardo Díaz Fernández
Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC)

Abstract

Plant-derived waste management represents a major challenge especially due to lignin recalcitrance. This phenolic polymer is a major carbon an energy reservoir, accounting for the 30% of the organic carbon in the biosphere, however it is usually inefficiently burned to produce energy and hence contributing to the production of green-house gases and global warming. On the other hand, more than 70% of the plastics made from fossil fuels end discarded or leaked into the environment with a huge negative impact in the environment and our societies. For these reasons, bioconversion processes capable of valorizing lignin into new sustainable plastic materials constitute green alternatives capable of integrating lignin and plastics in a closed-loop waste management model. Environmental biotechnology approaches can be used to design new microbial platforms capable of efficiently bioconvert lignin-derived molecules into platform chemicals. Taking this into consideration, the model bacterium Pseudomonas putida KT2440 has been used extensively for lignin valorization purposes as an optimal biocatalyst. Using synthetic biology strategies in this bacterium we have been able to design, implement, and evaluate new processes to channel lignin-derived monomers and dimers into novel chemical building blocks for the synthesis of bio-based plastics. More specifically, we have engineered a bioprocess to metabolize the biphenyl 5,5'-dehydrodivanillic acid (DDVA), an abundant model lignin dimer with C5–C5 linkage, into 5-carboxyvanillic acid (5CVA), which was recently shown to be a promising building block for the synthesis of new polyesters with enhanced properties. Synthetic DNA modules encoding metabolic and transport steps from the DDVA degradation pathway of the bacterium Sphingobium lignivorans SYK6, including a TonB-dependent outer-membrane transporter, were successfully engineered, and a KT2440-based recombinant biocatalyst reached a 5CVA yield of 30% in a resting cell process. On the other hand, metabolic engineering approaches were used to generate two additional P. putida KT2440-based biocatalysts for the bioconversion of two lignin derived monomers that have not been previously addressed, i.e., 4-hydroxyphenylacetic acid and homovanillic acid. These phenolic compounds were efficiently converted into a promising building block, a pyridine dicarboxylic acid, that can work as an analogue of the petrochemical terephthalate for the production of bio-based plastics. These bioconversions were further optimized and validated using alkaline lignin depolymerization mixtures. All these unprecedented results expand the current bacterial metabolic funnel to biologically valorize the wide spectrum of aromatic compounds derived from complex lignin depolymerization mixtures.

Financial support and acknowledgments

Funding was provided by grant PID2022-142540OB-I00 of the Spanish Ministry of Science, Innovation and Universities MICIU/AEI/10.13039/501100011033, by ERA CoBiotech grant PCI2019-111833-2 (MILIMO), by CSIC's PIE 202420E138, and by the RELAY project supported by a fellowship from the Fundación General CSIC's ComFuturo program which has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement no. 101034263.

Keywords

environmental microbiology • metabolic engineering • plant biomass valorization • bioplastics • lignin • synthetic biology

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

TOWARDS SUSTAINABLE CO_2 CONVERSION: INTEGRATING ELECTROCHEMICAL CO_2 REDUCTION AND MICROBIAL FERMENTATION TO PRODUCE 3-HYDROXYPROPIONIC ACID

Zainab Ul Kausar[1]; Albert Guisasola Canudas[1]; Juan Antonio Baeza Labat[1]; Mira Sulonen
1. Universitat Autònoma de Barcelona; 2. VTT Technical research center

Abstract

Carbon capture and utilization strategies seek to transform CO_2 into valuable products, providing a sustainable alternative to fossil-based production. In this study, CO_2 is electrochemically reduced to formate (eCO2RR), which serves as an energy source for Komagataella phaffii to produce 3-hydroxypropionic acid (3-HP), a valuable platform chemical used in biodegradable plastics and specialty chemicals. A key challenge in this integration is to ensure that the medium supports both electrochemical activity and microbial growth. To enhance sustainability, NH_4^+, an essential fermentation nutrient, is recovered from wastewater using a bioelectrochemical system (BES) and incorporated into the fermentation process. This proof of concept demonstrates CO_2 valorization and nutrient recovery for sustainable microbial production.

The eCO_2RR was carried out in a 1L single-chamber reactor using an indium-deposited graphite cathode and a platinode anode. NH_4^+ recovery was carried out in a 1L three-chamber BES with N-rich synthetic wastewater in the anode chamber 2. Electron flow facilitated NH_4^+ transport across a cation exchange membrane, where alkaline conditions in the cathode chamber partially converted NH_4^+ to NH3. NH3 was then recovered in a separate chamber using a gas diffusion electrode. Fermentation was conducted in 1L bioreactors with a genetically modified K. phaffii strain, utilizing methanol as a carbon source. To establish a baseline for process integration, a 3-step setup was tested, running each process separately under optimal conditions. Four 1L bioreactors were operated in parallel: (1) Control (commercial NH_4^+, no formate), (2) BES-recovered NH_4^+, (3) eCO_2RR-recovered formate, and (4) both BES-recovered NH_4^+ and eCO_2RR-recovered formate. The control reached 6.84 g/L of 3-HP. BES-recovered NH_4^+ increased titer by 6.4% (7.28 g/L), while formate from eCO_2RR boosted it by 16.4% (7.96 g/L). The fully integrated system (BES-recovered NH_4^+ + eCOvRR-recovered formate) achieved 8.16 g/L, demonstrating the potential of combining CO2 conversion and nitrogen recovery for enhanced 3-HP production. This trend was also reflected in 3-HP yield per methanol, with the fully integrated system reaching 0.26 g/g, an 18% increase over the control (0.22 g/g). To further optimize integration, a 2-step approach was explored, where eCO_2RR and fermentation were performed sequentially in the same reactor. Extensive medium optimization was required to balance electrochemical activity with microbial growth. The full presentation will also cover results from this strategy, including sequential and continuous operation, where eCO_2RR and fermentation occurred simultaneously. By directly linking eCO_2RR with microbial fermentation and incorporating BES-recovered NH_4^+, this approach enhances resource efficiency and advances sustainable bioproduction.

Financial support and acknowledgments

This work was supported by project 'innoVative blo-based chains for CO2 VALorisation as aDded-value organic acids' - VIVALDI (ID: 101000441) from the Horizon 2020 program of the European Commission.

Keywords

3 Hydroxypropionic acid • Ammonium recovery • CO_2 electroreduction

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

PROTEOME ANALYSIS OF THE PSEUDOMONAS PUTIDA ADAPTATION TO EPOXY RESIN SUBSTRATES

Álvaro Mourenza[1]; Sonia García-Alonso[1]; Ana Ibañez[1]; Lara Carretero-López[1]; Tonje M. B. Heggeset[2]; Marcia Barquero[1]; Mihai L. Lomanar[1]; Sonia Garrido-Chamorro[1]; Elías R. Olivera[1]; Carlos Barreiro[1]
1. Área de bioquímica y biología molecular, Departamento de biología molecular, Universidad de León; 2. Department of Biotechnology and Nanomedicine, SINTEF Industry S

Abstract
Epoxy resins are among the most important polymeric substances in the thermosetting category with a critical role in composite materials. These resins Epoxy resins from petroleum-derived monomers have exceptional characteristics, including tensile strength, elevated stiffness, and outstanding electrical strength, which makes them a preference material in industrial sectors such as aerospace, construction, and automotive. However, their highly crosslinked structure severely limits reprocessing and recycling options, leading to environmental accumulation. Addressing this challenge, the EU-funded ESTELLA project (Project No. 101058371, Call: HORIZON-CL4-2021-RESILIENCE-01-11) explores the potential microbial degradation of epoxy resins.

As a first approach in the EU-funded ESTELLA Project, different bacteria associated to a milled commercial epoxy matrix (HexFlow® RTM 6) were isolated. These bacteria were identified via MALDI-TOF MS (Bruker Biotyper) characterization, and their taxonomic classification was confirmed by 16S rDNA sequencing. One bacterium that was repetitively isolated from different epoxy resins samples was identified as Pseudomonas putida ULE23_002. The whole genome sequencing of this strain using MiniON and Illumina technologies revealed that it is composed of a circular chromosome of 6.59 Mb containing 6,065 putative coding sequences and with a GC content of 61.8%. Isogenic P. putida strain was cultured in a minimal medium containing samples of milled RTM 6 resin as only possible carbon and energy sources. Surprisingly, this strain survived for more than two months of incubation. However, no significative increase in the CFUs from the culture was observed. On the other hand, scanning electron microscopy of the solid RTM 6 particles showed cells attached to the surface forming a biofilm. The adaptation and survival mechanisms of this strain on the epoxy substrate are being analyzed using multi-omics approaches, including label-free proteomics and transcriptomics (RNAseq).

Our findings shed light on the strain's unique metabolic strategies to cope with and possibly degrade recalcitrant epoxy compounds. These results pave the way toward bio-assisted recycling processes for thermoset composites, highlighting the role of environmental isolates with metabolic flexibility.

Financial support and acknowledgments
ESTELLA project (Design of bio-based thermoset polymer with recycling capability by dynamic bonds for bio-composite manufacturing) (project nº: 101058371) funded by European Union by Horizon Europe program (call: HORIZON-CL4-2021-RESILIENCE-01-11); https://estellaproject.eu/

Keywords
Epoxy resin • Pseudomonas putida • Proteomics

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

SCALABLE PROTEIN PRODUCTION BY KOMAGATAELLA PHAFFII ENABLED BY ARS PLASMIDS AND CARBON SOURCE-BASED SELECTION

Florian Weiss[1]; Guillermo Requena-Moreno[2]; Arnau Gasset[2]; Miguel Angel Nieto-Taype[2]; Carsten Pichler[1]; Francisco Valero[2]; Anton Glieder[1]; Xavier Garcia-Ortega[2]
1. Christian Doppler Laboratory for Innovative Pichia pastoris host and vector systems, Institute of Molecular Biotechnology, Graz University of Technology, Graz, Austria; 2. Christian Doppler Laboratory for Innovative Pichia Pastoris Host and Vector Systems, Department of Chemical, Biological and Environmental Engineering, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Bellaterra, Spain

Abstract
The yeast Komagataella phaffii (formerly Pichia pastoris) is a widely used host for recombinant protein production. Traditionally, expression constructs are stably integrated into the genome, but this approach often leads to clonal variability due to differences in gene copy number and integration loci (NHEJ recombination), ultimately affecting protein yield. This variability limits the efficiency of high-throughput applications such as enzyme engineering, drug screening, and the comparative evaluation of genetic regulatory elements (new promoters). While circular episomal plasmids with autonomously replicating sequences (ARS) offer a promising alternative, they are inherently unstable in K. phaffii unless continuous selection pressure which is typically achieved through antibiotic resistance or auxotrophic markers, which are not ideal for scalable bioprocesses.

In this study, we developed a methanol-free, scalable episomal expression system using ARS plasmids that employ genes from the glycerol metabolism pathway—GUT1 (glycerol kinase 1) and TPI1 (triosephosphate isomerase 1)—as antibiotic-free selection markers. Expression both was driven by the Ashbya gossypii TEF1 promoter, chosen for delivering high transformation efficiencies and offering the best performance with the reporter protein (CalB, lipase B from Candida antarctica).

Initial screening in micro-scale deep-well plate cultivations showed high transformation efficiencies and robust expression of the reporter protein in both GUT1- and TPI1-based systems. In shake flask cultivations, the GUT1-based episomal strain outperformed a reference strain with chromosomally integrated expression cassette, showing a 46% increase in CalB activity. The TPI1-based strain also showed good performance, albeit slightly below that of the integrated control.

Most notably, in lab-scale bioreactor cultivations under methanol-free fed-batch conditions, the GUT1 episomal system achieved a 100% increase in CalB activity compared to the integrated system. These results highlight the potential of the GUT1 marker by enabling stable and reliable episomal-based protein expression system, allowing to achieve high titers without the need for antibiotic selection.

This work demonstrates, for the first time, a scalable and methanol-independent episomal expression system in K. phaffii. It also expands the molecular toolbox for this host with effective, selection-marker strategies based on native metabolic genes.

Financial support and acknowledgments
The authors kindly thank the Christian Doppler Gesellschaft and the industrial partners Novo Nordisk, Bionet, Aminoverse, BioGrammatics, SeSam Biotech, ZETA, and SolarBiotech for funding of the Christian Doppler Laboratory for Innovative P. pastoris host and vector systems, in which GRM, MAN, AG, FV and XGO are contributing as members of the UAB team. We are also grateful for the valuable discussion of all experiments and results with Kjeld Olesen & Sanne Jensen from Novo Nordisk A/S.

Keywords
Komagataella phaffii • Pichia pastoris • Episomal ARS plasmids • Methanol-free bioprocess • Carbon source marker

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

2,3-BUTANEDIOL PRODUCTION FROM NON-CONVENTIONAL CARBON SOURCES USING DIFFERENT MICROBIAL CHASSIS

Irene Cano; Isabel de la Torre: Miguel G. Acedos; Jorge Barriuso; José L. García
CIB-CSIC

Abstract

Promoting the transition toward a circular economy is essential in today's society to foster more sustainable and environmentally efficient industrial practices and address climate change. In this context, biotechnology and particularly the use of microorganisms to convert industrial residues into value-added products, has attracted growing interest. Research efforts have increasingly focused on exploring the versatility of microbial metabolism for such applications.

Acetate is a promising carbon source for microbial bioprocesses and can be derived from various origins, including lignocellulosic biomass and carbon dioxide through the action of acetogenic bacteria. However, its application has certain challenges, particularly due to its toxicity at high concentrations for microbes, the low energy content, and the scarce understanding of the microbial pathways involved in acetate metabolism for some biotechnological processes.

Our research focuses on the microbial production of 2,3-butanediol (2,3-BDO) in Escherichia coli W and Pseudomonas putida KT2440, using acetate as the primary carbon source. 2,3-BDO is a valuable secondary alcohol with a broad range of industrial applications, including as antifreeze agent and precursor in plastic manufacturing. Producing 2,3-BDO heterologously in non-pathogenic microbial hosts supported by metabolic engineering and process optimization presents a promising strategy for sustainable and scalable industrial production. Through the heterologous expression of the budABCoperon from Klebsiella oxytoca, one of the most efficient microorganisms for 2,3-BDO production, optimal conditions for the biosynthesis of this secondary alcohol in E. coli and P. putida have been established using acetate as carbon source. These production strategies were refined through bioreactor experiments and guided by genome-scale metabolic models (GSMMs).

Financial support and acknowledgments

This work has been funded by the European project CO2SMOS (H2020 101000790).

Keywords

2,3-butanediol • Acetate metabolism • Bioreactor optimization • Microbial bioprocesses • Circular economy • Value added compounds • Escherichia coli / Pseudomonas putida

TOWARD ENHANCED EXOPOLYSACCHARIDES PRODUCTION BY LEUCONOSTOC CITREUM CNTA 860: MULTIFACTORIAL SCREENING AND KINETIC VALIDATION

Nicolás Armendáriz[1]**;** Miguel Gastón[1]; Lara Pérez[1]; Leticia Ortiz[1]; Dante Fratebianchi[1]; Victoria E. Santos Mazorra[2]; Alberto García[2]
1. CNTA; 2. University Complutense of Madrid

Abstract

Exopolysaccharides (EPS) are valuable biopolymers used in food, pharmaceuticalsand biomaterials. However, insufficient understanding of the influence of processfactors limits the optimisation of their production. Previous studies havecharacterized EPS production by L. citreum under different media and conditions,particularly in food-related applications. In this study, we present the results of amultifactorial screening aimed at identifying influential factors for EPS production by L.citreum CNTA 860 as well as a fermentation kinetics experiment for futuremathematical modelling. Through this study, a framework of conditions for EPSprocess optimisation and scale-up is provided.

A two-level fractional factorial design was used to evaluate the effect of six operationalfactors on EPS production by L. citreum. Sixteen trials were carried out in ApplikonMiniBio bioreactors (400 mL). The factors and levels studied were: A) Temperature(25-30 °C), B) Agitation (50-600 rpm), C) Carbon/Nitrogen ratio (10-100 g C/g N), D) Initial sucrose concentration (70-150 g/L), E) Sucrose pulse frequency (0-12 h) and F) Inoculum concentration (106-108 CFU/mL), defined from previous experience with thestrain L. citreum CNTA 860.

Two main responses were analysed: culture viscosity (cP) and EPS concentration (g/L) measured at 6, 12, 19.5 and 24h. Statistical analysis was conducted with StatEase® software, applying ANOVA and regression models to identify the most influentialfactors.

As part of the study, a time course experiment was conducted over time under theexperimental conditions of experiment 3 of the two-level fractional factorial design,which yielded the highest viscosity and EPS concentration. This trial allowed recordingthe kinetic evolution of biomass, EPS, glucose, fructose, sucrose, mannitol, lactic acid,acetic acid and viscosity during the whole fermentation process. From this data, akinetic model will be developed in Python to simulate the dynamics of the system andfacilitate future scaling strategies.

MultifactorialScreeningDuringcultivation,agitation(B),initialsucroseconcentration(D)andInoculumconcentration (F) significantly influenced viscosity and EPS, with AE and BDinteractions relevant between 6-19.5 h. At later stages, D was the most importantfactor. The models for viscosity showed excellent fit ($R^2 > 0.93$). In EPS, the fit wasmore irregular but consistent at key stages.Kinetic EvolutionThe fermentation kinetics under the conditions of experiment 3 of the two-levelfractional factorial design are shown in Figure 1. EPS concentration reached its firstmaximum before the viscosity peaked, this behaviour indicates a non-simultaneousrelationship which is interesting to understand the evolution of the properties of thesystem. Figure 1. Fermentation kinetics.

As a next step, a kinetic model describing the evolution of compounds will bedeveloped in Python, inspired by previous kinetic modelling strategies applied tobioprocesses. The goal is to create a predictive tool that contributes to processoptimisation, facilitates decision making for process scale-up and improves the overallefficiency of the bioprocess through simulation-based strategies.

Financial support and acknowledgments

The work developed has been possible thanks to the financing of the Government of Navarra

Keywords

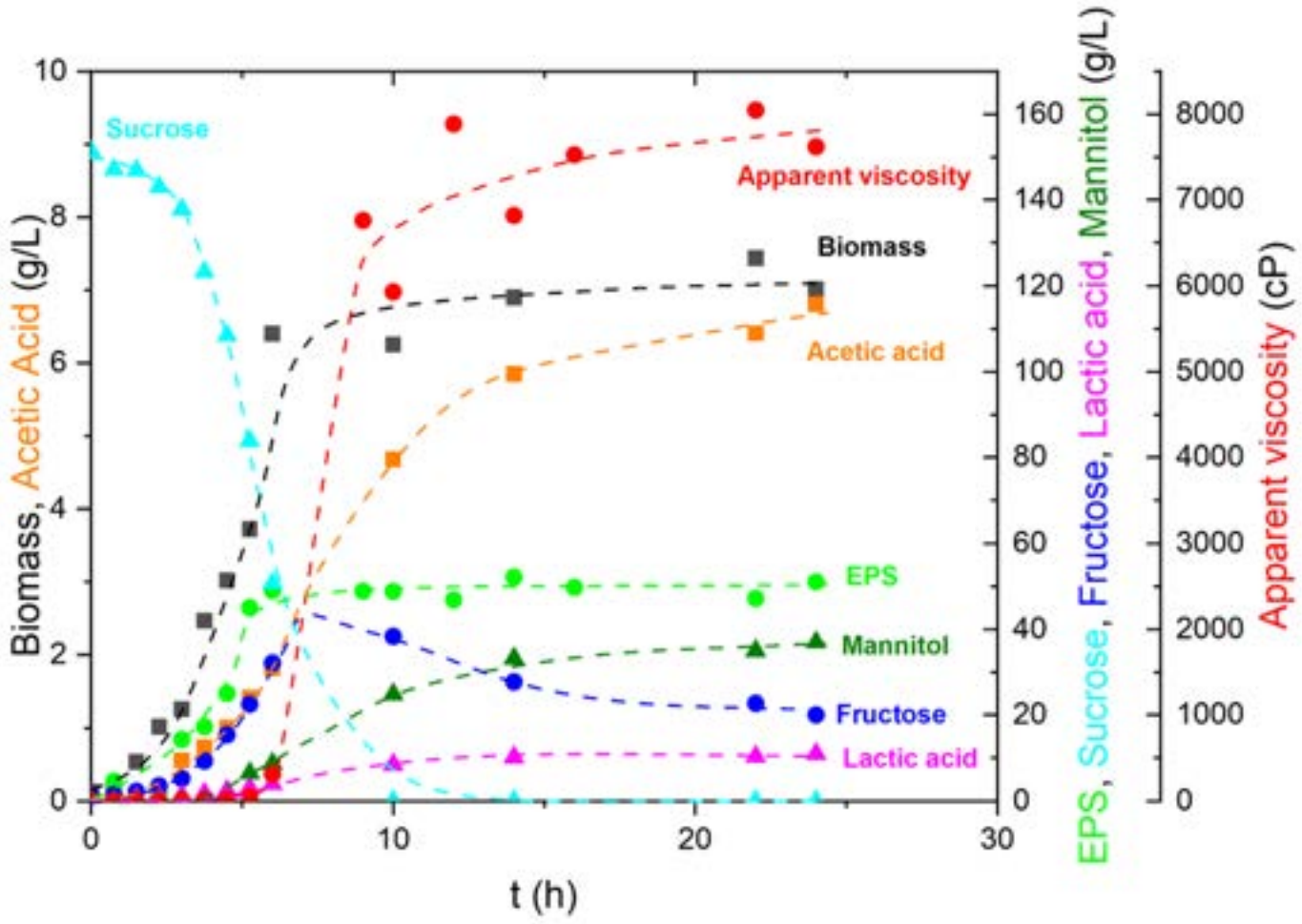
Exopolysaccharides (EPS) • Leuconostoc citreum • Multifactorial screening • Fermentation kinetics • Kinetic modeling • Process optimisation

continue –>

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

ORAL COMMUNICATIONS

Industrial Biotechnology



July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

OPTIMIZATION OF CELL-SPECIFIC PERFUSION RATE IN CONTINUOUS PERFUSION CULTURE OF CHO CELLS PRODUCING MABS

Manuel Alonso Moreda; Paula Pérez Mateos; Pol Pérez Rubio; Laura Cervera Gracia; Francesc Gòdia Casablancas
Departament d'Enginyeria Química, Biològica i Ambiental. Universitat Autònoma de Barcelona

Abstract

CHO cell culture has become the dominant biomanufacturing platform meeting the growing demands and associated quality standards of the monoclonal antibody (mAb) market. Even most of the actual manufacturing processes still rely on fed- batch, there is renewed interest in continuous perfusion due to its potential for higher productivity and process efficiency.

In this context, we investigate the optimal Cell Specific Perfusion Rate (CSPR) necessary to maintain high cell density in culture, while minimising media consumption, key factors for scalable and cost-effective continuous bioprocessing. Pseudo-perfusion experiments at Erlenmeyer flask scale testing various CSPR levels highlighted the importance of nutrient availability. The results indicated that a minimum CSPR of 50 pL/(cell·day) is essential to maintain high cell viability and achieve steady-state conditions. However, at a viable cell density of 25·106cells/mL, cultures exhibited nutrient limitations that constrained growth, even as they achieved the highest specific and volumetric productivities. In contrast, perfusion at bioreactor scale did not show glucose limitations. The difference in glucose consumption rates could be attributed to the continuous feeding available at the bioreactor setup. Moreover, the culture behaved quite differently at higher cell densities. At 35·106cells/mL the specific glucose consumption rate was reduced, and the metabolism favoured growth over mAb production. Hence, a scale-down pseudo-perfusion model was used to identify CSPR values to be used in perfusion at different cell concentration and correctly implemented in a bench- scale bioreactor reaching steady-state culture at viable cell densities of 25·106 and 35·106 cells/mL minimizing the lactate formation and medium consumption.

Keywords

Perfusion Culture • CHO Biomanufacturing • Cell Specific Perfusion Rate (CSPR) • mAb production

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

A GENETIC FRAMEWORK OF GENES INVOLVED IN CALLUS GROWTH

Fuensanta Verdú Navarro[1]; Ángela Espín Veracruz[2]; Alberto Gila Navarro[2]; Pedro J. Navarro Lorente[3]; David Borraz Moron[3]; Eduardo Salas Fernández[3]; Julia Weiss[2]; Marcos Egea Cortines[2]
1. Bioprocess Pilot Plant, Bionet, Spain; 2. Instituto de Biotecnología Vegetal, Universidad Politécnica de Cartagena, Spain; 3. División de Sistemas e Ingeniería Electrónica (DSIE), Universidad Politécnica de Cartagena, Spain

Abstract
The capacity to form totipotent tissues from developed plants has been a major cornerstone in plant genetics and biotechnology. It allows both transgenic approaches and the use of cell cultures as platforms for biomolecule production. Despite the use of in vitro culture for over 100 years, the basic concepts of breeding, or the genetic analysis of callus growth have remained unexplored. We developed a screening method based on artificial vision and IA to classify calli size. We screened a total of 27648 calli comprising 1728 wild type and 25920 mutants from 2880 T-DNA lines of Arabidopsis thaliana. We identified 190 mutants with larger calli than WT and 2456 with smaller calli. We further identified 19 lines that were significantly larger and 988 smaller than the plate WT. We performed a GEA based on the GO assigned to the identified loci and found that smaller calli were affected in many different pathways and did not show enrichment for a given process, while larger calli appeared affected in genes coding for hormone biosynthesis. We found that vegetative size of larger mutants classified further into those with overall larger biomass, and a second group with equal or lower biomass than wild type indicating that some of the loci identified may be specific for in vitro growing conditions. Our work is the first to identify genes involved in callus growth and opens new possibilities to improve plant biotechnology in a crucial step in many processes.

Financial support and acknowledgments
This work is funded by Proyecto PID2021-127933OB-C21 financed by MICIU/AEI/10.13039/501100011033 and FEDER to MEC and JW, Catedra BIONET in Biotechnology and project AGROALNEXT from Plan de Recuperación y Resiliencia. Grant DIN2020-011559 funded by MICIU/AEI/10.13039/501100011033 and by “European Union NextGenerationEU/PRTR”to FVN.

Keywords
TDNA mutants • Arabidopsis • AI • Increase growth • In vitro culture • Callus culture

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

DEVELOPMENT OF MICROALGAE CHLOROPLASTS AS A GREEN CHASSIS FOR SYNTHETIC BIOLOGY AND PRODUCTION OF VALUABLE RHAMNOLIPID BIOSURFACTANTS

Marçal Gallemí[1]; Xavier Melero-Cobo[1]; Xavier Ortiz[2]; Marc Carnicer[1]; Elena Monte[3]; Antoni Planas[1]; Pablo Leivar[1]
1. Laboratory of Biochemistry, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain; 2. Environmental Laboratory, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain; 3. Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, Barcelona, Spain

Abstract

RhamnoLipids (RL) are a class of glycolipids conformed by a glycosyl head group (one or two rhamnoses) and a 3-(hydroxyalkanoyloxy)alkanoic acid (HAA) fatty acid tail. They are preferred biosurfactants thanks to their excellent physicochemical and biological activities, and enhanced environmental biodegradability. Despite their interest in industry, RL use is currently limited due to their challenging and costly production, mostly obtained as a mixture of RLs from the pathogenic bacteria Pseudomonas aeruginosa. Aided by the development of novel synthetic biology tools (SynBio), unicellular photosynthetic microalgae are emerging as important sustainable hosts for industrial biotechnology, as they can use photosynthetic light to efficiently transform CO_2 into complex and valuable bioproducts of interest. In particular, the chloroplast organelle is being developed as a SynBio chassis as it contains its own genome (the plastome) and some interesting advantages such as high recombinant protein titers and a diverse and dynamic metabolism. Recently, we have developed a modular cloning toolkit for chloroplast expression in Chlamydomonas reinhardtii (MoCloro).

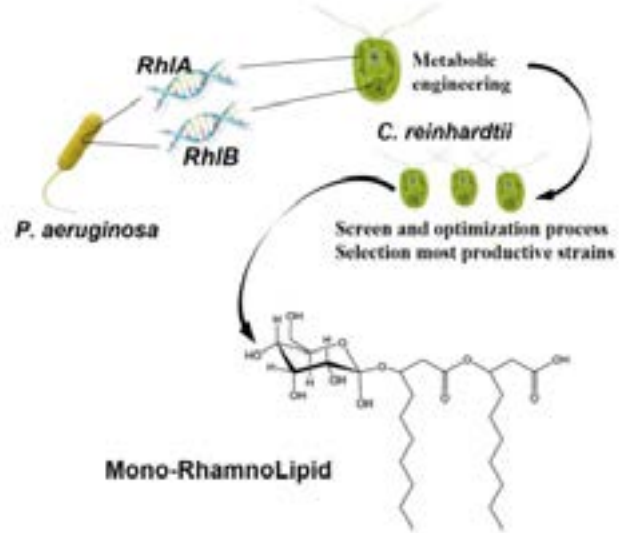
Previous studies in our laboratory expressed P.aureginosa RhlA acyltransferase in C. reinhardtii. This enzyme catalyzes the condensation of two 3-hydroxyacyl acid (3-HA) precursors to produce HAA. HAA congeners of varying carbon length (e.g. C10-C10, C10-C8, C10-C12) were identified by GC-MS and UHPLC-QTOF, and quantified by GC-FID. These results indicated that RhlA enzyme is active in the chloroplast and is able to access the 3-HA intermediaries of the fatty acid synthase cycle to produce a new pool of HAA. We are using the MoCloro for simultaneous expression of RhlA and RhlB, a P.aureginosa rhamnosyltransferase that transfers a rhamnosyl unit to HAA to produce target mono-RL. We aim to improve the expression of those transgenes and the total production of RL to contribute to the development of an alternative clean, safe and cost-effective platform for the sustainable production of RLs in microalgae.

Financial support and acknowledgments

This work was supported by MICIU/AEI, Spain. M.G. acknowledges a Beatriu de Pinòs postdoctoral fellowship from AGAUR, Generalitat de Catalunya and XM-C acknowledges a predoctoral fellowship from Institut Químic de Sarrià.

Keywords

Rhamnolipid • Chlamydomonas • Microalgae • Cell factory



Panel ID BC1 • Abstract ID 13

RECOVERY OF POLYPHENOLS FROM THE OLIVE OIL INDUSTRY AND THEIR ENZYMATIC MODIFICATION FOR ADAPTATION TO END-USERS: THE LIFE CYCLOPS PROJECT

Francisco J. Plou Gasca; David Rodríguez-García; Lucía Fernández-Arrojo; Carlos Uceda-Domínguez; Jose Luis González-Alfonso
Instituto de Catálisis y Petroeloquímica, ICP-CSIC, 28049 Madrid

Abstract

Polyphenols are a group of natural compounds found in various plant-based sources, known for their diverse biological activities. They play a key role in shielding cells from the damaging effects of reactive oxygen species (ROS), which contribute to conditions like neurodegeneration, inflammatory diseases, diabetes, and rheumatoid arthritis. Including foods rich in polyphenols in the diet is vital for promoting overall health and preventing chronic illnesses, making them an indispensable part of a balanced and healthy lifestyle.

The European project LIFE CYCLOPS (http://life-cyclops.eu) focuses on creating a sustainable technology to recover and utilize polyphenols from waste produced by the olive oil and wine industries. This initiative stands out for its environmentally friendly approach, using water instead of organic solvents for extraction processes, thereby improving ecological compatibility. The project aims to process 100% of the olive pomace (alperujo) produced at Borges Agricultural & Industrial Edible Oils (BAIEO) in Spain. It promotes a circular economy and zero-waste model by transforming these residues into high-value-added products for industries such as pharmaceuticals, nutraceuticals, and cosmetics.

Advanced analytical methodologies including High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) and Two-Dimensional Nuclear Magnetic Resonance Spectroscopy (2D-NMR) have been employed to systematically profile the phytochemical composition of olive pomace extracts. Initial findings identified hydroxytyrosol and tyrosol as dominant phenolic constituents, both recognized for their significant antioxidant properties and potential health benefits.

To tailor polyphenols for specific applications, glycosylation serves as an effective strategy to enhance their water solubility and stability, potentially boosting bioavailability and therapeutic efficacy. Methods using enzymes are more effective than chemical synthesis for producing polyphenol glycosides, offering precision and sustainability. Sucrose phosphorylases (SPs) catalyze the transfer of the glucosyl group of sucrose to diverse acceptor molecules. This enzymatic activity enables the synthesis of modified compounds such as oligosaccharides and phenolic derivatives, demonstrating the versatility of SPs in glycoside modification reactions.

Financial support and acknowledgments

This work was supported by Grant 101074544 “LIFE Program” (LIFE21-ENV-ES-CYCLOPS) funded by European Union.

Keywords

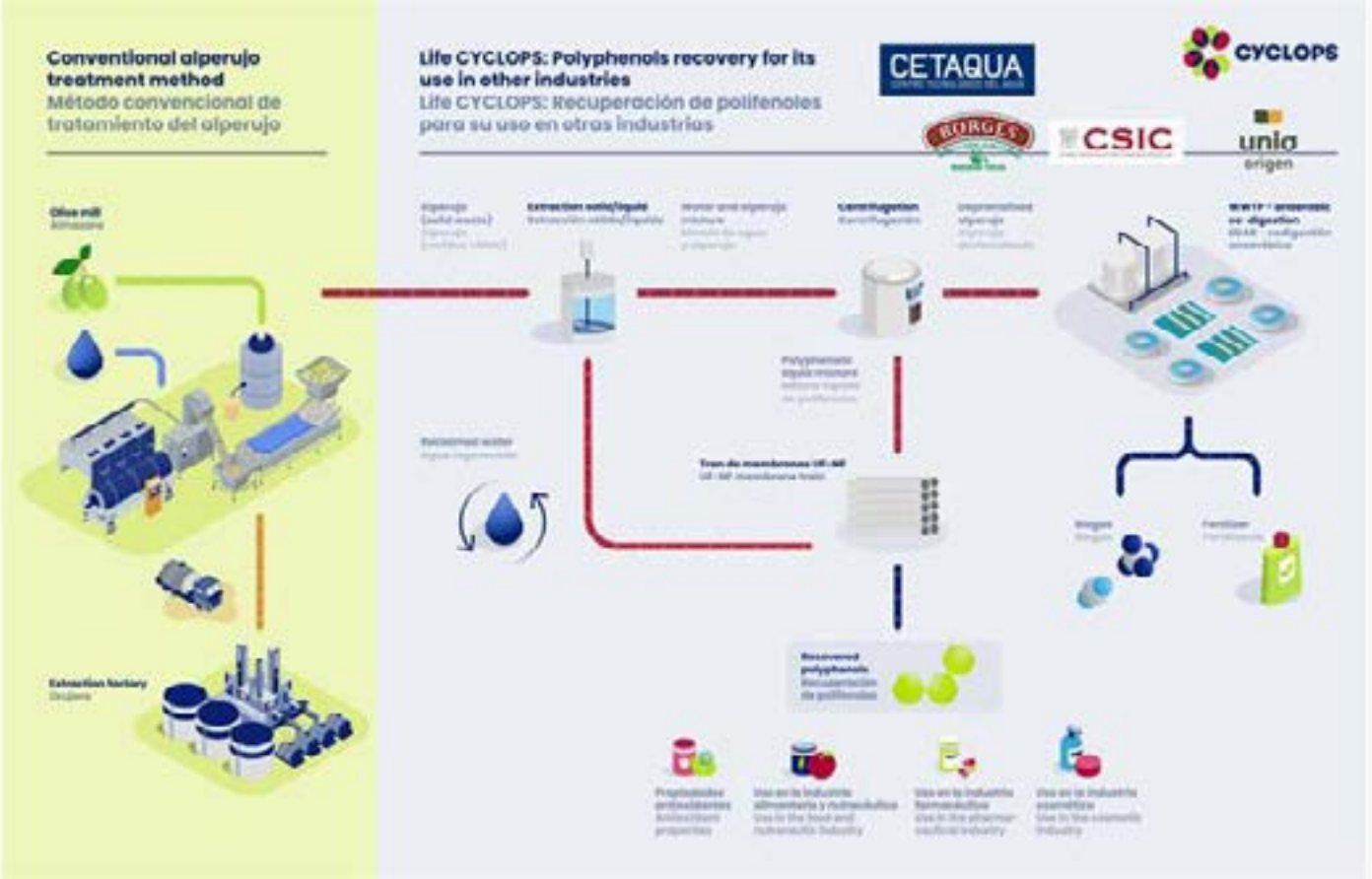
Olive pomace • Agro-food residues • Polyphenols • Glycosidases • Hydroxytyrosol • Glucosylation

continue –>

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

POSTER COMMUNICATIONS

Biocatalysis



Panel ID BC2 • Abstract ID 52

IMMOBILIZATION OF A VERSATILE NAD(P)H-DEPENDENT REDUCTIVE AMINASE FROM RHODOCOCCUS ERYTHROPOLIS ON AGAROSE-BASED SUPPORTS

Uriel A. Ruiz; Juan C. Almada; Miguel Marco Martín; Susana Velasco-Lozano
Universidad de Zaragoza - Instituto de Síntesis Química y Catálisis Homogénea (ISQCH-CSIC), Zaragoza, Spain

Abstract

Reductive amination of ketones is a widely utilized and highly versatile strategy for amine synthesis, enabling the efficient coupling of diverse ketones with primary or secondary amines. This transformation is particularly valuable in the synthesis of pharmaceuticals, agrochemicals, and fine chemicals. A key challenge in this context remains the mild activation of alcohols for nucleophilic substitution. Among various synthetic methods, reductive amination is especially significant for the production of chiral amines.Reductive aminases (RedAms) represent a promising class of enzymes that catalyze the reductive amination of carbonyl compounds with high stereoselectivity and low amine loading requirements. A recently identified RedAm from Rhodococcus erythropolis (RytRedAm) has shown exceptional activity with a broad substrate scope, functioning efficiently with both NADPH and NADH as cofactors.

This enzyme has been employed successfully in both crude extract and purified forms, demonstrating its versatility in biocatalytic applications.To further enhance the operational stability, reusability, and overall efficiency of RytRedAm, we investigated its immobilization on various agarose-based solid supports. The development of robust heterogeneous RedAm biocatalysts through immobilization aims to improve process sustainability and expand the practical use of this enzyme in synthetic biotransformations.

Financial support and acknowledgments

This work has been funded by ERC-St (PIEZOZYMES-101041192) and Gobierno de Aragón grupo E37_23 R.SVL thanks ARAID for the sponsoring.

Keywords

Reductive Aminase • Inmobilization • Agarose-based solid supports

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

Panel ID BC3 • Abstract ID 53

BIOCATALYTIC CONVERSION OF ALCOHOLS TO AMINES USING A CO-IMMOBILIZED HYDROGEN-BORROWING ENZYMATIC CASCADE

Miguel Marco-Martín; Uriel A. Ruiz; Juan C. Almada; Susana Velasco-Lozano
Universidad de Zaragoza - Instituto de Síntesis Química y Catálisis Homogénea

Abstract

Amines are widely used as key chemical intermediates in the production of active pharmaceutical ingredients, fine chemicals, agrochemicals, polymers, dyes, pigments, emulsifiers, and plasticizers. However, their natural availability is limited, and their industrial synthesis largely relies on metal-catalyzed hydrogenation processes, which often require additional protection and deprotection steps. Hydrogen borrowing offers a more sustainable and redox-neutral alternative, enabling key bond formations through a single-step process that combines oxidation and reduction without the use of waste-generating additives. This approach has proven effective in the enzymatic synthesis of enantiopure chiral amines. Nevertheless, most hydrogen-borrowing redox biocascades reported to date employ soluble enzymes, which pose challenges for practical applications due to limited recovery, recyclability, and operational stability. To overcome these barriers and broaden the applicability of biocatalytic hydrogen borrowing under preparative conditions, the development of immobilized enzyme systems is essential. In this study, we report the co-immobilization of a previously described bienzymatic system composed of an NADPH-dependent alcohol dehydrogenase (ADH) and an NADPH-dependent reductive aminase (RedAm). This system enables the direct transformation of alcohols into primary amines via a hydrogen-borrowing cascade, where the NADPH consumed by the ADH is efficiently regenerated by the RedAm, thus eliminating the need for external cofactors or sacrificial donors. The coupling of these enzymes supports a streamlined, cofactor-balanced synthesis of amines under mild conditions.

To construct a robust heterogeneous biocatalyst, we screened a variety of agarose-based microbeads functionalized with different chemistries to determine optimal conditions for co-immobilizing both enzymes. This screening led to the identification of an effective immobilization strategy that preserved the activity and stability of both enzymes while enabling their reuse in multiple cycles.

Financial support and acknowledgments

This work has been funded by ERC-St (PIEZOZYMES-101041192) and Gobierno de Aragón grupo E37_23 R.SVL thanks ARAID for the sponsoring.

Keywords

Biocatalysis • Enzyme co-immobilization • NADPH-dependent alcohol dehydrogenase • NADPH-dependent reductive aminase

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BC4 • Abstract ID 89

MULTIENZYMATIC PRODUCTION OF L-ERYTHRULOSE FROM GLYCEROL AND FORMATE USING IMMOBILIZED ENZYMES.

Camila Clavero; Kírian Bonet-Ragel; Marina Guillén; Oscar Romero
Bioprocess Engineering and Applied Biocatalysis Group, Department of Chemical, Biological and Environmental Engineering, Universitat Autònoma de Barcelona

Abstract

Formaldehyde is a key one-carbon building block in the synthesis of high-value compounds in the chemical and pharmaceutical industries, due to its ability to form C–C bonds from a non chiral C1 substrate. This enables the production of molecules with asymmetric carbon centers, such as D-threose, glyceraldehyde, and L-erythrulose. A sustainable route for formaldehyde production involves its biocatalytic generation from CO_2 via sequential reduction to formate and then to formaldehyde, catalyzed by formate dehydrogenase (FDH) and formaldehyde dehydrogenase (FaldDH), respectively. This enzymatic approach is highly selective and operates under mild conditions, representing a promising strategy for CO_2 valorization. In this work, we explored a multi-enzymatic cascade for the synthesis of L-erythrulose starting from glycerol and formate as substrates, with in situ regeneration of the NADH cofactor. Three enzymes were individually immobilized in a single step from crude cell lysates onto nickel-functionalized agarose: glycerol dehydrogenase (GlyDH), formaldehyde dehydrogenase from Burkholderia multivorans (BmfaldDH), and the fructose-6-phosphate aldolase (FSA A129S). After 24 hours of reaction, a 59% conversion of formate and accumulation of 12.6 mM L-erythrulose were achieved.

These findings underscore the significant potential of immobilized multienzyme systems as sustainable and efficient route for transforming CO_2 into high-value molecules like erythrulose, paving the way for novel bioconversion strategies.

Financial support and acknowledgments

Camila Clavero acknowledges the support of the National Agency for Research and Development (ANID)/ Scholarship Program / DOCTORADO BECAS CHILE 2023-72230235.All authors acknowledges MEPLABCO2 Project (TED2021-129732A-I00), funded by MCIN/AEI/10.13039/501100011033 and by the European Union “NextGenerationEU”/ PRTR” and AGAUR of Generalitat de Catalunya for 2021 SGR00143.

Keywords

Multi-enzymatic • Carbon capture & utilization • Industrial waste valorization

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BC5 • Abstract ID 96

ADVANCING PETASE FOR GREEN RECYCLING

Jesús Laborda Mansilla[1]; José Luis González[1]; Ivan Mateljak[2]; Mikel Dolz[1]; Valeria A. Risso[3]; José M. Sanchez-Ruiz[3]; Francisco Plou[1]; Miguel Alcalde[1]; Eva Garcia-Ruiz[1]

1, Departamento de Biocatálisis, Instituto de Catálisis y Petroleoquímica, CSIC, Madrid, España; 2. EvoEnzyme S.L. Parque Científico de Madrid, Madrid, España; 3. Departamento de Química Física, Facultad de Ciencias, Universidad de Granada, Granada, España

Abstract

Polyethylene terephthalate (PET) is a widely used polyester in the production of bottles, packaging, and textile fibers, valued for its low cost and physicochemical properties. However, its resistance to degradation and the limitations of current recycling methods have led to a massive accumulation of waste, which, together with other plastics, has caused a significant environmental impact. A promising alternative to conventional recycling systems is the use of biocatalysts capable of breaking down PET into its monomers, terephthalic acid (TPA) and ethylene glycol (EG), under mild reaction conditions and with high specificity.

This study focuses on the design and optimization of new variants of Ideonella sakaiensis PETase, aiming to enhance its catalytic activity and adaptability for industrial applications. To achieve this, we combined two complementary strategies: Ancestral Sequence Reconstruction (ASR) and directed evolution.

Through ASR, we generated ancestral enzymes structurally similar to modern PETase but with substantial amino acid sequence divergence. These differences resulted in more robust scaffolds and significant improvements in thermal stability. One of the most promising resurrected enzymes was selected as a parental template for a directed evolution campaign, aimed at enhancing its PET hydrolyzing activity under mild reaction conditions.

A High-Throughput Screening (HTPS) system was developed to identify mutants with improved activity. Across multiple iterative rounds of mutagenesis and selection, we obtained variants with substantial functional enhancements. After this progressive evolution process, an evolved PETase variant exhibited a shifted optimal pH closer to neutrality, representing a key improvement for industrial implementation. This adaptation enables the enzyme to work efficiently under less aggressive conditions, improving its stability through the reaction and potentially reducing the environmental impact and operational costs of PET recycling processes.

Overall, this combined approach led to the development of an improved PETase variant with enhanced physicochemical properties, better suited for practical applications in PET biodegradation. The results demonstrate the potential of harnessing evolutionary strategies to develop sustainable biotechnological solutions to address the global plastic waste challenge.

Financial support and acknowledgments

This study is funded by the Comunidad de Madrid project 2019-T1/BIO-13207.

Keywords

PET hydrolases • Ancestral Sequence Reconstruction • Directed Evolution • Plastic Biodegradation • Protein Engineering

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

Panel ID BC6 • Abstract ID 111

ENZYMATIC EPOXIDATION OF HEMP OIL USING IMMOBILIZED LIPASE B FROM CANDIDA ANTARCTICA

Guillermo Rodríguez Alonso; Amín Salami; Javier Rocha Martin; Isabel de la Mata; Miguel Arroyo
Universidad Complutense de Madrid

Abstract

Vegetable oils are gaining increasing attention as renewable feedstocks for the synthesis of high-value chemical intermediates. Among the various modifications they can undergo, epoxidation of unsaturated triglycerides is of particular interest due to the versatility of epoxides in industrial applications, including plasticizers, stabilizers, polyurethanes, lubricants, and epoxy resins.

While traditional chemical epoxidation of vegetable oils involves harsh conditions and generate environmentally harmful byproducts, enzymatic epoxidation using lipases has emerged as an efficient and eco-friendly method. Within the context of a circular bioeconomy, hemp seed oil stands out as a sustainable and promising raw material due to its advantageous fatty acid composition and increasing availability across Europe.

In this work, epoxidation of hemp oil has been performed using immobilized lipase B from Candida antarctica (Novozym® 435) due to its robustness and versatility in non-aqueous media, and its excellent performance in previous epoxidations of other vegetable oils. The epoxidation reactions were carried out using hydrogen peroxide as the oxygen donor, both in the presence and absence of toluene as solvent, to evaluate solvent-free conditions as a greener alternative.

Reaction progress and product formation were monitored through Fourier-transform infrared spectroscopy (FTIR), as well as by iodine value and oxirane oxygen content determinations. The results confirm the capability of Novozym® 435 to catalyze the epoxidation of hemp oil and highlight the potential of this biocatalytic system for the development of environmentally friendly epoxidation processes.

Financial support and acknowledgments

This work was supported by Grant TED2021-131462B-I00 from the Spanish Ministerio de Ciencia, Innovación y Universidades.

Keywords

hemp oil • lipase • CALB • Epoxidation

Panel ID BC7 • Abstract ID 113

CHARACTERIZATION OF A NEW FUNGAL CHITIN DEACETYLASE FOR ENZYMATIC PRODUCTION OF DEACETYLATED BIOMOLECULES

Laura Barahona Pérez[1]; Beatriz Gracia-Gómez[1]; María Martínez-Ranz[1]; Peter Elias Kidibule[2]; María Fernández Lobato[1]

1. Department of Molecular Biology, Centre for Molecular Biology Severo Ochoa. University Autónoma of Madrid, (CSIC-UAM), Madrid, Spain; 2. Faculty of Chemistry, Biotechnology and Food Science, NMBU - Norwegian University of Life Sciences, Norway

Abstract

Polysaccharide-based biopolymers are recently receiving attention as they can be obtained from renewable sources, possess a wide range of biological activities and can be used in various biotechnological applications. Among them, chitin, composed of N-acetyl-D-glucosamine units linked by β-(1→4) glycosidic bonds, is the most abundant polysaccharide in marine environments, being extensively available from the aquatic waste processing industry. Chitosan, a deacetylated form of chitin consisting of randomly distributed β-(1→4)-linked N-acetyl-Dglucosamine and D-glucosamine units, is unique among biomolecules due to its high nitrogen content (6.89 %) and its polycationic nature under acidic conditions (pH ←5.0) which allows it to interact with other polyanionic biological structures. In nature, chitosans of different pattern and degree of deacetylation can be found in the cell wall of several fungi. However, industrial chitosans are mainly produced by chemical deacetylation of chitin, showing a random distribution of deacetylated units and inconsistent physico-chemical characteristics. In this context, enzymatic production of chitosan is gaining industrial interest as it provides a greener alternative and generates molecules with a defined pattern of deacetylation, which is closely linked to their different bioactivities.

In this work, a potential chitin deacetylase (ChDA) from a zygomycete fungus has been cloned and heterologously expressed in Pichia pastoris, resulting in a 34 kDa metalloprotein that is dependent on cobalt ion. The enzyme showed maximum deacetylase activity at 40-50 ºC and pH 6.5-7.5 and retained 50 % of activity after being incubated at 65 ºC for 45 minutes. ChDA showed activity on chitin oligomers (faCOS) with degree of polymerization (DP) → 2, chitosans with different deacetylation degrees, colloidal chitin and acetylxylan, but not on peptidoglycan or hyaluronic acid. The acetylation degree of the reaction products was analysed using mass spectrometry and nuclear magnetic resonance techniques. The recombinant enzyme fully deacetylated faCOS with DP > 2, mono-deacetylated di-N-acetylchitobiose, and reduced the degree of acetylation of colloidal α-chitin from more than 97 % to 85 % in 24 h only. Multiple structural alignment with other characterized CE4 enzymes revealed that ChDA presents the typical structure (β/α)7-8 of a chitin deacetylase with an open binding cleft at the active site and allowed to study the relationship between structure and function of this type of enzymes. Further studies on structural determinants in this family of proteins will allow the refinement of enzymatic chitosan production, with great potential in medicine, agriculture, and food industry.

Financial support and acknowledgments

This work was supported by the Project GLYCOENZ-GREEN (PID2022-136367OB-C31/C32), funded by MCIN/AEI/10.13039/501100011033 and FEDER, a Way of Making Europe. Also, by Project SUST_CHIT_BIOPR (TED2021-129288B-C21/22) funded by MCIN/AEI/10.13039/501100011033 and by the European Union Next Generation EU/PRTR.

Keywords

Chitosan • Chitin • Chitooligosaccharides

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BC8 • Abstract ID 114

NOVEL BACTERIAL ARYL-ALCOHOL OXIDASES AS SUSTAINABLE BIOCATALYSTS: FROM SEQUENCE MINING TO FUNCTIONAL CHARACTERIZATION

Paula Cinca-Fernando[1]; Christian Ascaso-Alegre[2]; Emma Sevilla[3]; Marta Martínez-Júlvez[1]; Juan Mangas-Sánchez[4]; Patricia Ferreira[1]

1. Department of Biochemistry and Molecular and Cellular Biology / Institute for Biocomputation and Physics of Complex Systems, University of Zaragoza; 2. Department of Organic Chemistry, University of Zaragoza; 3. Department of Biochemistry and Molecular and Cellular Biology, University of Zaragoza; 4. IUQOEM, Department of Organic and Inorganic Chemistry, University of Oviedo

Abstract

Aryl-alcohol oxidases (AAOs) are flavin-dependent enzymes of the glucose-methanol-choline (GMC) oxidoreductase superfamily that catalyze the oxidation of activated primary alcohols into the corresponding aldehyde, generating hydrogen peroxide. Their ability to selectively oxidize a broad range of aromatic and aliphatic alcohols makes them attractive biocatalysts for greener chemical synthesis, including the production of fine chemicals, pharmaceuticals, and polymer precursors. While extensively studied in fungi, recent evidence suggests the presence of AAO-like sequences in other biological kingdoms, such as bacteria, with yet unexplored structural and functional potential.

In this study, we conducted an extensive bioinformatic screening of fungal, bacterial, and arthropod genomes using known AAOs as query sequences. Candidate proteins were selected based on the conservation of GMC-characteristic motifs and key catalytic residues. Maximum-likelihood phylogenetic analysis confirmed the separation of distinct AAO clades, supporting evolutionary divergence across kingdoms. Structural predictions using AlphaFold, combined with active-site analysis and substrate pocket inspection, supported the annotation of these sequences as putative AAOs and guided the prioritization of candidates for experimental validation. The full set of putative AAOs was further grouped into distinct structural and evolutionary types based on conserved motifs and active-site architecture, revealing convergent strategies and potential functional specialization across biological kingdoms.

Two bacterial AAOs, designated SdAAO and ShAAO (1), were successfully expressed recombinantly and purified for functional and structural characterization. Both enzymes displayed broad substrate specificity, efficiently oxidizing a variety of aromatic and aliphatic alcohols with catalytic efficiencies up to 4970 min^{-1} mM^{-1}. Thermal denaturation assays revealed moderate thermostability, with melting temperatures of 50.9 °C for ShAAO and 48.6 °C for SdAAO. Crystal structures confirmed the conserved GMC fold but revealed a notably wide-open active-site entrance compared to fungal homologs, a feature that may correlate with their enhanced substrate promiscuity and catalytic performance.

Altogether, our findings expand the known diversity of AAOs and provide the first detailed characterization of this enzyme class in bacteria. These results underscore the potential of cross-kingdom genome mining to discover novel enzymatic functions and support the development of new biocatalysts for sustainable oxidation chemistry.

Financial support and acknowledgments

We would like to thank the Agencia Estatal de Investigación (AEI), the Ministerio de Ciencia y Universidades (Ministry of Science and Universities, MICIU), and the EU for the financial support (TED2021-130803B-I00 MICIU/AEI /https://doi. org/10.13039/501100011033 NextGenerationEU/PRTR; PID2022-136369NB-I00 funded by MCIN/AEI/https://doi.org/10.13039/501100011033 and FEDER).

Keywords

Aryl-alcohol oxidases • Aldehydes • Enzyme diversity • Green chemistry • Biocatalysis

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BC9 • Abstract ID 116

A NOVEL CHEMOENZYMATIC CASCADE TO ACCESS CHIRAL B-HYDROXY ESTERS AND B-HYDROXY AMIDES FROM ALKYNES

Pablo López-Fernández; Marta Menéndez-González; Vicente Gotor-Fernández; Juan Mangas-Sánchez
Organic and Inorganic Chemistry Departament. School of Chemistry, University of Oviedo. Instituto Universitario de Química Organometálica "Enrique Moles"

Abstract

The implementation of catalytic and atom-economy processes into synthetic routes to access chiral compounds stand out as a key aim in the design of sustainable new chemical products under safe and mild conditions. We have recently found that L-cysteine (L-Cys) is an efficient and cost-effective catalyst for the hydration of activated alkynes. Using sub stoichiometric loadings, we were able to obtain a broad set of β-ketosulfones, amides and esters under aqueous conditions and in a very effective and regioselective manner from the corresponding activated alkynes. At a mere cost of less than €0.5 per gram of catalyst, this approach exhibits significant promise as a more economical and environmentally friendly alternative to existing strategies. Furthermore, the mild and biocompatible reaction conditions enable its seamless integration with biotransformations, providing the possibility to explore chemoenzymatic approaches to synthesize chiral.

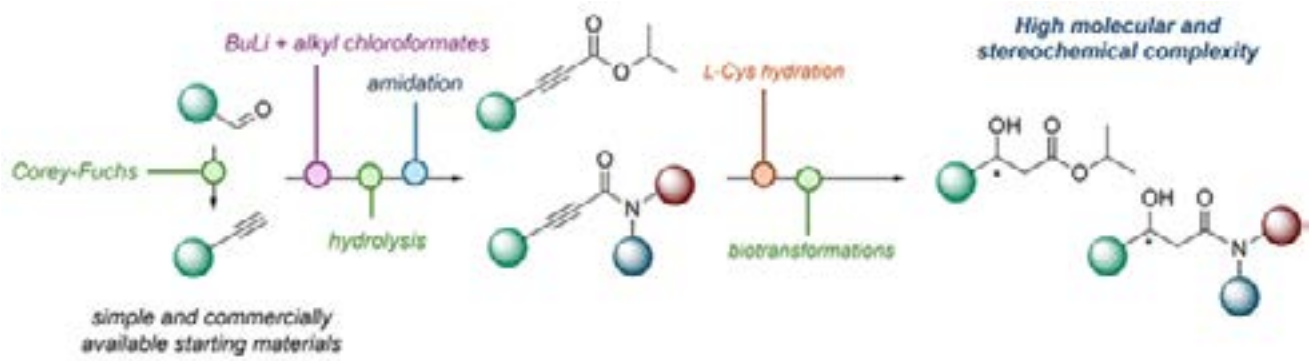
In this contribution, we present our recent results on the synthesis of chiral β-hydroxy esters from different aryl and alkyl propiolic esters via the one-pot combination of organocatalytic hydration and asymmetric reduction using ketoreductases. In parallel, we have also initiated the study of cascade processes aimed at the synthesis of structurally diverse hydroxy amides, versatile intermediates for the synthesis of chiral 1,2- and 1,3-amino alcohols, which are present in many important pharmaceutical ingredients.

Financial support and acknowledgments

We would like to thank the Agencia Estatal de Investigación (AEI) and the Ministerio de Ciencia e Innovacion (Ministry of Science and Innovation, MCIN) for the financial support through grants PID2020-113351RA-I00/AEI/10.13039/501100011033 (J.M.-S.) and PID2022-137893OB-I00 (V.G.-F.). J.M.-S. also thanks AEI and EU for a Ramón y Cajal Fellowship RYC2021-032021-I. The technical support of the Scientific-Technical Services of the University of Oviedo is acknowledged. We also thank Prof. Wolfgang Kroutil (University of Graz, Austria) for the donation of heterologously expressed alcohol dehydrogenases.

Keywords

biocatalysis • organocatalysis|asymmetric synthesis • β-hydroxy esters • β-hydroxy amides • chemoenzymatic cascade



Panel ID BC10 • Abstract ID 117

ARTIFICIAL CELL-FREE SYSTEMS FOR THE SUSTAINABLE PRODUCTION OF ACETOIN FROM BIOETHANOL

David Muñoz Sánchez; Óscar Romero Ormazabal; Marina Guillén Montalbán
Universitat Autònoma de Barcelona

Abstract

Biocatalysis enables selective, efficient and green chemical transformations under mild conditions, offering a sustainable alternative to conventional chemical approaches for the synthesis of bio-based products. In this framework, the development of enzymatic routes for the production of industrially relevant molecules from renewable feedstocks has gained increasing interest. Acetoin (3-hydroxy-2-butanone), a chiral compound mainly used as a flavour in the food industry and as a building block for other chemicals, is one such promising target product.

In this work, we developed two promising alternative multi-enzymatic systems for the sustainable synthesis of natural acetoin from bioethanol in a one-pot reactor strategy. Both multi-enzymatic systems share a key reaction enzyme, a pyruvate decarboxylase from Zymobacter palmae (ZpPDC), that catalyzes the condensation of acetaldehyde into acetoin.

The first system integrates an alcohol dehydrogenase from Saccharomyces cerevisiae (ScADH), that oxidizes ethanol into acetaldehyde, and a NADH oxidase from Streptococcus pyogenes (SpNOX), that regenerates the cofactor NAD^+, in combination with ZpPDC for acetoin synthesis (Fig. 1). This system was described, characterized and optimized for the first time in literature. The optimal reaction parameters were found to be pH 7.5, 25 °C, and 1 mM NAD^+. The use of purified enzymes helped prevent undesired side reactions from the crude extracts. Enzyme ratio optimization revealed that the best performance was achieved using a ScADH:ZpPDC ratio of 0.5 and a SpNOX:ZpPDC ratio of 1.27, with an initial ZpPDC activity of 100 $U \cdot mL^{-1}$. To overcome oxygen transfer limitations associated with cofactor regeneration, air bubbling was applied, resulting in a faster reaction and significantly improved performance metrics. Finally, bioethanol was tested successfully as a substrate to produce natural acetoin. Under these conditions, 99.5% conversion and 92.7% yield were obtained, as well as a productivity of 319.8 mg acetoin$\cdot L^{-1} \cdot h^{-1}$.

Despite the excellent performance achieved with the cofactor-dependent system, the limited operational stability of SpNOX is challenging for process efficiency and scalability. To address these issues, an alternative system was developed based on an alcohol oxidase from Phanerochaete chrysosporium [5] (PcAOX) and a catalase from bovine liver (CAT), enabling ethanol oxidation and hydrogen peroxide decomposition without requiring external cofactors, coupled with ZpPDC (Fig. 1). This system was also tested with sparged air and bioethanol, resulting in similar results: 100% conversion, 89.0% yield and 392.9 mg acetoin$\cdot L^{-1} \cdot h^{-1}$.

Altogether, this study demonstrates the potential of biocatalysis to enable efficient one-pot systems for the production of bio-based compounds.

Financial support and acknowledgments

This study is part of the DEMUBI Project (PID2022-139725OA-I00), funded by MCIN/AEI/10.13039/501100011033 and FEDER, EU. This work is included within the research group of Bioprocess Engineering and Applied Biocatalysis (Generalitat de Catalunya, 2021, SGR 00143). David Muñoz acknowledges his predoctoral fellowship (PIF) from the Universitat Autònoma de Barcelona. The authors thank Dr. Marco Fraaije (University of Groningen) for kindly providing the plasmid containing the PcAOX gene, and Dr. Nataša Božic (University of Belgrade) for generously supplying with bioethanol samples.

Keywords

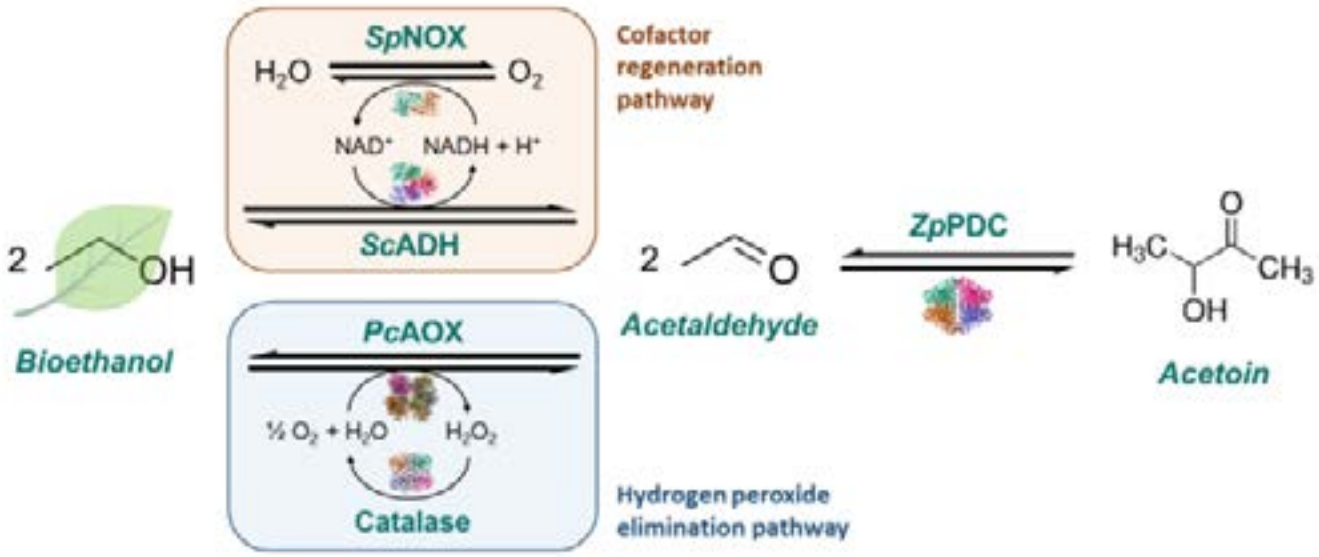
Multi-enzymatic system • Acetoin • Bio-based chemical

continue –>

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

POSTER COMMUNICATIONS

Biocatalysis



July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BC11 • Abstract ID 121

DESIGNING SOLUBLE PORE-FORMING ENZYMES FOR BIOPOLISHING SYNTHETIC TEXTILES

Miguel Luengo; Martin Floor, Victor Guallar
BSC

Abstract

Microplastic pollution from synthetic textiles—such as polyester, nylon, and acrylic—poses a growing environmental threat, with an estimated 200,000 to 500,000 tonnes of microfibers entering marine ecosystems annually. These fibers are released not only during washing but throughout a garment's lifecycle, contributing significantly to plastic contamination. While enzymatic biopolishing is widely used to enhance the quality of natural fibers, such as cotton, and reduce fiber shedding, its application to synthetic fibers remains unexplored due to the lack of suitable enzymes.In this study, we investigate the potential of engineering soluble, pore-forming protein scaffolds for biopolishing synthetic fibers. Inspired by our prior work that introduced PETase-like activity into membrane nanopores, we hypothesize that the geometry and architecture of pore-forming folds may be co-opted to host hydrolytic active sites for synthetic polymer processing. To this end, we conducted a systematic bioprospecting of the Protein Data Bank (PDB) to identify candidate pore-forming proteins with suitable aqueous solubility and specific structural features, including pore radius and surface accessibility.

Candidate scaffolds were filtered based on pore geometry and evaluated using ProteinMPNN, which provides sequence design scores that reflect the likelihood of successful expression and structural stability. We then applied a simulation-guided design algorithm to introduce a catalytic triad (Ser-His-Asp) into these scaffolds, aiming to confer serine hydrolase activity. Solubility and surface accessibility were prioritized to ensure compatibility with aqueous formulations commonly used in textile processing.

This work represents a conceptual advance toward the development of enzymes tailored for the biopolishing of synthetic textiles. By targeting surface-accessible polymer cleavage rather than complete depolymerization, our approach aims to reduce microfiber release at the source, providing a sustainable strategy to mitigate textile-derived microplastic pollution and promote circularity in the textile industry.

Financial support and acknowledgments

Proyecto Nacional DeNaPol

Keywords

enzyme engineering • biocatalysis • detergents

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

Panel ID BC12 • Abstract ID 124

ONE-POT SYNTHESIS OF NITRILES FROM PRIMARY ALCOHOLS VIA A CHEMOENZYMATIC CASCADE

Enol de Prado Fernández[1]; Juan Mangas Sánchez[1]; Patricia Ferreira Neila[3]; Paula Cinca Fernando[3]; Vicente Gotor Fernández[4]

1. Universidad de Oviedo, Organic and Inorganic Chemistry Department; 2. Instituto Universitario de Química Organometálica "Enrique Moles", University of Oviedo. Oviedo, Spain; 3. Department of Biochemistry and Molecular and Cellular Biology and Institute of Biocomputation and Physics of Complex Systems (BIFI, GBsC-CSIC Joint Unit), University of Zaragoza, Zaragoza, Spain; 4. Organic and Inorganic Chemistry Department, Instituto Universitario de Química Organometálica "Enrique Moles", University of Oviedo. Avenida Julián Clavería 8, 33006 Oviedo, Spain

Abstract

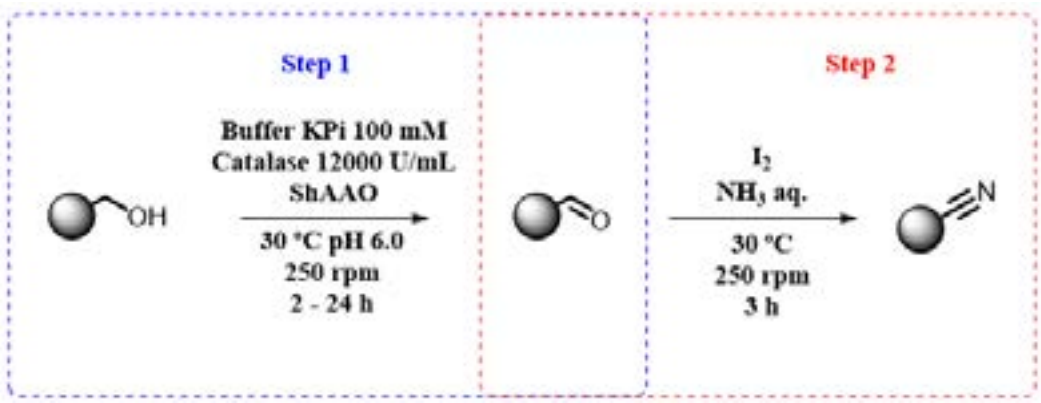
The nitrile group is a highly versatile functional moiety in synthetic chemistry, serving as a key intermediate for the preparation of diverse heterocycles and functional groups including amines, amides, carboxylic acids, and esters. Beyond their role as synthetic precursors, nitriles are also highly valuable end-products themselves, frequently present in fine chemicals as well as numerous pharmaceutical, agrochemical agents or fragrances. Aldehydes have proven to be valuable starting materials for nitrile synthesis, with numerous methodologies reported for such transformations. However, these compounds are often toxic, labile and prone to degradation so the use of surrogates, such as primary alcohols, to generate aldehydes in-situ provides many practical advantages.4Aryl-alcohol oxidases are a family of FAD-containing enzymes that catalyze the oxidation of a wide range of primary alcohols, including aliphatic, benzylic, and cinnamyl derivatives. In this work, we propose the straightforward chemoenzymatic synthesis of nitriles (Scheme 1) by integrating the oxidation of primary alcohols (step 1) using a newly discovered bacterial AAO from Streptomyces hiroshimensis, with a second oxidation process (step 2) using an iodine–ammonia system in aqueous media that enables the formation of the final nitriles through imine oxidation under mild reaction conditions.

Financial support and acknowledgments

We would like to thank the Agencia Estatal de Investigación (AEI), the Ministerio de Ciencia y Universidades (Ministry of Science and Universities, MICIU), and the EU for thefinancial support (TED2021-130803B-I00 MICIU/AEI/https://doi. org/10.13039/501100011033 NextGenerationEU/PRTR; PID2022-136369NB-I00 funded by MCIN/ AEI/https://doi. org/10.13039/ 50110 00110 33 and FEDER) and PID2022-137893OB-I00 (V.G.-F.). J.M-S also thanks the AEI for a Ramón y Cajal Fellowship (RYC2021-032021-I). The technical support of the Scientific-Technical Services of the University of Oviedo is acknowledged.

Keywords

Biocatalysis • aryl-alcohol oxidase • nitriles • enzymes



July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BC13 • Abstract ID 126

CHARACTERIZATION OF A FUNGAL GH84 ENZYME REVEALS DUAL ACTIVITY AND STRUCTURAL FEATURES RELEVANT TO GLYCOENGINEERING

María Martínez-Ranz[1]; Laura Barahona[1]; Beatriz Gracia-Gómez[1]; Marina Minguet-Lobato[2]; Peter Elias Kidibule[3]; María Fernández-Lobato[1]

1. Department of Molecular Biology, Centre of Molecular Biology Severo Ochoa, CSIC-UAM, Madrid, Spain; 2. Institute of Catalysis and Petrochemistry, CSIC, Madrid, Spain; 3. Department of Chemistry Biotechnology and Food Science, Norwegian University of Life Sciences, Norway

Abstract

Glycoside hydrolase family 84 (GH84) comprises enzymes with N-acetyl-β-D-glucosaminidase activity, involved in the hydrolysis of terminal N-acetylglucosamine (GlcNAc) moieties of O-linked oligosaccharides and N-glycans via a two-step mechanism involving two aspartate residues: one serving as the catalytic acid/base and the other as the polarizing residue. These enzymes are implicated in cellular processes such as protein modification, nutrient metabolism, mRNA transcription, signal transduction, and cytoskeletal remodelling. Given their ability to cleave GlcNAc residues, GH84 enzymes hold potential for modifying chitin-derived oligosaccharides, known as chitooligosaccharides (COS). Chitin is an abundant, insoluble polymer composed of β-1,4-linked GlcNAc units, widely found in shellfish waste. Its degradation, either by chemical methods or by more environmentally friendly enzymatic processes, yields water-soluble COS, which have been extensively studied for their bioactive properties, including roles in plant defense, antimicrobial activity, and applications in agriculture and biomedicine.

In this study, we report the cloning, heterologous expression, and biochemical characterization of an about 83 kDa GH84 protein (OGA83A) from an opportunistic fungus, produced in Komagataella phaffii. The enzyme was successfully expressed in the extracellular medium after 120 hours of methanol induction and when p-nitrophenyl-N-acetyl-β-D-glucosaminide (pNP-GlcNAc) was used as a substrate, the recombinant enzyme retained over 80% of its hydrolytic activity across a pH range of 4.0 to 6.0 and temperatures between 53 °C and 63 °C.

Hydrolytic activity of OGA83A toward various GlcNAc-containing substrates was assessed, showing a marked preference for pNP-GlcNAc, with a catalytic efficiency of 44.23 ± 0.39 $mM^{-1} \cdot s^{-1}$, making it among of the most efficient GH84 enzymes reported so far. Beyond its hydrolytic capacity, OGA83A also exhibited transglycosylation activity and production of COS was confirmed by MALDI-TOF and HPLC analyses. Sequence alignment with representative GH84 enzymes revealed conserved family residues, and its structural modeling with AlphaFold3 and docking with GlcNAc-derived substrates pointed to residues likely involved in its dual activity.OGA83A not only proved to be a promising biocatalyst for the sustainable synthesis of COS but could also represent a valuable molecular tool for studying glycan processing in fungal-pathogen interactions and biocontrol strategies.

Financial support and acknowledgments

The Spanish Ministry of Science and Innovation (TED2021-129288BC22, PID2022-136367OB-C32), and an Investigo Programme Contract (CAM-UAM) supported this work.

Keywords

GH84 • N-acetyl-β-D-glucosaminidase • heterologous expression • transglycosylation • p-nitrophenyl-N-acetyl-β-D-glucosaminide

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BC14 • Abstract ID 140

CHARACTERIZATION OF TWO NOVEL GH75 CHITOSANOLYTIC ENZYMES

Beatriz Gracia Gómez[1]**;** María Martínez Ranz[1]; Laura Barahona[1]; Marina Minguet Lobato[2]; Peter Elias Kidibule[3]; Francisco José Plou[2]; María Fernández Lobato[1]

1. Department of Molecular Biology, Centre of Molecular Biology Severo Ochoa, CSIC-UAM, Madrid, Spain; 2. Institute of Catalysis and Petrochemistry, CSIC, 28049, Madrid, Spain; 3. Department of Chemistry Biotechnology and Food Science, Norwegian University of Life Sciences, Norway

Abstract

Chitin is the most widely distributed renewable aminocarbohydrate in nature. It is composed of repeating N-acetyl-D-glucosamine (GlcNAc) units and is a fundamental component of fungal cell walls, arthropod exoskeletons, and mollusc endoskeletons. Chitosan is a chitin derivative obtained by deacetylation and composed of D-glucosamine (GlcN) and GlcNAc units linked by B-(1-4) glycosidic bonds. Chitooligosaccharides (COS) are oligomers obtained from chitin or chitosan by physicochemical methods and enzymatic hydrolysis. The solubility of COS in water allows their antimicrobial, antiviral, antioxidant, neuroprotective, and antitumor properties to be exploited. This has attracted significant interest from various industrial sectors, thereby increasing their demand. Chitosan can be hydrolyzed by different enzymes, including chitosanases (EC 3.2.1.132) belonging to the glycoside hydrolases (GH) 3, 5, 7, 8, 46, 75 and 80 families. This study focused on the characterization of two novel GH75 chitosanolytic enzymes, ChitoA and ChitoB, from an ascomycete fungi. Both enzymes were successfully produced in Pichia pastoris. The activity of ChitoA demonstrated optimal ranges of hydrolytic activity at 50-60 ºC and pH 3-4,5. Additionally, it retained 50 % of its activity after incubation for 90 minutes at 70 ºC. ChitoB shows maximum activity at 50 ºC and pH 3-5 and retained 50 % of its activity after 90 minutes at 50 ºC. Neither of the two chitosanases hydrolyzed chitin and both show a preference for chitosan with a degree of deacetylation of 75-85 %. These data enhance our understanding of the GH75 family and could be utilized to expand the industrial applications of chitosanases.

Financial support and acknowledgments

Financed by Science, Innovation and Universities Ministry (Proyect PID2022-136367OB-C31/-C32) and Statal Investigation Agency (Proyect TED2021-129288B-C22).

Keywords

Chitosanase • Chitosan • Chitooligosaccharides (COS) • Glycoside hydrolases (GH)|GH75 • Carbohydrate active enzymes • Enzymatic characterization

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

Panel ID BC15 • Abstract ID 152

GENETICALLY ENCODED CLICK CHEMISTRY FOR FUTURE HETEROGENEOUS BIOCATALYSIS

Amín Salami; Javier Rocha
Universidad Complutense de Madrid

Abstract

Enzyme processes in chemical synthesis are gaining attention as an efficient alternative to traditional chemical methods, offering faster, safer, and more sustainable reactions. Genetically encoded click chemistry provides a powerful approach for covalently immobilize enzymes or even linking separately produced proteins in vitro, making it a promising strategy for the designing of future generation heterogeneous biocatalysts.In this preliminary study, we explore the use of the recently developed conjugation system SpyCatcher/SpyTag, to facilitate the site directed immobilization of a protein on solid supports. This system is based on the spontaneous formation of specific isopeptide bonds under mild conditions via the split protein domain CnaB2 from Streptococcus pyogenes and it can be genetically fused to protein termini.

To validate this approach, two SpyCatcher constructs were immobilizated in glyoxyl agarose beads, differing in one of them having an extra poly-Lys tag, a crucial aminoacid for the covalent immobilization chemistry involved. In parallel, the SpyTag partner was genetically fused to the green fluorescent protein (GFP) and then purified. Incubating both partners together, allows us to reveal the distribution of the SpyCatcher in the porous support via Confocal Laser Scanning Microscopy (CLSM).

In addition, all recombinant peptides and proteins were fused to poly-His tags too for one step purification via metal affinity chromatography.

The results suggest that this strategy provides a viable platform for directed immobilization of enzymes through a specific region of the protein, highlighting its potential in biocatalytic applications.

Financial support and acknowledgments

This work was supported by Grant CNS2022-135135

Keywords

biocatalysis • click-chemistry

Panel ID BC16 • Abstract ID 153

COMPUTATIONAL IDENTIFICATION OF PET-BINDING DOMAINS DERIVED FROM CARBOHYDRATE-BINDING MODULES FOR ENHANCED AFFINITY TO AMORPHOUS PET

Ivan Dimitri; Oscar Romero; Marina Guillen; Martin Floor

1. Department of Chemical, Biological and Environmental Engineering, Universitat Autònoma de Barcelona, Barcelona, Spain; 2. Barcelona Supercomputing Center, Barcelona, Spain

Abstract

Conventional mechanical and thermal recycling methods for polyethylene terephthalate (PET) often yield lower-quality products with increased crystallinity, limiting their reuse primarily to low-value applications (Thomsen et al., 2023). Considering that 84% of the environmental impact of PET bottles derives from virgin resin production, more sustainable recycling alternatives are urgently needed (Ingrao & Wojnarowska, 2023). Enzymatic hydrolysis of PET using PETases offers an environmentally friendly approach; however, the high surface glass transition temperature and restricted mobility of amorphous PET chains significantly impede enzymatic access and degradation.

This approach aims to identify domains suitable for fusion to PETases to enhance amorphous PET accessibility and reduce the need for high-temperature processing (Thomsen et al., 2023). Carbohydrate-Binding Modules (CBMs), known for their affinity toward amorphous polysaccharides, offer a promising scaffold for engineering binding domains targeting synthetic polymers such as PET (Hashimoto, 2006). To explore this potential, an in silico bioprospecting pipeline was developed combining structural similarity searches with FoldSeek, domain annotation and selection with InterProScan, and sequence prioritization through an inverse folding approach with ProteinMPNN (Sumida et al., 2024).

A total of 1,091 protein domains were screened and subjected to Protein Energy Landscape Exploration (PELE) simulations against an amorphous PET dimer. The top 50 candidates, selected based on binding free energies (−46.21 to −63.61 kcal/mol), underwent molecular dynamics (MD) simulations in triplicate to preliminarily evaluate the stability of the protein–PET complexes. Further refinement using ligand RMSD and interaction energy analyses reduced the set to 10 candidates, each of which underwent 10-replicate MD simulations, consistently exhibiting high structural stability and persistent interactions with PET chains.

This computational pipeline represents a novel in silico bioprospecting approach that not only evaluates the predicted binding interactions between candidate domains and PET, but also incorporates predictions of protein expressibility and physicochemical properties — essential factors for the practical success of subsequent experimental validation. These candidates are now being evaluated experimentally as potential fusion partners to PETases for low-temperature PET hydrolysis applications.

Financial support and acknowledgments

This work was supported by funding from the Spanish Ministry of Science and Innovation (PID2022-139725OA-I00, project DEMUBI) and by the Generalitat de Catalunya (2021 SGR 00143).

Keywords

Polyethylene terephthalate (PET) • Carbohydrate-Binding Modules (CBMs) • Protein engineering • Bioprospecting • Protein Energy Landscape Exploration (PELE) • Molecular dynamics simulations • ProteinMPNN

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BC18 • Abstract ID 157

ENGINEERING UNSPECIFIC PEROXYGENASES FOR REGIO- AND ENANTIOSELECTIVE HYDROXYLATION OF FATTY ACIDS: INSIGHTS FROM LONG-TIMESCALE MD SIMULATIONS

Martin Floor[1]; Alejandro González-Benjumea[2]; Mireia Martínez-Sugrañes[1]; Miguel Alcalde[3]; Elvira Romero[4]; Victor Guallar[1]; Angel T. Martínez[4]; Ana Gutiérrez[2]

1. Barcelona Supercomputing Center (BSC); 2. Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC; 3. Instituto de Catálisis y Petroleoquímica (ICP), CSIC; 4. Centro de Investigaciones Biológicas "Margarita Salas" (CIB), CSIC

Abstract

Unspecific peroxygenases (UPOs) have emerged as versatile biocatalysts for the oxyfunctionalization of non-activated C–H bonds in fatty acids and related substrates. Their capacity to perform selective hydroxylation reactions without the need for expensive cofactors makes them highly attractive for biotechnological applications. In this work, we integrate experimental assays with advanced computational simulations to dissect the regio- and enantioselectivity of arachidonic acid hydroxylation catalyzed by fungal UPOs.

We focus on wild-type rCciUPO and rAaeUPO, along with engineered variants A77L, A77T, and A77N of rAaeUPO designed to modulate the geometry of the heme access channel. Experimental results show that the A77L variant ("Fett") achieves 92% (S)-selectivity in the synthesis of 19-hydroxyarachidonic acid, coupled with high ω-1 regioselectivity and minimal overoxidation. These findings position A77L as a promising candidate for the sustainable synthesis of bioactive lipid mediators.

To rationalize these observations, we performed molecular dynamics simulations of more than 10 μs per system, identifying catalytically competent poses based on geometric and energetic criteria. We found that the A77L mutation restricts substrate access and imposes torsional constraints on the lipid chain, resulting in fewer reactive conformations but enhanced (S)-enantioselectivity. Notably, the narrower channel suppresses the formation of keto-overoxidation products by sterically disfavoring post-hydroxylation binding modes. Energetic analyses revealed that interactions with residues T242, E245, and D70 play a central role in modulating substrate positioning via stabilization or repulsion of the carboxylate moiety.

Our study highlights how conformational entropy, local residue interactions, and active-site geometry converge to shape the selectivity profile of UPO-catalyzed oxidations. Beyond arachidonic acid, these insights are transferable to other n-6 fatty acids (C18–C22), as confirmed by experimental assays on linoleic, γ-linolenic, dihomo-γ-linolenic, and adrenic acids.

This integrative approach provides a mechanistic foundation for rational UPO engineering, enabling the selective production of pharmacologically relevant lipid derivatives under mild, cofactor-independent conditions. Ongoing work includes the design of next-generation variants and the application of enhanced sampling techniques to further elucidate reactivity landscapes.

Financial support and acknowledgments

Grant TED2021-129264B-C33 funded by MICIU/AEI/10.13039/501100011033Funded by the European Union under Grant Agreement No 101135119

Panel ID BF1 • Abstract ID 18

TRANSCRIPTIONAL REGULATION OF GABA GENES IN STREPTOCOCCUS THERMOPHILUS ST 8.1

Baltasar Mayo
IPLA-CSIC

Abstract

Due to the purported health benefits of γ-aminobutyric acid (GABA) for consumers, its production by the lactic acid bacterium Streptococcus thermophilus is of significant applied interest in the dairy industry. While the composition and structure of the GABA operon (gadBC) in S. thermophilus are well characterized, the regulatory mechanisms governing the expression of gad genes remain poorly understood. This study investigated the transcriptional regulation of gadB and gadC, which encode glutamate decarboxylase and the glutamate/GABA antiporter, respectively, in S. thermophilus St 8.1, a wild strain isolated from raw milk. Gene expression was analyzed in both a rich medium (LM17) and a chemically defined medium (CDM), under conditions simulating those of fermented dairy product manufacturing and ripening processes. Concurrently, GABA production from monosodium glutamate (MSG) was assessed in both media. In LM17, GABA yield was influenced by several factors, including temperature, pH, MSG and NaCl concentrations, as well as the type and concentration of the carbohydrate. In CDM, the presence of MSG led to differential upregulation of gadB and gadC, as determined by quantitative reverse transcription PCR (qRT-PCR). gadB expression increased after 4–6 hours of incubation (pH ≈5.0), peaking at 8 hours with an 8-fold increase. In contrast, gadC exhibited a bimodal expression pattern: a 2.5-fold increase at 2 hours (pH ≈6.0) and a second peak at 6 hours, reaching a 3-fold increase at 8 hours. In CDM, transcription of gad genes and GABA production were enhanced by NaCl supplementation but reduced in the presence of lactose. These results suggest that the expression of gad genes is pH-responsive and potentially subject to catabolite repression. Supporting this hypothesis, a conserved cre-like sequence was identified upstream of the gadC start codon. Understanding the regulatory pathways controlling gad gene expression could enable the rational use of S. thermophilus as a starter culture for producing GABA-enriched fermented dairy products.

Financial support and acknowledgments

The work was supported by a grant (Ref. PID2022-141271NB-I00) funded by MCIN/AEI/10.13039/501100011033 and by the ERDF A way of making Europe project. A PhD grant from the Severo Ochoa Program of Asturias Principality was awarded to J.R. (BP19-098). J.A.V's stay at IPLA-CSIC was sponsored by a scholarship from the Programa de Movilidad Académica de la Asociación Universitaria Iberoamericana de Posgrado (AUIP).

Keywords

Streptococcus thermophilus • GABA • Starters • Transcriptional regulation

Panel ID BF2 • Abstract ID 103

UNLOCKING THE BIOCHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION OF AN ARTISANAL KOMBUCHA FROM SOUTHERN EUROPE

Amanda Laca[1]**;** Ares Fernández[1]; Marta Sánchez[1]; Ana Isabel Díaz[2]; Inés Calvete-Torre[2]; Adriana Laca[1]; Aberlardo Margolles[2]; Lorena Ruiz[2]

1. Department of Chemical and Environmental Engineering, University of Oviedo, Oviedo, Spain; 2. Dairy Research Institute of Asturias (IPLA-CSIC), Oviedo, Spain - Functionality and Ecology of Beneficial Microbes (MicroHealth) Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Asturias, Spain

Abstract

Kombucha, an ancient fermented tea beverage originating in China, has recently seen a remarkable increase in global popularity, partly associated with its potential effects supporting gut health and boosting immunity (Prajapati et al., 2024). However, kombucha properties depend on several variables, such as the type of raw material, time of infusion, starter culture microbial community, and fermentation parameters (Vargas et al., 2021). Additionally, nowadays, there is a growing demand of artisanal products, since the interest of consumers in “natural foods” has increased during the last years (García-Barón et al., 2025).

In this work, the production of a kombucha at small scale has been evaluated from a biochemical and microbiological perspective. The fermentation process of an artisanal kombucha elaborated in a winter season in Spain has been followed and, in addition, different final products have been analysed. Several compounds, including total carbohydrates, total reducing sugars, total phenolic compounds, flavonoids, condensed tannins, acetic acid, ethanol and, also, antioxidant activity have been determined by spectrophotometry and chromatography. Besides, viable microorganisms in the final products have been quantified by means of plating on different culture media (MRS, PCA, GM17 and YGC). Identification of representative isolates was performed through sequencing of 16S rRNA and ITS regions for bacteria and yeast, respectively. The results showed that, since artisanal kombucha is produced through an uncontrolled fermentation process, there are a notable variation between final products batches. In general, it was found that the most abundant microorganisms were yeast, such as Pichia membrifaciens, Brettanomyces anomalus, Brettanomyces bruxelensis and Zygosaccharomyces bailli, although some bacteria like Acetobacter musti were also detected. Regarding the ethanol content, most of the samples showed a value lower than 1%, whereas the amount of total carbohydrates ranged between 15 and 54 g/L and the concentration of tannins and flavonoids were 70-130 mg/L and 37-54 mg/L, respectively. The values of these compounds were in the same order of magnitude as those found in literature for this fermented beverage.Finally, it is important to remark that, although consumers generally positively appreciate the unique flavours attributed to artisanal products; according to the results obtained here, the standardization of some parameters, such as temperature, inoculum and/or time of fermentation, would help to obtain a more homogenous product without losing its artisanal character.

Financial support and acknowledgments

This work has been supported by CSIC grants PIE-202370E063 and PIE202370E168.

Keywords

kombucha • antioxidant • artisanal • fermentation • microbiota

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BF3 • Abstract ID 133

DECAYING MICROBIOTA OF PORPHYRA SP. AS A SOURCE OF XYLANASES FOR THE PRODUCTION OF NOVEL XYLOOLIGOSACCHARIDES (XOS) FROM ALGAE

Andrea Luaces; Marta Diéguez; Andrea Rodríguez; Clara Fuciños; Ana María Torrado; María Luisa Rúa
Biochemistry Laboratory, Faculty of Science-Ourense, University of Vigo, Spain

Abstract

The increasing consumer demand for health-promoting food has driven interest in prebiotics, particularly xylooligosaccharides (XOS), known for their strong prebiotic activity at low doses compared to fructooligosaccharides (FOS) and galactooligosaccharides (GOS). Conventionally, XOS are derived from terrestrial lignocellulosic biomass. However, marine algae offer an alternative source of xylans with distinct structural features, such as beta-(1,3) or mixed beta-(1,3)/beta-(1,4) linkages, as found in species like Porphyra sp. and Palmaria palmata. These differences may yield XOS with unique chemical and functional properties, supporting their development as next-generation prebiotics.

To isolate xylanase-producing microorganisms from the microbiota associated with Porphyra sp. to obtain novel beta-(1,3)-linked XOS with favourable characteristics for food applications

Algal decay conditions were optimized to enrich xylanase-producing microorganisms. Xylanase activity was detected on beta-(1,3)-xylan agar plates using Congo Red staining. Positive microbial consortium was identified via 16S rRNA gene sequencing and cultured at 30 °C for 6 days in media containing mixed xylan (0.2%–2%). Growth (OD600), pH, sugar consumption, enzymatic activity (30 °C, pH 7, 24 h), and XOS production were evaluated at 24-hour intervals. XOS were analysed via HPLC using a Rezex RNO-Oligosaccharide Na+ (4%) column, with beta-(1,4)-XOS standards from Megazyme (Ireland) and beta-(1,3)-XOS standards prepared in-house.

The consortium showed positive xylanase activity: M17 (Tenacibaculum, Vibrio, Gilvimarinus). M17 demonstrated that growth increases markedly with substrate concentration, indicating that this variable is critical (OD600 = 3.3 at 2% xylan). Furthermore, complete sugar consumption and XOS production within 24 hours were observed. Enzymatic assays revealed that M17 had the highest activity on mixed xylan—double that observed on beta-(1,4)-xylan—and lower activity on beta-(1,3)-xylan (20% relative to beta-(1,4)). HPLC confirmed the presence of beta-(1,4)-XOS (X2, X3), beta-(1,3)-XOS (X2) and mixed XOS (X3, X4, X5, X6) reflecting the mixed linkage composition of the substrate. These results suggest that M17 harbours xylanases with broad substrate specificity or tolerance to different glycosidic bonds.

This study demonstrates the potential of marine algae-associated microbiota as a source of novel xylanases capable of producing structurally diverse XOS. The microbial consortium M17 showed robust growth, efficient sugar utilization, and high enzymatic activity on marine-derived xylans, enabling the production of beta-(1,3), beta-(1,4) and mixed XOS. These findings support the use of marine microbial enzymes for the development of innovative prebiotic ingredients with unique structural and functional properties.

Financial support and acknowledgments

Grant BLUE-XOS project “Marine macroalgae as emerging sources of xylooligosaccharides and study of their prebiotic effects (PID2021-126194OB-C21)” and Grant “FPU23/00762”.

Keywords

algae • xylooligosaccharides • prebiotic • xylanases • beta-(1,3) and beta-(1,4) bonds

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BF4 • Abstract ID 137

CLEAN ENZYMATIC VALORIZATION OF MARINE BIOMASS: NOVEL XYLOOLIGOSACCHARIDES (XOS) FROM PALMARIA PALMATA

Sonia Iglesias; Marta Diéguez; Sandra González-Rodríguez; Andrea Rodríguez-Sanz; Clara Fuciños; Ana Torrado; María Luisa Rúa
Universidade de Vigo

Abstract

XOS are functional xylooligosaccharides currently obtained from cereals, composed of xylose units linked by beta-(1,4) bonds, with degrees of polymerization ranging from 2 to 6. They are widely recognized for their strong potential as emerging prebiotics compared to currently marketed alternatives due to their high bifidogenic power, which is evident at the lowest effective doses (in the range of 0.7–2.8 g/day). This supports greater digestive tolerance and reduced laxative and flatulence effects. Additionally, XOS provide a mild sweetness without bitter or metallic tastes, offer interesting technological properties, and are of plant-based origin.

Exploring new sources of XOS is of growing interest. Marine macroalgae offer a promising and underexplored alternative to terrestrial biomass. In this context, the red seaweed Palmaria palmata is of particular interest, as it contains a distinctive xylan composed of mixed beta-(1,3)/beta-(1,4) linkages. This structural feature may lead to the generation of novel XOS containing beta-(1,3) bonds and potentially unique functional properties, reinforcing the value of marine resources in the development of next-generation prebiotics.

This work presents a clean process for obtaining novel XOS from the red seaweed Palmaria palmata, consisting of three steps: (i) aqueous xylan extraction at mild temperature, (ii) ultrafiltration/diafiltration using a 100 kDa cut-off membrane for concentration and purification, and (iii) enzymatic hydrolysis with Amano 4, a commercial enzymatic preparation containing beta-(1,4)-xylanase activity.

The results demonstrate that this strategy enables the production of a final hydrolysate rich in novel XOS containing beta-(1,3) linkages with potential prebiotic activity. The process avoids the formation of degradation products and yields a hydrolysate with low salt content. Consequently, the resulting XOS can be directly formulated into food products with minimal need for further purification.This study contributes to the valorization of underutilized marine resources within the framework of the blue bioeconomy and proposes an innovative approach for producing high value-added functional ingredients from algal biomass.

Financial support and acknowledgments

Financial support from BLUE-XOS project “Marine macroalgae as emerging sources of xylooligosaccharides and study of their prebiotic effects (PID2021-126194OB-C21)” and Predoctoral Contract “AUGA131H6450211”.

Keywords

XOS • Palmaria palmata • xylanases • mixed beta-(1,3)/beta-(1,4) xylan • xylooligosaccharides • prebiotic • algae

Panel ID BF5 • Abstract ID 151

VANILLIN ENZYMATIC SYNTHESIS FROM FERULIC ACID: P. PASTORIS CELL FACTORY STUDY

Raúl Benages[1]**;** Javier Sebastián[1]; Albert Carceller[2]; Miquel García[2]; Enrique Vázquez[2]; Marina Guillén[2]; Francisco Valero[2]; José María Bueno[1]

1. Hausmann SL; 2. Universitat Autònoma de Barcelona

Abstract

Vanillin is a compound of high commercial relevance in the flavor and fragrance industry due to its broad application across food products and the growing market demand for its natural form. The elevated value of natural vanillin, coupled with the environmental burden of packaging and transportation associated with its import, underscores the need for sustainable, biosynthetic production methods.

The ENZIVANIL project aims to develop a biotechnological route for vanillin synthesis from ferulic acid from renewable resources, involving using a multi-enzymatic system composed of a ferulic acid decarboxylase (FDC) and a coniferyl alcohol oxidoreductase (CCO). This study reports on the heterologous expression of these enzymes in *Pichia pastoris*.

Recombinant expression constructs were designed to express *Bacillus pumilus* FDC (BpFDC) and *Caulobacter segnis* CCO (CsCCO2), including a Q390A mutant variant. The expression system employed the methanol-inducible AOX1 promoter, secretion signal, C-terminal His-tag, and Zeocin resistance. Plasmid integration in *P. pastoris* X-33 was confirmed for both CCO2 variants by colony PCR, whereas no integration was detected for FDC using this method.

Despite successful integration of CCO2 genes, no vanillin production was detected, and SDS-PAGE analysis revealed a predominant 75 kDa band instead of the expected 55KDa, suggesting undesired protein processing, potentially due to glycosylation or misfolding. Deglycosylation assays and protein identification via sequencing revealed this band to be a host-derived chaperone, not CCO2, indicating that *P. pastoris* struggled to produce active CCO2 due to protein misfolding.

In contrast, even though no plasmid integration was confirmed by colony PCR, FDC activity was confirmed in all 24 tested P. pastoris clones, with clone F9 showing the highest activity after 96 hours. Further optimization in high cell density fed-batch fermentations significantly enhanced FDC production. A methanol-salt pulse strategy achieved 155 µM/h activity representing a 40-fold increase over shake flasks but suffered from instability due to suspected proteolysis. A strategy based on an exponential feeding approach improved stability but showed enzyme degradation after 6 hours. Finally, implementing a glycerol-based biomass accumulation phase before methanol induction led to a substantial increase in enzyme yield (394 U within 6 h) with specific activity maintained at 1600 U/mg.

While FDC was successfully expressed at high levels, ongoing difficulties with functional CCO2 expression in *P. pastoris* led to the strategic decision to explore *Escherichia coli* as an alternative host for both enzymes.

Financial support and acknowledgments

This work is supported by CDTI and EFDR (ENZIVANIL; IDI-20210755). The UAB group is a member of the 2021 SGR 00143 Department of Research and Universities of Generalitat de Catalunya.

Keywords

vanillin • enzymatic synthesis • Pichia pastoris • enzyme production

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

Panel ID BF6 • Abstract ID 155

COMBINED EFFECT OF LACTOBIONIC ACID AND LACTOBACILLUS CASEI AGAINST STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI. APPLICATION IN VEGETABLE JUICES

Ismael Marcet; Saliha Oussaid; Manuel Rendueles; Mario Díaz
Universidad de Oviedo

Abstract

Consumers are increasingly attracted to natural and fresh products. However, the quality and safety of these products are easily degraded by microorganisms that pre-exist or arise during the various manufacturing or storage processes. Organic acids, lactic acid bacteria and their metabolites are potential natural antimicrobial agents that respond to market requirements. Additionally, organic acids can serve as prebiotics and thus can stimule the growth, increase metabolism and enhance antagonistic efficacy of probiotic bacteria. The objective of this work was to evaluate the efficacy of lactobionic acid, Lactobacillus casei and their combination to control Staphylococcus aureus and Escherichia coli growth in carrot and pear juices. The agar diffusion method showed a strong antagonistic effect of L. casei with diameter of inhibition of 52.33 ± 1.53 and 58.66 ± 3.055 mm for E. coli and S. aureus, respectively.

The minimal bactericidal concentration obtained with the acid in both juices was 4 times lower than that obtained in nutrient broth medium (MBC=25 mg/ ml). In general, growth of both strains was greater in carrot juice, and S. aureus showed more sensitivity than E. coli to each treatments. The inhibitory effect was greater when the two treatments were applied simultaneously, with total bacterial elimination was obtained by combining L. casei and 1/16 MBC of LBA. Thus, these results encourage the use of L. casei -LBA combined process as alternative to chemical preservatives in fruit and vegetable-based beverages.

Financial support and acknowledgments

AGENCIA DE CIENCIA, COMPETITIVIDAD EMPRESARIAL E INNOVACION ASTURIANA -SEKUENS- (SEK-25-GRU-GIC-24-010).AGENCIA ESTATAL DE INVESTIGACION (Mº. ECON. IND.) (MCIU-22-PID2021-125942OB-I00).

Keywords

Lactobionic acid • Lactobacillus casei • Antibacterial effect • Vegetal juices

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BM1 • Abstract ID 50

COMBINED USE OF IN SITU AND EXPOSURE APPROACHES FOR THE ASSESSMENT OF AQUATIC ENVIRONMENTAL HEALTH

Laura Pérez-González[1]; Chiara Trombini[2]; Verónica I. Luna-Guerrero[1]; Julián Blasco[2]; José Alhama[1]; Marina Barbudo-Lunar[1]
1. Universidad de Córdoba; 2. ICMAN

Abstract

Pollution is a major global issue that impacts both human well-being and ecosystems. Aquatic ecosystems are the final destination of a large number of pollutants due to the presence of nearby anthropogenic activities, such as agriculture, industry or urban development. Microorganisms show a sensitive response to the presence of contaminants and have been proposed as good bioindicators of environmental pollution. Their quick response to pollutants leads to changes in abundance, diversity and functionality. In this work, we have studied sediment samples from two coastal areas of the Bay of Cadiz, supposed to be pristine: “Río San Pedro” (S1) and “Puente Zuazo” (S2), and two from Guadalquivir Estuary, previously described as areas impacted by chemical pollutants: “Puerto Bonanza” (S3) and “Brazo de la Torre” (S4) (Southwest Atlantic coast, Spain). Sediments from these locations were analysed directly (EvS1-EvS4, respectively) or indirectly by exposing aquatic microcosms (ExS1-ExS4, respectively). The bacteriome associated with the sediments was identified and analysed by 16S rRNA metagenomics. The highest number of identifications at phyla level corresponded to Proteobacteria in both EvS and ExS, but the abundance of other phyla varied depending on the approach. In both EvS and ExS approaches, Proteobacteria, Firmicutes and Planctomycetes levels were significantly increased in polluted sites in comparison to the clean ones. The same pattern was observed in Cyanobacteria, Actinobacteria, Chlamydiae and Spirochaetes but only in ExS, whereas Nitrospirae increment in polluted sites was only evident in EvS samples. Flavobacteriaceae and Nostocaceae families were the most abundant in both approaches. Forty-eight families were significantly altered in the EvS study, but only forty were in ExS. According to the pattern observed at the phyla level, most of the families were increased in the impacted sites in EvS and ExS (thirty-four and twenty-six, respectively). Twenty families were commonly altered in both approaches but not always with the same tendency. We also found significant changes in alpha and beta diversity patterns. Alpha indexes increased in EvS4 compared to the clean sites. Beta diversity by Aitchison distance showed that the communities of the clean sites S1 and S2 were closer together and that the impacted sites S3 and S4 were further apart. A total of 23 functions were associated with the microbiome through FAPROTAX analysis, of which 20 were common to the two approximations, EvS and ExS. In conclusion, Metagenomics is a very useful tool in ecotoxicological studies to understand pollution-associated changes in the environmental microbiome.

Financial support and acknowledgments

MICINN PID2019-110049RB-I00 and Junta de Andalucía PCM_00118. Fundings to BIO187 and RNM306 groups (PAIDI, UCO/CSIC). Research Support Services (SCAI) of the University of Córdoba. M. Barbudo-Lunar received a predoctoral contract from the University of Cordoba (“Plan Propio”) and the “Ministerio de Ciencia e Innovación” (FPU).

Keywords

Aquatic enviroments • Microbiome • Microcosms • Environmental samples • Pollution biomarkers

Panel ID BM2 • Abstract ID 62

SUBSTRATE SPECIFICITY MAPPING OF A RECOMBINANT SERINE PROTEASE WITH COLLAGENOLYTIC ACTIVITY FOR BIOTECHNOLOGICAL APPLICATIONS

Hamilton Cabral; Camila Langer Marciano
School of Pharmaceutical Sciences of Ribeirao Preto/University of Sao Paulo

Abstract

Filamentous fungal proteases have gained prominence in the market due to their ease of handling, high efficiency, and diverse industrial applications. Proteases represent approximately 60% of the enzyme market due to their critical roles in various industries, including food, detergents, dairy, silk degumming, waste management, and therapeutics. Amount proteases collagenases are peptidases involved in collagen degradation, with critical roles in physiological processes, microbial pathogenicity, and biotechnological applications. Their ability to generate bioactive peptides, provide wounds and burns debridement, and offer cost-effective therapies for collagen-related disorders underscores their industrial and medical relevance. This study aims to produce, purify, and determine the kinetic parameters of recombinant collagenase from Purpureocilium lilacinum (rSerPepCol_Pl) expressed in Komagataella phaffii. The recombinant strain was cultured and induced with 1% methanol for protein expression following the EasySelectTM Pichia Expression Kit. Purification involved ammonium sulfate precipitation followed by hydrophobic interaction chromatography (HiTrapTM Phenyl FF equilibrated with 100 mM NaH2PO4, 2M (NH4)2SO4, pH 7.0, and eluted in 100 mM NaH2PO4, pH 7.0). To elucidate substrate specificity and catalytic efficiency was used FRET-based substrates with systematic substitutions at positions P1, P2, P3, P'1, P'2, and P'3 with variations including acid (Asp and Glu), basic (His, Lys, and Arg), neutral (Asn, Gln, and Tyr), or hydrophobic residues (Ala, Ile, Leu, Val, Gly, Pro, Phe, and Trp). Kinetic assays (0.02 µM enzyme, 20 mM HEPES pH 7.5, 40°C) monitored fluorescence (Vmax, Km, Kcat, Kcat/Km) were derived via Michaelis-Menten kinetics. The rSerPepCol_Pl demonstrated high catalytic efficiency with alanine in S1 (80.723 ± 1.475 mM^{-1} s^{-1}) and S2 (10.181 ± 0.061 mM−1 s−1), while glycine at S'1 conferred a high turnover rate (0.290 ± 0.010 s−1). In contrast, proline in S1, S'2, and S'3 showed strong binding, possibly making hydrolysis more difficult. These findings highlight the enzyme's specialization for collagen degradation and its versatility in cleaving alternative substrates. The rSerPepCol_Pl showed robust activity and specificity, with a promising biocatalyst for applications in biomedicine and industrial biotechnology. Future work will explore its structural determinants and optimization for targeted collagen hydrolysis and biotechnological applications.

Financial support and acknowledgments

São Paulo Research Foundation, FAPESP, Proc. n° 2024/18725-6

Keywords

Catalytic efficiency • Collagenolytic Activity • Peptidases • Serine Protease

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BM3 • Abstract ID 63

BIOPROCESS INTENSIFICATION FOR 3-HYDROXIPROPIONIC ACID PRODUCTION FROM METHANOL USING ENGINEERED KOMAGATAELLA PHAFFII

Giovanni Covaleda-Cortés; Sílvia Àvila-Cabré, Arnau Gasset, Xavier García-Ortega, José Luis Montesinos-Seguí, Pau Ferrer, Francisco Valero

Department of Chemical, Biological and Environmental Engineering, Universitat Autònoma de Barcelona, Bellaterra, Spain

Abstract

Green methanol is increasingly recognized as an attractive feedstock to produce value-added biochemicals. It can be produced in a renewable manner, e.g., by direct electrochemical reduction of CO_2, offering a viable alternative to fossil resources. Thus, it provides a unique opportunity to positively impact sustainability. The methylotrophic yeast Komagataella phaffii (syn. Pichia pastoris) has been recently engineered for 3-hydroxypropionic acid (3-HP) production. 3-HP is a key building block listed among the top value-added products to be obtained from biomass by the Department of Energy of the United States. 3-HP can be converted to acrylic acid and other chemicals of interest such as 1,3-propanediol, as well as to biopolymers.However, challenges remain in developing fermentation processes allowing for techno-economically viable and environmentally sustainable bioprocesses. In this study, we have implemented and compared different fed-batch operational strategies and conditions based on a preprogrammed exponential feeding of methanol to attain a constant specific growth rate or a closed-loop feedback control of methanol concentration, using a reference K. phaffii engineered with the synthetic b-alanine pathway to produce 3-HP. The latter strategy resulted in better overall process performance, reaching a 3-HP concentration of 45.7 gP/L in the supernatant, a yield on methanol of 0.105 gP/gS and a volumetric productivity of 0.47 gP/(L·h) in a fed-batch cultivation at 5-L scale. Additionally, we investigated the use of formate as an auxiliary substrate (electron donor) in methanol fed-batch cultures, demonstrating improved process efficiency (final product concentration, yield on methanol and volumetric productivity increase of about 15%).

Overall, the results obtained in this study highlight the potential of K. phaffii as a methanol-based platform cell factory, paving the way for further bioprocess intensification for sustainable chemicals production.

Financial support and acknowledgments

This work has been supported by the VIVALDI project, funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000441, and the project PID2019-104666GB-I00 from the Spanish Ministry of Science and Innovation. The authors receive support from the Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) of the Catalan Government (grant 2021-SGR-00143). SAC was supported by a FI scholarship (2022FI_B1_00173) from AGAUR.

Panel ID BM4 • Abstract ID 66

THE MI-HY BIOELECTROCHEMICAL PLATFORM FOR GREEN ENERGY AND CIRCULAR HYDROPONICS ECOSYSTEMS

David Sanz; Ignacio Baquedano; Daniel González; Alicia Prieto; Jorge Barriuso
CIB-CSIC

Abstract

Climate change and global warming, driven by excessive reliance on fossil fuels, represent some of the most critical and complex challenges of our time. Addressing them requires transformative and interdisciplinary approaches capable of reshaping the current energy paradigm. Bioenergy—encompassing not only biofuels but also electricity produced by microorganisms in Microbial Fuel Cells (MFCs)—offers a sustainable, low-carbon solution. MFCs exploit the metabolic activity of electrogenic microorganisms that oxidize organic substrates at an electrode, generating an electric current in the process.

The Mi-Hy project, funded under the EU Horizon Europe program, introduces a novel bioelectrochemical system that integrates hydroponic plant cultivation with microbial fuel cell technology. For the first time, the project leverages wastewater pollutants and root exudates—organic compounds naturally released by plant roots—as the electron donors for MFCs. Hydroponics provides a controlled environment where plants convert atmospheric CO_2 and nitrogen into biomass while excreting bioavailable carbon compounds. These exudates are captured in a prosthetic rhizosphere in the MFCs, enabling simultaneous biomass production, wastewater treatment, and energy generation.

To maximize system performance, Mi-Hy employs metabolic engineering to design symbiotic microbial communities capable of forming efficient, stable biofilms on the MFC anode. These communities include electrogenic bacteria and fungi that act synergistically to enhance substrate consumption, electron transfer and long-term operation. Preliminary experiments using synthetic consortia of *Shewanella oneidensis, Pseudomonas putida*, and the fungus *Ophiostoma piceae*, show very good results in terms of electricity-generating capacity and cooperative behavior within the MFC environment.

The system is supported by a digital monitoring infrastructure that allows smart, data-driven control of energy output, nutrient cycles, and biofilm dynamics. This next-generation hybrid platform has significant potential for applications in urban and peri-urban agriculture, precision gardening, on-site wastewater treatment, and the decentralized production of bioelectricity and high-value biocompounds.

In line with the EU Missions on Climate Adaptation and Smart Cities, Mi-Hy promotes a co-creative vision by involving designers, scientists, policymakers, and citizens in developing future applications—advancing toward more sustainable, resilient, and nature-integrated urban ecosystems.The Mi-Hy project has been funded by the European Innovation Council (EIC) (HORIZON-EIC-2022-PATHFINDERCHALLENGES-01) under grant agreement ID 101114746.

Financial support and acknowledgments

The Mi-Hy project has been funded by the European Innovation Council (EIC) (HORIZON-EIC-2022-PATHFINDERCHALLENGES-01) under grant agreement ID 101114746.

Keywords

Microbial Fuel Cells (MFCs) • Synthetic Microbial Consortia • Electrogenic Microorganisms • Hydroponics • Wastewater Valorization

Panel ID BM5 • Abstract ID 68

METABOLIC ENGINEERING OF BACILLUS SUBTILIS TOWARD THE EFFICIENT AND STABLE PRODUCTION OF C30-CAROTENOIDS

Pedro Picart Faiget
Universitat de Barcelona

Abstract

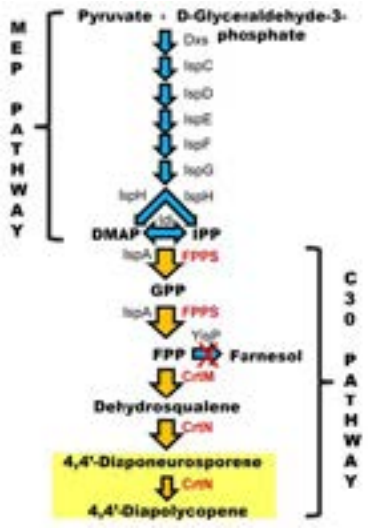
Terpenoids are a large and diverse class of natural compounds, with over 70,000 known chemical structures, offering significant potential for applications in the pharmaceutical, cosmetic, and food industries, as well as in the production of biofuels. Traditional extraction from plant sources or chemical synthesis of terpenoids is often technically challenging, involving processes that processes are not sustainable or ecological, and typically yields low to moderate product concentrations. Additionally, is generally dependent on the seasons and geographic areas, which cannot always be standardized. In contrast, their biotechnological production using microbial hosts represents a sustainable and environmentally friendly alternative, capable of achieving high yields at reasonable costs under mild process conditions.

Bacillus subtilis, a microorganism generally recognized as safe (GRAS), has a high growth rate, and is easy to genetically manipulate and cultivate, with a wide substrate range. Remarkably, it is among the highest isoprene producers (the simplest terpenoid) reported in bacteria, making it an attractive microbial chassis for terpenoid biosynthesis. Therefore, in this study, we explored the potential of B. subtilis as a heterologous host for the production of diaponeurosporene, a yellow C30 carotenoid with strong antioxidant properties and immune-stimulating activity.To this end, the crtM (dehydrosqualene synthase) and crtN (dehydrosqualene desaturase) genes from Staphylococcus aureus were chromosomally integrated into B. subtilis in multiple copies to ensure overexpression. Subsequently, intracellular levels of the precursor farnesyl diphosphate (FPP), the substrate of the CrtMN enzymes, were increased through overexpression of the farnesyl diphosphate synthase (FPPS) gene from Bacillus megaterium, along with the deletion of the gene encoding farnesyl diphosphate phosphatase (yisP), an enzyme involved in undesired FPP consumption (see Figure).

The stepwise combination of these genetic modifications led to a progressive increase in diaponeurosporene production, ultimately yielding a safe, stable, and efficient B. subtilis strain capable of endogenously producing the yellow carotenoid without the use of inducers. Notably, the strain is plasmid-free and does not rely on antibiotic resistance markers. We anticipate that this engineered strain will serve as a robust platform for further metabolic engineering and fermentation process optimization targeted at establishing a commercially viable bioproduction process.

Keywords

Bacillus subtilis • carotenoids • metabolic engineering



July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BM6 • Abstract ID 84

EXPLORING PHO4-REGULATED RESPONSES IN KOMAGATAELLA PHAFFII UNDER LOW PHOSPHATE AND HIGH PH CONDITIONS

Marcel Albacar; Antonio Casamayor; Abdelghani Zekhnini; Asier González; Joaquín Ariño
Institut de Biotecnologia i Biomedicina, Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain

Abstract

Previous research from our laboratory demonstrated that in Saccharomyces cerevisiae alkaline stress triggers an inorganic phosphate (Pi) starvation response, highlighting a link between the two stresses. The transcriptional response to mild alkalinization of Komagataella phaffii (formerly Pichia pastoris), a yeast extensively used to produce heterologous proteins of industrial and therapeutic interest, has been only recently reported, and this work revealed that the PHO89 gene (encoding Na+-Pi-cotransporter), was strongly induced by alkaline pH. Notably, the PHO89 promoter (pPHO89) has been successfully used to drive the expression of industrially relevant enzymes in a pH-dependent manner and under Pi limitation, suggesting again a link between alkaline pH and Pi starvation. In fungi, the transcriptional responses to low Pi are mediated by the Pho4 transcription factor and deletion of this gene renders S. cerevisiae cells unable to grow under Pi limitation or pH >7.0. However, little is known in K. phaffii about the molecular mechanisms underlying these processes and how are they interconnected. Since K. phaffii appears to contain two putative PHO4 genes (PHO4-A and PHO4-B) we generated single and double mutant strains using CRISPR/Cas9 techniques. Phenotypic characterization shows that pho4-A cells are very sensitive to Mn2+, whereas pho4-B cells are not. In contrast, pho4-B cells are highly sensitive to the cell wall damaging agent SDS, but the pho4-A strain is not. However, the double mutant displays an even higher SDS sensitivity and is unable to grow at 37°C, suggesting a role for both genes in cell wall integrity. Whereas single mutants are not sensitive to alkaline pH, the pho4-A pho4-B strain fails to grow at pH > 7.0. Furthermore, growth tests under Pi limitation and flow cytometry assays using mutant strains carrying a pPHO89-GFP reporter suggest that PHO4-B is critical for a proper Pi starvation response, but PHO4-A is not. Currently ongoing RNA-seq experiments will elucidate how alkaline stress and Pi starvation transcriptional responses are linked in K. phaffii and the relative contribution of Pho4-A and Pho4-B.

Financial support and acknowledgments

Supported by grants PID2020-113319RB-I00 and PID2023-150535OB-I00 (Ministerio de Ciencia, Innovación y Universidades & Agencia Estatal de Investigación) to JA and AC, and grant 2023 PROD 00006 (AGAUR, Generalitat de Catalunya) to JA.

Keywords

Komagataella phaffii • alkaline pH • stress response • phosphate starvation

Panel ID BM7 • Abstract ID 98

CONSTRUCTION OF A SUPER-SECRETING BACILLUS SUBTILIS STRAIN VIA SYSTEMATIC PATHWAY MODULATION USING CRISPR-CAS9

Jordi Ferrando Núñez
Universitat de Barcelona

Abstract

The Gram-positive bacterium Bacillus subtilis is a well-established workhorse in industrial biotechnology for the mass production of heterologous proteins. In fact, approximately 60% (in weight) of the commercially available enzymes are produced by Bacillus species. However, despite its widespread use, its ability to secrete a broad range of proteins efficiently is hampered by bottlenecks at nearly every stage of protein production, from transcription and translation to folding and secretion. These constraints significantly reduce the yield of extracellular enzymes, especially at industrial scales. Additionally, with rising environmental concerns, there is a critical need for the construction of sustainable antibiotic-free microbial cell factories. Within this context, this study introduces for first time a systematic, step-by-step CRISPR-Cas9 genome engineering framework for building a stable, high-efficiency, and environmentally friendly B. subtilis strain designed for industrial enzyme production.

Using amylase (AmyQ) as a model protein, we demonstrate that optimizing transcription through promoter selection and gene copy number is crucial for boosting expression. However, once the levels of the gene-specific message have saturated the microbial cell's processing capacity, it is paramount to consecutively target the various components of the post-transcriptional machinery, focusing on chaperones, folding processes, translocon systems, membrane stress factors, and metabolic loads. Step by step, each of these elements was fine-tuned to relieve downstream bottlenecks and maximize the final ability of B. subtilis to secrete the extracellular enzyme (see Figure). Finally, after further fermentation optimization, our engineered strain achieved a 57.9-fold increase in AmyQ secretion compared to the parental strain.

To our knowledge, this is the first report that describes an improvement in amylase extracellular production levels in B. subtilis by merely using the CRISPR-Cas9 system, rendering an industrial strain devoid of plasmids and antibiotic selection markers and bypassing the need for expensive inducers. Although the impact of gene modifications within the secretory pathway of heterologous proteins might be variable, probably depending on each specific gene, we believe the strategy presented in this work to obtain the maximum secretion levels from multiple copy gene insertion, along with combinational Sec pathway analysis, a promising approach that will facilitate the construction of robust, ecologically safe, industrial strains of B. subtilis in forthcoming years.

Financial support and acknowledgments

This work was supported by the Pla de Doctorats Industrials del Departament de Recerca i Universitats de la Generalitat de Catalunya with the support of Gestio d'Ajuts Universitaris de Recerca, with grant number 2021 DI 77, awarded to J.F.N.

Keywords

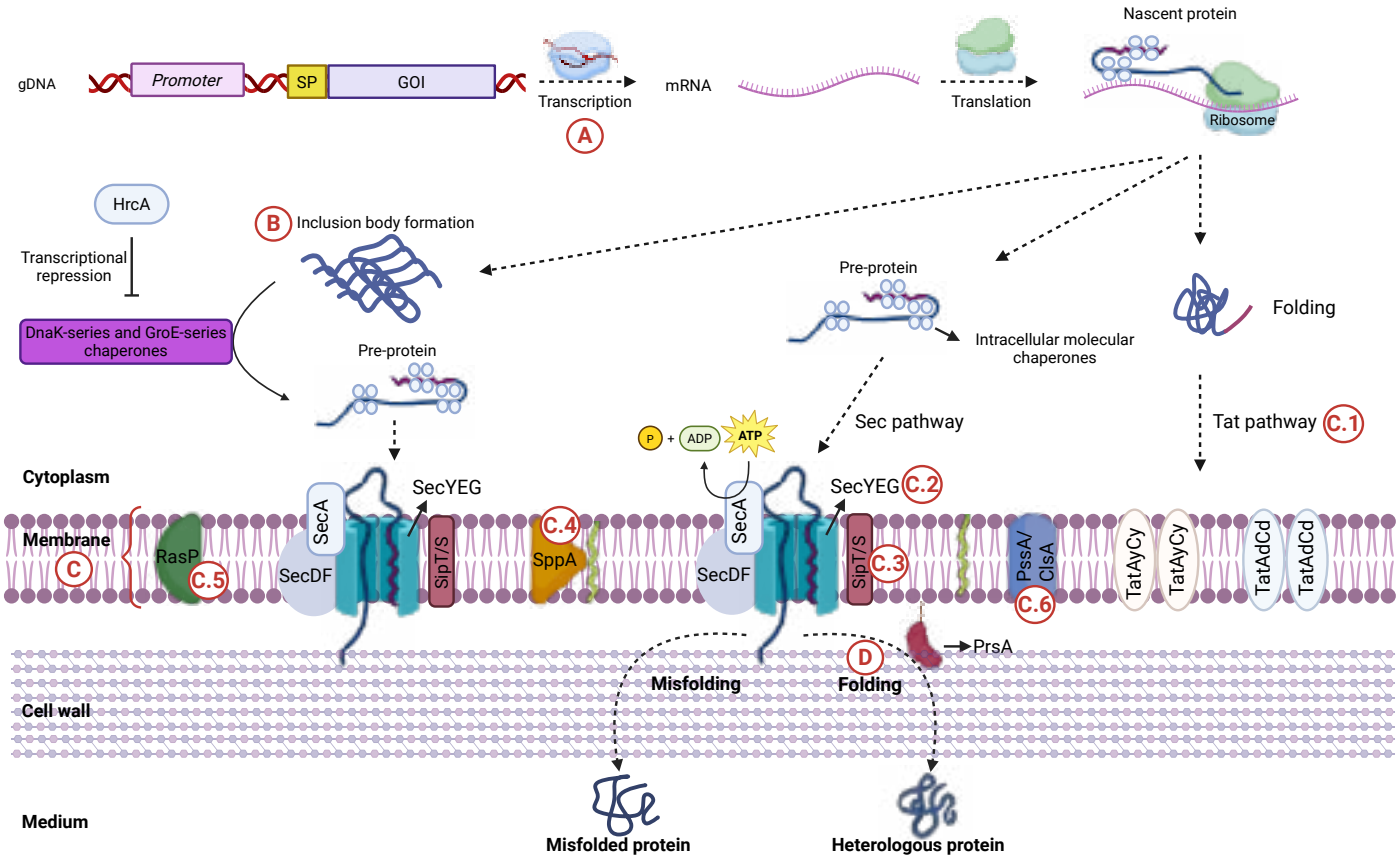
Bacillus subtilis • CRISPR-Cas9 • amylase

continue –>

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

POSTER COMMUNICATIONS

Microbial Biotechnology



Panel ID BM8 • Abstract ID 104

INDUSTRIAL BY-PRODUCTS FROM THE CHOCOLATE AND DAIRY SECTOR AS SUBSTRATES FOR THE OBTENTION OF SINGLE CELL PROTEIN

Amanda Laca; María Peláez; Adriana Laca
Department of Chemical and Environmental Engineering, University of Oviedo, Oviedo, Spain

Abstract

The challenge of feeding a growing global population in a sustainable way has led to increased interest in alternative protein sources like Single Cell Protein (SCP). Producing animal and plant-based proteins entails many environmental impacts, including greenhouse gas emissions, deforestation and biodiversity loss and, in addition, requires large amounts of water and land (Fanzo & Miacho, 2023). In this context, SCP, which is derived from microorganisms such as algae, yeasts, fungi, and bacteria, arises as an interesting option due to its many advantages over the traditional protein sources. For example, SCP production needs less land, water, and energy, this protein source presents numerous nutritional benefits and it can be produced employing as substrate residual organic streams (Pipliya et al., 2025). The agri-food sector generates large quantities of organic wastes derived from production and processing processes, which entails significant environmental, social, and economic problems.

This work aims to contribute to the circular economy strategy by valorising wastes from the agro-food industry to obtain SCP that could be used in human food. Hence, the possibility of obtaining SCP through the fermentation of wastes from the chocolate and/or dairy industry employing Kluyveromyces marxianus (CECT1446), a specie recognized as GRAS and QSP, has been addressed. Cocoa bean shell (CBS) and/or lactose were used as substrate and three different fermentations summarised in Figure 1 were carried out. The best yield was obtained when CBS was employed as unique source of carbon and nitrogen, achieving a production of approximately 20 g of SCP per kg of dried CBS.Figure 1. Overview of the processes of fermentation carried out in this work.

Financial support and acknowledgments

The authors are grateful to Chocolates Lacasa for providing the cocoa bean shell employed in this work.

Keywords

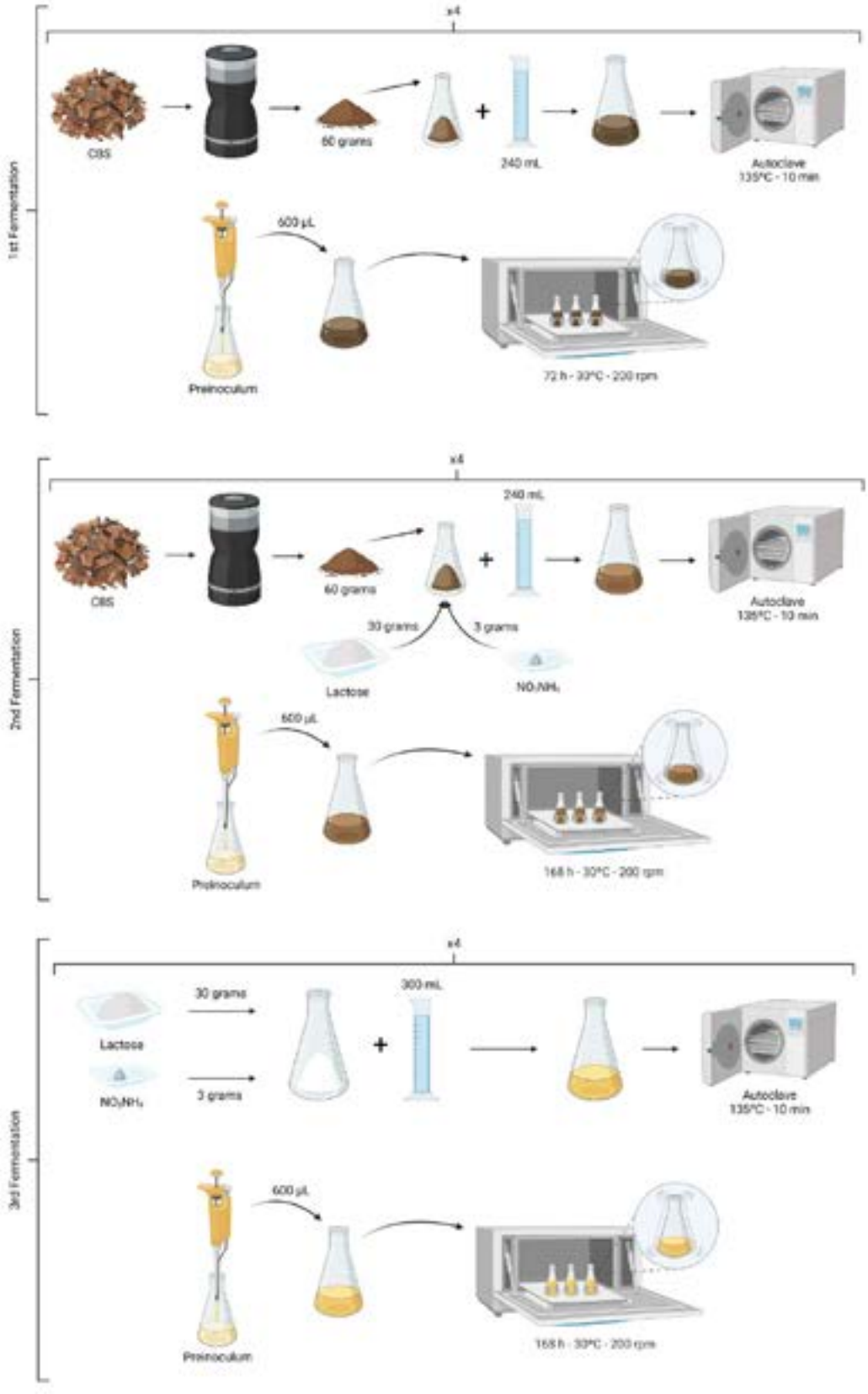
cocoa bean shell • lactose • Kluyveromyces marxianus • fermentation|single cell protein

continue –>

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

POSTER COMMUNICATIONS

Microbial Biotechnology



Panel ID BM9 • Abstract ID 109

COMBINATORIAL ALKALI-RESPONSIVE HYBRID PROMOTERS AS TOOLS FOR HETEROLOGOUS PROTEIN EXPRESSION IN SACCHAROMYCES CEREVISIAE

Abdelghani Zekhnini; Antonio Casamayor; Joaquín Ariño
Institut de Biotecnologia i Biomedicina, Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona

Abstract

The demand for potent and easily inducible promoters to produce heterologous proteins in Saccharomyces cerevisiae has not yet been adequately met. In this organism alkalinization triggers a robust and well-characterized transcriptional response that includes activation of the calcium–dependent calcineurin-Crz1 and the phosphate-responsive PHO pathways. Here we present the construction of random libraries containing multiple combinations of Crz1- and Pho4-binding sequences and we show that these elements are able to promote efficient expression of GFP by simple addition of KOH to the medium. The expression in Crz1 or Pho4-deficient cells allowed us to define the relative contribution of these elements to GFP production. We also show that addition of a single-copy of a 60-bp fragment of the ENA1 promoter containing a Stp1/2 site further enhances expression. Finally, we demonstrate that these constructs drive potent expression of secretable laccase, an enzyme of industrial interest in processing lignin biopolymers, and that the level of expression can be adjusted by modifying the pH of the medium. In conclusion, our work presents a novel expression system whose induction is simple, cheap and easy to monitor, and that could be an attractive alternative to current expression platforms for both research and industrial protein production purposes.

Financial support and acknowledgments

Work supported by grants PID2020-113319RB-I00 and PID2023-150535OB-I00 (AEI, Ministerio de Ciencia, Innovación y Universidades) to JA and AC, and 2023 PROD 00006 (AGAUR, Generalitat de Catalunya) to JA.

Keywords

Hybrid promoters • Saccharomyces cerevisiae • Alkaline pH • Research protein production • Industrial protein production

Panel ID BM10 • Abstract ID 118

DE NOVO BIOSYNTHESIS OF PLANT FLAVONOIDS FROM CO_2 VIA ENGINEERED MULTISPECIES MICROBIAL CONSORTIA

Erika González[1]; Fabián Moreno[1]; Juan Nogales[2]
1. Systems Biotechnology Group, Department of Systems Biology, Centro Nacional de Biotecnología, CSIC, Madrid, Spain; 2. Systems Biotechnology Group, Department of Systems Biology, Centro Nacional de Biotecnología, CSIC, Madrid, Spain.; bCNB DNA Biofoundry (CNBio), CSIC, Madrid, Spain

Abstract

The sustainable production of high-value natural compounds is a key goal of modern metabolic engineering. Flavonoids stand out due to their pharmacological properties and applications in food, cosmetics, and medicine. However, their biosynthesis involves complex, energy-demanding pathways that are difficult to replicate in microbial monocultures. These pathways often compete with essential processes such as fatty acid and aromatic amino acid biosynthesis, leading to metabolic burden and reduced yields.

To overcome these challenges, synthetic microbial consortia have emerged as a promising solution. By distributing metabolic tasks across species, they offer improved robustness, scalability, and efficiency. In this study, we developed a modular co-culture system of autotrophic and heterotrophic microbes to mimic plant-like flavonoid biosynthesis from CO_2. At its core, the cyanobacterium Synechococcus elongatus fixes atmospheric CO_2 and converts it into sucrose.

The secreted sucrose supports two heterotrophic partners: recombinant Escherichia coli engineered to produce p-coumaric acid (pCA), a key intermediate, and Pseudomonas putida strains optimized for biosynthesizing naringenin (NAR), a central flavanone precursor. This division of labor reduces metabolic stress and allows independent optimization of each organism.

Consortium performance was enhanced by tuning environmental parameters such as nitrogen availability, inoculation ratios, and cell densities. These adjustments significantly improved productivity and stability. This strategy demonstrates the potential of autotrophic–heterotrophic co-cultures for carbon-neutral biosynthesis of complex compounds.

Our results also highlight cyanobacteria as efficient biofactories for carbon fixation and sucrose secretion, enabling light-driven microbial production. This model supports further integration of autotrophic systems into scalable, sustainable synthetic biology platforms.In conclusion, combining cyanobacterial CO_2 fixation, sucrose export, and heterotrophic flavonoid biosynthesis within a synthetic consortium represents a promising route to green biotechnology. Leveraging metabolic modularity and interspecies cooperation, this system lays the groundwork for efficient, low-cost production of flavonoids and other valuable natural products.

Financial support and acknowledgments

This research received funding from the European Union's Horizon 2020 research and innovation program under grant agreement numbers no. 101081782 (deCYPher), as well as PID2022-139247OB-I00 (Rob3D) projects funded by MCIN/AEI/10.13039/501100011033 and European Union (Next Generation EU/PRTR) funding, a way to make Europe.

Keywords

Synthetic biology • microbial-consortia • flavonoids

Panel ID BM11 • Abstract ID 120

SYSTEMS METABOLISM ENGINEERING OF ESCHERICHIA COLI W FOR IMPROVED PRODUCTION OF THE INDUSTRIALLY RELEVANT FLAVONOID (S)-EQUOL

Tomás Tejera[1]; Blas Blázquez[2]; Bruno Manta[3]; Juan Nogales[4]; Cecilia Ortiz[5]

1. Laboratorio de Biotecnología, Universidad ORT Uruguay, Montevideo, Uruguay. Laboratorio de Genómica Microbiana, Institut Pasteur de Montevideo, Montevideo, Uruguay; 2. Department of Systems Biology, Centro Nacional de Biotecnología, CSIC, Madrid, Spain. CNB DNA Biofoundry (CNBio), CSIC, Madrid, Spain; 3. Laboratorio de Genómica Microbiana, Institut Pasteur de Montevideo, Montevideo, Uruguay; 4. Department of Systems Biology, Centro Nacional de Biotecnología, CSIC, Madrid, Spain. CNB DNA Biofoundry (CNBio), CSIC, Madrid, Spain; 5. Laboratorio de Biotecnología, Universidad ORT Uruguay, Montevideo, Uruguay

Abstract

(S)-Equol is a biologically active metabolite derived from soy isoflavones, noted for its potential health benefits and increasing relevance in the development of nutraceuticals and functional foods. However, its chemical synthesis remains inefficient and environmentally detrimental. Engineered Escherichia coli strains offer a sustainable alternative, enabling the microbial valorisation of underutilised agro-industrial by-products, such as defatted soy flour, into high-value compounds.

We describe a multidimensional strategy for optimising E. coli-based whole-cell biocatalysts for efficient (S)-equol production. This approach combines host strain engineering with modular synthetic biology tools to enhance both the biosynthetic capacity and operational flexibility of the system. At the metabolic level, central carbon fluxes were rewired to improve intracellular NADPH regeneration, thereby increasing the availability of this key cofactor for the reductive biosynthetic pathway. Preliminary results confirm that the engineered background supports enhanced NADPH generation, contributing positively to biosynthetic efficiency.In parallel, synthetic operons encoding key enzymes were constructed using the Golden Standard modular cloning framework, which enabled rapid prototyping and systematic variation of genetic design parameters. We developed a set of genetic circuits differing in plasmid copy number and promoter architecture, including chemically inducible and substrate-responsive regulatory systems. Early characterisation of selected variants indicates that fine-tuning expression levels is critical for balancing productivity and cellular fitness. Inducible control not only mitigates metabolic burden during biomass accumulation but also allows on-demand activation of the biocatalytic module. This regulatory flexibility facilitates the implementation of resting-cell biotransformation processes, wherein biomass is first generated under growth-optimised conditions and subsequently converted into an efficient production platform via pathway induction. Such decoupling improves process performance and simplifies downstream processing, particularly for scalable applications. Moreover, the modular nature of the system allows the exploration of context-dependent circuit configurations and adaptation to different feedstocks or bioprocess constraints.This work highlights the value of integrating rational strain engineering with tuneable expression architectures to construct robust, efficient, and sustainable microbial biocatalysts. While the proposed strategy directly advances (S)-equol production, it also contributes to the broader development of circular biotechnological processes grounded in the valorisation of agricultural residues. Beyond (S)-equol, this integrative framework offers a blueprint for designing next-generation microbial platforms tailored to the biosynthesis of a wide range of high-value compounds.

Financial support and acknowledgments

This project was supported by the Sectorial Innovation Fund for Agriculture (Innovagro) and the National Graduate Scholarships Program of the National Agency for Research and Innovation (ANII) of Uruguay under grant agreements numbers FSA_1_2024_1_180126 and POS_NAC_2023_1_177389, respectively. This research also received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 101081782 (deCYPher) and funds from PID2022-139247OB-I00 (Rob3D) project funded by MCIN/AEI/10.13039/501100011033 and European Union (Next Generation EU/PRTR) funding, a way to make Europe.

Keywords

Synthetic biology • Metabolic engineering • Whole-cell biocatalysis

Panel ID BM12 • Abstract ID 127

ANTIBIOFILM AND ANTIVIRULENCE EFFECTS OF A QUORUM QUENCHING ACYLASE AND AN ENDOLYSIN ON PSEUDOMONAS AERUGINOSA

Lucía Mei Vivó Vindel[1]**;** Daniel Baratto[1]; Inés Pareja-Cerbán[2]; Ana Saborido[1]; Javier Rocha[1]; Beatriz Maestro[1]; Jesús Miguel Sanz[2]; Isabel de la Mata[1]

1. Universidad Complutense de Madrid (UCM), Universidad Complutense de Madrid (UCM); 2. Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC)

Abstract

Antimicrobial resistance (AMR) is an increasingly concerning public health problem, primarily driven by the excessive and inappropriate use of antibiotics. Consequently, antimicrobial therapies become ineffective in both humans and animals, allowing pathogenic microorganisms to persist within their hosts. Among emerging alternative strategies, the use of quorum quenching enzymes and antibacterial lysins, has gained attention as promising alternatives and/or complements to conventional antimicrobial chemotherapy.Among pathogens, Pseudomonas aeruginosa is one of the most common causes of nosocomial infections as well as responsible for infections in immunocompromised individuals. Its pathogenicity is largely regulated by quorum sensing (QS), cell-to-cell communication mechanism that promotes biofilm formation and the production of virulence factors. Currently, the inhibition of QS, called quorum quenching (QQ), is one of the most interesting strategies for inhibiting virulence mechanisms of this notable opportunistic Gram-negative bacterium.

In addition, phage therapy, particularly the use of phage-derived lysins (known as “enzybiotics”) is of great interest due to their ability to lyse bacterial cell walls, leading to host cell lysis and subsequent bacterial death. In this work, Pseudomonas aeruginosa PAO1 DSMZ 22644, a type strain widely employed as a model organism in pathogenicity and antimicrobial strategy research, was used to evaluate the interference of the quorum quenching enzyme aculeacin A acylase from Actinoplanes utahensis (AuAAC) on quorum sensing-regulated processes: (i) biofilm adherence capacity, by crystal violet staining; (ii) biofilm development in the presence of immobilized enzyme on chambered glass slides, by interference and epifluorescence microscopy; and (iii) production of virulence factors (i.e pyocyanin and protease activity).

For biofilm adherence studies, a novel and optimized method was developed using 96-pin Peg lids, which allow for reliable and reproducible quantification of biofilm formation directly on the pins. Similarly, the effect of the VL1L endolysin, from the P. aeruginosa bacteriophage VL-1, was also assessed in this system.

The results show that treatment of both enzymes significantly inhibited biofilm formation and led to a marked reduction in the production of virulence factors, highlighting their potential as promising tools for the development of alternative antimicrobial strategies.

Financial support and acknowledgments

This work was supported by Grant PID2022-132090B-C22 from the Spanish Ministerio de Ciencia, Innovación y Universidades.

Keywords

Pseudomonas aeruginosa PAO1 DSMZ 22644 • Aculeacin A Acylase • VL1L endolysin • Quorum Sensing • Quorum Quenching

Panel ID BM13 • Abstract ID 129

DEVELOPMENT OF STARCH-BASED BIOPLASTICS FUNCTIONALIZED WITH MICROORGANISMS FOR AGRICULTURAL APPLICATIONS

Ramón Gómez-Fernández; Sara Pérez-Gutiérrez; Ana Ibáñez; Javier Rúa; María Rosario García-Armesto; María Fernanda Vasco-Cárdenas; Laura A.Aguilar; Elías R. Olivera; Carlos Barreiro
Área de Bioquímica y Biología Molecular. Facultad de Veterinaria. Campus de Vegazana. Universidad de León

Abstract

Los plásticos se han integrado profundamente en la sociedad moderna gracias a su versatilidad y durabilidad. Sin embargo, su persistencia en el medio ambiente plantea una grave preocupación ecológica, especialmente en el contexto europeo, donde la acumulación de residuos plásticos representa uno de los desafíos científicos y tecnológicos más acuciantes. Entre los sectores que contribuyen a este problema, la agricultura desempeña un papel importante, especialmente mediante el uso generalizado de plásticos en prácticas como el acolchado y la construcción de invernaderos. Si bien la plasticultura mejora la productividad y la protección de los cultivos, la eliminación de estos materiales después de la cosecha es problemática. Los métodos convencionales de reciclaje se ven obstaculizados por la contaminación con residuos del suelo y vegetales, lo que hace que la recuperación y la reutilización de plásticos agrícolas sean altamente ineficientes. Además, la división de los plásticos en el campo contribuye a la liberación de micro y nanoplásticos, que tienen efectos perjudiciales para la salud del suelo, las comunidades microbianas y los ecosistemas en general. Para abordar estas limitaciones, se necesitan estrategias innovadoras para la transición de los plásticos convencionales a materiales que se degradan de forma natural y segura en el entorno agrícola. El proyecto BioPac (ref. TED2021-131864B-C21), financiado por el Ministerio de Ciencia e Innovación de España, tiene como objetivo desarrollar plásticos biodegradables de nueva generación que incorporan microorganismos beneficiosos, seleccionados y optimizados por su capacidad para promover la degradación de plásticos. En este trabajo, se han integrado con éxito dos cepas microbianas en una matriz bioplástica a base de almidón . Se han optimizado las condiciones de crecimiento para garantizar la viabilidad, y las evaluaciones preliminares confirman la incorporación exitosa de los microbios, así como la biodegradación del material puramente derivado del almidón. Estudios en curso evalúan la formulación de prototipos de bioplásticos mejorados, complementados con compuestos plastificantes para mejorar la resistencia mecánica y la durabilidad, y se evaluará su biodegradabilidad. Este enfoque interdisciplinario, que combina la biotecnología y la ciencia de los materiales, abre nuevas vías para la producción de bioplásticos funcionalizados adaptados para uso agrícola. Estos materiales ofrecen un doble beneficio: minimizan el impacto ambiental del uso de plástico y mejoran la salud de las plantas. El proyecto BioPac ejemplifica el potencial de la biotecnología para impulsar innovaciones sostenibles y circulares en la agricultura.

Financial support and acknowledgments

Proyecto BioPAC (Desarrollo de bioplásticos bioactivos y de vida útil controlado) (Ref. n.º TED2021131864BC21), financiado por el MCIN (Ministerio de Ciencia e Innovación)/AEI (Agencia Estatal de Investigación)/10.13039/501100011033 (Identificador Digital de Objetos) y el Plan de Recuperación, Transformación y Resiliencia de la Unión Europea (RETC).

Keywords

Biodegradation • Bioplastics • Microbial functionalization

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BM14 • Abstract ID 130

SUSTAINABLE ALTERNATIVES TO SYNTHETIC FUNGICIDES: THE BIOBIVE APPROACH

Ana Ibáñez; Olga Galán; Ainara L. Díez-Díez; Ramón Gómez-Fernández; Elías R. Olivera; Carlos Barreiro
Área de Bioquímica y Biología Molecular. Facultad de Veterinaria. Campus de Vegazana. Universidad de León

Abstract

Fungal pathogens affecting horticultural crops represent a persistent and costly challenge in modern agriculture, often managed through the use of synthetic pesticides. While effective, these chemical treatments carry significant environmental and health risks, driving the urgent need for safer and more sustainable alternatives. The EU-funded project BioBIVE (no. 101130442; https://biobive.eu) addresses this need by developing biodegradable delivery systems for the controlled release of bioactive agents aimed at managing or reducing fungal diseases in three key European crops: tomatoes, strawberries, and carrots.

The project combines three types of biodegradable platforms (bioplastic mulch films, biochar-based formulations, and sprayable biodegradable mulching) with diverse bioactive agents (including basic substances, microbial antagonists, and seaweed extracts) selected for their antifungal properties and compatibility with biodegradable matrices. This integrated approach seeks to enhance disease control efficacy while promoting sustainability and circularity in agricultural practices.In vitro bioassays indicate that certain basic substances, particularly chitosan and horsetail extract (Equisetum arvense), exhibit strong inhibitory effects against a range of phytopathogenic fungi, including Sclerotinia sclerotiorum, Phytophthora cactorum, and Pythium ultimum, among others. These findings underscore the potential of these natural agents as effective and environmentally benign alternatives to synthetic fungicides.In parallel, thermogravimetric analyses (TGA) were conducted to assess the thermal stability of candidate bioactive substances under conditions relevant to their incorporation into biodegradable matrices. Remarkably, most of the basic substances analyzed retained over 90% of their mass at temperatures above 100 °C, suggesting they are thermally robust and suitable for integration into thermally processed delivery systems. However, further analyses are required to determine whether thermal treatment allows them to retain their biocidal activity.

By combining natural bioactivity with biodegradable technology, BioBIVE proposes an innovative path forward for crop protection: one that reduces chemical inputs, limits environmental damage, and aligns with EU strategies for sustainable agriculture. This work contributes to the broader goal of transitioning toward eco-sustainable plant protection solutions and resilient agroecosystems in Europe and beyond.

Financial support and acknowledgments

BioBIVE project (No. 101130442), funded by the EU under the Horizon Europe Framework Programme(https://cordis.europa.eu/project/id/101130442; www.biobive.eu).

Keywords

Antifungal activity • Basic substances • Biopesticides

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

Panel ID BS1 • Abstract ID 10

TOPICAL OPHTHALMIC NANOFORMULATION OF ASTAXANTHIN: PHYSICOCHEMICAL CHARACTERIZATION AND IN VITRO INTRAOCULAR DIFFUSION FOR RETINAL THERAPY

Marisol Alejandra Luviano García
Universidad de Guadalajara

Abstract

Oxidative stress is a key driver of retinal neurodegeneration in age-related macular degeneration (AMD), the leading cause of irreversible vision loss in the elderly. Astaxanthin (ASTAX), a potent antioxidant with neuroprotective properties, has demonstrated the ability to mitigate oxidative damage in retinal cells. However, its clinical application is severely hindered by poor aqueous solubility and limited ocular bioavailability. To address these challenges, we developed a topical ophthalmic liposomal nanoformulation of ASTAX designed to enhance corneal permeability and retinal delivery.

The formulation was developed using a self-assembling PEGylated lipid system, achieving an encapsulation efficiency >98%. Physicochemical characterization confirmed a nanoscale particle size, low polydispersity, and near-neutral zeta potential, ensuring stability and minimal electrostatic interactions. The formulation exhibited a physiologically compatible pH and osmolarity, and transmission electron microscopy (TEM) validated its uniform spherical morphology (Fig. 1A-C).

In vitro intraocular diffusion studies using excised rabbit corneas demonstrated a significant enhancement in ASTAX permeation with the liposomal formulation compared to free ASTAX (Fig. 1D-F), supporting its potential for effective transcorneal transport and retinal bioavailability. These findings establish the feasibility of this nanocarrier system for non-invasive ocular drug delivery, addressing critical limitations in antioxidant-based retinal therapies.

With optimized physicochemical properties and enhanced intraocular permeability, this formulation represents a promising candidate for in vivo pharmacokinetic and therapeutic validation in AMD models, advancing the development of targeted, non-invasive neuroprotective strategies for retinal diseases.

Financial support and acknowledgments

This research was supported by the Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT). The authors gratefully acknowledge the Centro Universitario de Ciencias Exactas e Ingenierías (CUCEI), Universidad de Guadalajara, and the Escuela de Medicina y Ciencias de la salud, Tecnológico de Monterrey, campus Guadalajara for providing the research facilities and technical support necessary for this study.

Keywords

Topical ophthalmic delivery • Astaxanthin nanoformulation • Intraocular diffusion • Retinal neuroprotection

continue –>

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

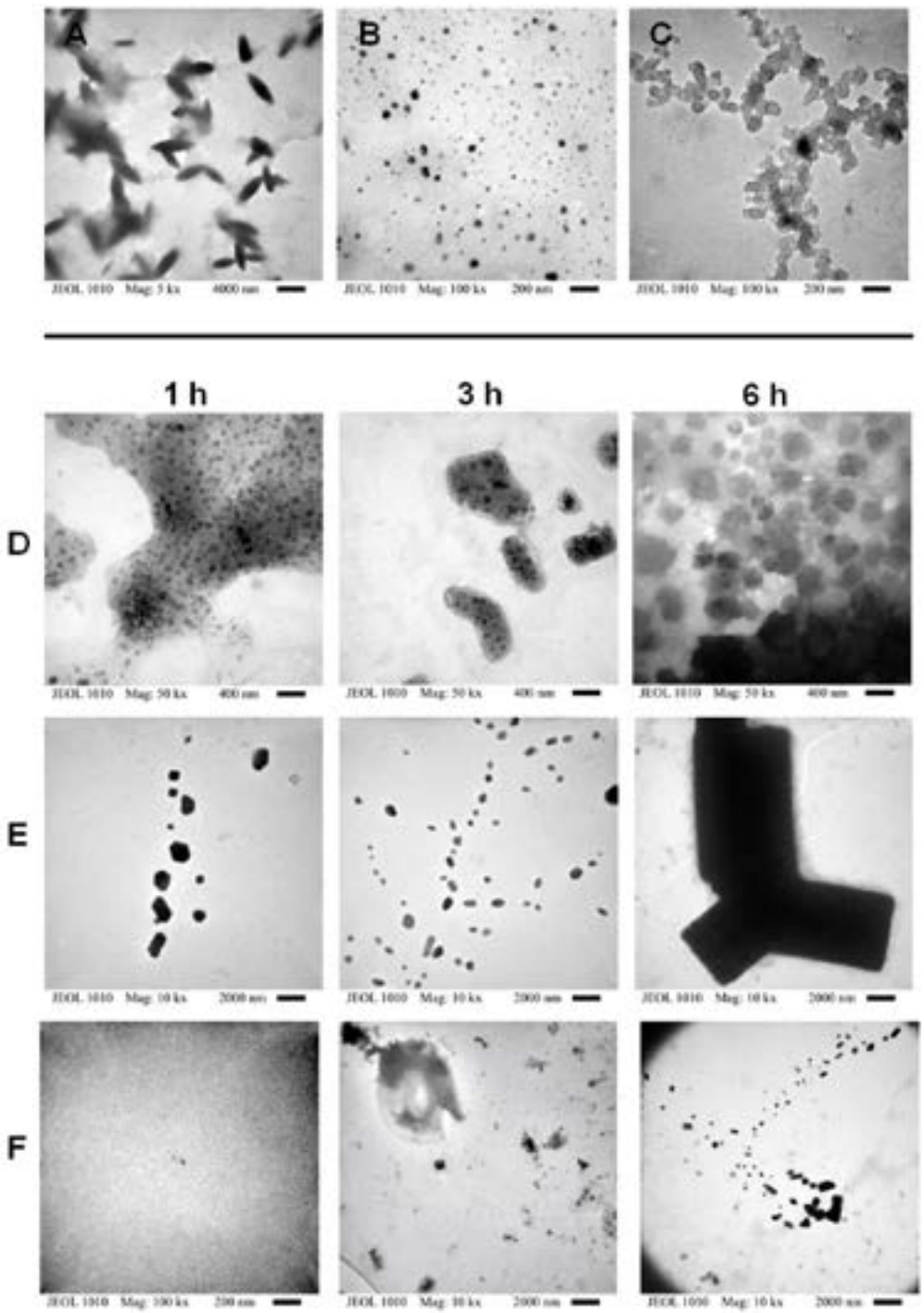


Fig.1 TEM micrographs of formulation characterization and intraocular diffusion samples over time: (A-C) Morphological characterization of formulations: (A) free ASTAX, (B) empty liposomes, and (C) ASTAX-liposomes. (D-F) Receptor chamber samples over time: (D) Vesicular structures corresponding to ASTAX-liposomes, (E) ASTAX crystals released from liposomes, and (F) ASTAX crystals from free ASTAX.

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BS2 • Abstract ID 35

STRUCTURAL BASIS OF SPECIFICITY OF A FUNGAL B-FRUCTOFURANOSIDASE IN COMPLEX WITH OLIGOSACCHARIDES

Marta Ruiz[1]; Egle Narmontaite[2]; María Fernández Lobato[2]; Francisco J. Plou[3]; Julia Sanz Aparicio[1]
1. Department of Crystallography & Structural Biology. Institute of Physical-Chemistry "Blas Cabrera", CSIC, Madrid, Spain; 2. Centre of Molecular Biology Severo Ochoa (CBMSO), CSIC. Madrid, Spain. 3 Institute of Catalysis, CSIC. Madrid, Spain; 3. Institute of Catalysis, CSIC. C/Marie Curie 2, Madrid, 28049, Spain

Abstract

Glycosyl hydrolases (GH) represent a fundmental group of key enzymes in carbohydrate metabolism responsible for hydrolyzing glycosidic bonds in oligo- and polysaccharides. Within this group, invertases or B-fructofuranosidases from the GH32 family (EC 3.2.1.26) have gained significant relevance due to their dual functionality: (1) the synthesis of fructooligosaccharides (FOS), compounds widely recognized for their prebiotic effects on human health, and (2) their specific hydrolytic activity on B-D-fructofuranoside substrates, releasing B-fructose at non-reducing ends. These properties explain their biotechnological relevance and broad applications, ranging from sweetner production to the development of bioactive molecules for the pharmaceutical industry.

In this context, the fungal PILNV B-fructofuranosidase GH32 is a highly glycosylated enzyme that exhibits broad susbtrate specificity, recognizing and cleaving B-glycosidic bonds, specifically targeting small substrates with B(1-2) and B(1-6) linkages, generating inverted sugars. Additionally, it catalyzes the reverse reaction via transglycosylation, producing short-chain FOS such as 6-kestose, neokestose, 1-kestose or blastose. In this work, the structural determination of the PILNV B-fructofuranosidase has been conducted by X-ray crystallography, including its native form and the inactivated mutant D81A, in complex with fructose, sucrose and raffinose. The results elucidate the structural basis for modulating GH32 substrate specificity, offering novel tools to enhance bioactive compound synthesis via enzyme engineering.

Financial support and acknowledgments

This work was supported by Project GLYCOENZ-GREEN (PID2022-136367OB-C31/2/3)

Keywords

X-ray crystallography • Fructooligosaccharides • Glycosyl hydrolase family 32 • β-fructofuranosidase

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BS3 • Abstract ID 92

HETEROLOGOUS EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF ARENIN: A NOVEL KUNITZ-TYPE PEPTIDE FROM DRYOPHYTES ARENICOLOR

Enrique Hidalgo Vázquez
Tecnologico de Monterrey, Escuela de Ingeniería y Ciencias, Centro de Biotecnología-FEMSA

Abstract

Amphibian-derived secretions have emerged as a rich source of bioactive molecules with therapeutic potential against cancer, metabolic syndromes, and inflammatory conditions. Arenin is a cysteine-rich Kunitz-type serine protease inhibitor originally isolated from the skin secretion of Dryophytes arenicolor. In this study, the heterologous expression, purification, and comprehensive in vitro bioactivity evaluation of recombinant arenin are assessed, highlighting its multifunctional therapeutic promise.Recombinant arenin was produced by cloning a codon-optimized gene into Escherichia coli, with expression induced at 30°C and 37°C to evaluate the effects of temperature on solubility and yield. Proteins were purified by affinity chromatography and quantified for downstream assays. Bioactivity was assessed using human dermal fibroblasts (HDFa), colorectal (Caco-2) and breast (MCF-7) cancer cell lines, insulin-resistant HepG2 hepatocytes, and RAW 264.7 macrophages. Assays included cell viability (MTT), wound healing (scratch), glucose uptake, intracellular ROS quantification, and nitric oxide production, under both standard and stress-inducing conditions.

At 30 °C, the expressed arenin accumulated predominantly in the soluble fraction, while at 37 °C the formation of inclusion bodies was favored. Both conditions yielded arenin at →80% purity, with final recoveries of 8.36 ± 0.63 mg and 14.16 ± 1.02 mg per 1000 mL culture, respectively,In HDFa, viability was unaffected across a wide concentration range and was enhanced at the highest dose (1 mg/mL), suggesting pro-regenerative capacity. Scratch assays in serum-deprived and hyperglycemic conditions showed near-complete wound closure (98.61 ± 3.09% and 100.0 ± 0.20%, respectively) within 72 h. Regarding its effect on cancer cell lines, arenin displayed selective cytotoxicity in Caco-2, reducing viability to 60.20 ± 3.40% at 31.25 µg/mL. In contrast, in MCF-7, viability responses were non-linear, indicative of dose-dependent receptor crosstalk. Arenin enhanced glucose uptake in HepG2 cells, increasing it from 23.97 ± 2.79% (insulin-resistant control) to 51.38 ± 2.95% at 250 µg/mL. In RAW 264.7 macrophages, it modulated nitric oxide (NO) production in a concentration-dependent manner.Altogether, these findings support the therapeutic versatility of arenin, encompassing protease inhibition, pro-regenerative wound healing activity, metabolic enhancement, antioxidant defense, and selective anticancer effects.

Financial support and acknowledgments

The authors acknowledge the support provided by SECIHTI through Enrique De Jesús Hidalgo Vázquez scholarship CVU: 1095127. The authors also express their gratitude for the partial financial support received from Tecnológico de Monterrey through the “Challenge-Based Research Funding Program 2022” (Project ID: IJXT070–22EG57001).

Keywords

Arenin • Amphibian • Kunitz-type peptide

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BS4 • Abstract ID 112

IMPACT OF SUGARS IN PROTEIN-BASED BIOPHARMACEUTICAL DRUG FORMULATIONS TO PROTECT THEM AGAINST FREEZE DRYING DERIVED STRESSES

Noel Arias Salas[1]; Marina Guillen Montalban[1]; Oscar Romero Ormazábal[1]; Maria Aguilar[2]; Jaume Vallet Xicoy[2]; Antoni Casablancas Mira[1]
1. Universitat Autònoma de Barcelona; 2. Compliance Consulting and Engineering Services, S.L.

Abstract

Protein-based biopharmaceuticals, such as enzymes and antibodies, are gaining prominence in the drug market due to their high specificity and potency. However, their limited stability in solution complicates manufacturing and global distribution. Lyophilization (freeze-drying) addresses this issue by removing water through sublimation under vacuum and low temperatures. Rabbit muscle lactate dehydrogenase (RmLDH) is an oxidoreductase enzyme that plays a crucial role in the final step of anaerobic glycolysis. RmLDH has been extensively used as a model protein for lyophilization and desiccation studies, due to its commercial availability, its sensitivity to lyophilization-induced stresses and its mammalian origin.

This study aimed to investigate the protective effects of sucrose, trehalose, and hydroxypropyl Beta cyclodextrin (HP B CD) on RmLDH during freeze-drying and subsequent room-temperature storage. Additionally, the impact of these sugars on the cake appearance after lyophilization was assessed.Following freeze-drying, RmLDH without excipients experienced a 30% loss in enzymatic activity. In contrast, trehalose and HP B CD significantly mitigated this loss, limiting it to 19% and 16%, respectively. After 30 days of storage at room temperature, formulations with sucrose and trehalose maintain activity levels comparable to those just after lyophilization, while the excipient-free samples activity further decreased to 30%. An additional benefit of the sugar formulations was the production of non-collapsed cakes, indicating improved structural integrity.These results underscore the importance of optimized protein formulations, with trehalose and HP B CD acting as effective lyoprotectants during freeze-drying.

Financial support and acknowledgments

Acknowledgments: This work is supported by AGAUR Doctorate Industrial 2022 DI 55 and AGAUR of Generalitat de Catalunya for 2021 SGR00143.

Keywords

lyophilization • proteins • formulation • trehalose • sucrose • Hydroxypropyl B Cyclodextrin

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BS5 • Abstract ID 139

TARGETED PROTAC THERAPY FOR HER2-OVERPRESSING BREAST TUMORS

Celia Nieto[1]; Elena Díaz-Rodríguez[2]; Milena A. Vega[1]; Atanasio Pandiella[2]; Eva M. Martín del Valle[1]
1. Departamento de Ingeniería Química y Textil, Facultad de Ciencias Químicas, Universidad de Salamanca; 2. Instituto de Biología Molecular y Celular del Cáncer, Salamanca

Abstract

Today, about 15-20% of the more than two million cases of breast cancer (BC) diagnosed each year are characterized by the overexpression of the human epidermal growth factor receptor 2 (HER2). Since it controls cell growth and migration, the amplification of this receptor has been traditionally linked to poor clinical prognosis. Over the past two decades, various HER2-targeted therapies have been developed, but HER2+ BC remains a fatal disease, especially in advances stages and when resistances emerge. Hence, novel therapeutic strategies for this subtype of BC are still needed and, in this regard, some PROteolysis Targeting Chimeras (PROTACs) have gained recognition, like MZ1.

MZ1, when combined with the monoclonal antibody trastuzumab (Tz), has shown marked ability to inhibit HER2+ BC proliferation. However, this compound, which has high molecular weight and remarkable polarity, does not have very promising pharmacokinetics or selective antiproliferative activity. Thus, in this work, a targeted MZ1 vehicle based on polydopamine nanoparticles (PDA NPs) was developed to solve these drawbacks. PDA NPs have certain inherent antitumor capacity, and that is why they (180 nm) were loaded, in addition to MZ1 (39 µg/mg), with Tz (37 µg/mg) to target HER2-overexpressing cancer cells and achieve synergistic effects. The resulting drug delivery system (DDS) was deeply characterized (DLS, TEM, SEM, FTIR), and its antitumor activity was successfully determined in vitro with different HER2-overexpressing breast cancer cell lines, conventionally grown and forming spheroids. Both, colorimetric and live/dead assays were performed, revealing similar results: PDA NPs loaded with MZ1 and Tz (PDA NPs@MZ1-Tz) managed to significantly reduce the survival rate of the treated cells after 72 hours, and they completely disaggregated HER2+ BC spheroids after the same period time. Likewise, when in vivo assays were also carried out with a murine xenograft model that was daily injected with PDA NPs@MZ1-Tz (0.5 mg/kg), a reduction in tumor growth after 21 days was shown despite their great aggressiveness. In fact, PDA NPs@MZ1-Tz were more effective than equivalent doses of MZ1 and Tz freely administered, so transporting MZ1 in Tmab-vectored PDA NPs could be an excellent strategy to enhance MZ1 pharmacokinetics and specificity, and to improve current HER2+ BC therapy.

Financial support and acknowledgments

This research work was financially supported by the Spanish Ministry of Science and Innovation (PDC2021-121393-I00).

Keywords

Breast cancer • HER2 • Polydopamine nanoparticles • PROTAC

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BS6 • Abstract ID 144

SUSTAINABLE SYNTHESIS OF ANTIMICROBIAL POLYHYDROXYALKANOATES IN GENETICALLY OPTIMIZED PSEUDOMONAS PUTIDA KT2440

Celia Parrado; Marina Rodríguez-Carreiro; M. Auxiliadora Prieto
Centro de Investigaciones Biológicas - Margarita Salas (CIB-MS)

Abstract

Over the past decades, the development of biomedical devices has significantly enhanced patient care and quality of life. However, their susceptibility to infections caused by opportunistic microorganisms that adhere to implant surfaces and proliferate through the formation of structured biofilms remains a major drawback. These biomaterial-associated infections (BAIs) are particularly difficult to treat, as biofilm-embedded bacteria display increased antibiotic tolerance. Among resistant pathogens, methicillin-resistant Staphylococcus aureus (MRSA) is especially concerning due to its resistance to all b-lactam antibiotics, high prevalence, pathogenicity and remarkable capacity to acquire further resistance mechanisms, making it a major public health threat. A promising strategy to prevent BAIs involves coating implant surfaces with antimicrobial polymers. Polyhydroxyalkanoates (PHAs), natural polyesters produced by microorganisms under unbalanced growth conditions, exhibit notable biomedical potential due to their biodegradability, biocompatibility and absence of teratogenic and carcinogenic effects. Although PHAs lack intrinsic antimicrobial activity, functionalization with thioester groups in their side chains leads to the production of PHACOS, a second-generation PHA that exhibits antimicrobial activity against S. aureus, including MRSA strains. This biopolymer is synthesized by Pseudomonas putida KT2440 under co-feeding conditions using fatty acids and the PHACOS precursor 6-acetylthiohexanoic acid (6-ATH). With the aim of improving process sustainability and economic feasibility, biopolymer production was evaluated using the MT9 strain, a genetically engineered derivative of P. putida KT2440 designed to synthesize PHAs from carbon sources non-related to fatty acids such as sugars. Additionally, the production experiments were explored using 6-ATH synthesized in the laboratory. P. putida MT9 cultures were carried out using glucose as primary carbon source and co-fed with 6-ATH. The resulted biopolymer was purified via Soxhlet extraction using CHCl3 as solvent and subjected to monomer composition analysis. Results confirmed the successful chemical synthesis of the precursor 6-ATH and the effective production of PHACOS using glucose as the main carbon source, supporting the feasibility of this approach for the sustainable development of antimicrobial biomaterials.

Financial support and acknowledgments

This work has been supported by the “Ayudas para contratos predoctorales para la formación de doctores” fellowship (PREP2023-001825), granted by the Spanish Ministry of Science and Innovation; the JAE Intro 2024 research initiation fellowship (JAEINT24_EX_0273), awarded by the Spanish National Research Council (CSIC); and the OPENVIRO (PID2023-146557OB-C21) project funded by AEI/10.13039/501100011033. Authors would also like to thank the Complutense University of Madrid for the support provided during this project.

Keywords

Antibiotic resistance • Polyhydroxyalkanoates • Functionalized PHA • Pseudomonas putida KT2440 • Sustainable production

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

Panel ID BS7 • Abstract ID 146

MODELING NANOPARTICLE DYNAMICS ACROSS BIOLOGICAL BARRIERS: FROM CIRCULATION TO TUMOR CELL INTERNALIZATION

Alvaro González Garcinuño; Eva Martín del Valle
University of Salamanca

Abstract

Understanding how nanoparticles travel through blood vessels and distribute within tumor tissues is vital for advancing nanotechnology-based clinical treatments. Although nanoparticle transport in the bloodstream has been extensively studied, a complete evaluation of all stages leading to their internalization by target cells remains lacking.

This study introduces a comprehensive computational model that simulates the entire journey of nanoparticles—from transport in the bloodstream and passage through the endothelium to diffusion within the tumor stroma and eventual cellular uptake. Uniquely, the model incorporates the role of nanoparticle surface affinity, capturing ligand-receptor interactions crucial for targeted delivery. Through multiple case studies, the model identifies key nanoparticle design parameters that may help reduce reliance on in vivo experimentation. Results highlight the importance of a low Stokes number for effective dispersion in capillary networks. Additionally, surface functionalization with ligands significantly enhances targeting efficiency within the tumor stroma, reducing the proportion of nanoparticles that do not reach the intended cells by half.

This work presents a novel, end-to-end computational framework for simulating nanoparticle distribution following systemic administration, including their specific recognition and internalization by target cells.Keywords: nanoparticle tracking, transport modeling, tumor microenvironment, affinity, computational fluid dynamics (CFD).

Panel ID BS8 • Abstract ID 147

NANOTECHNOLOGY APPLIED TO CANCER: THERMAL PHOTOTHERAPY WITH GOLD NANORODS TARGETING A TUMORAL BIOMARKER

Benjamín Ignacio Riquelme Díaz[1]; Ivo Campos[2]; Marcelo Kogan[3]; María Carmen Molina[4]: Claudia Altamirano[5]

1. Laboratory of Animal Cell Culture, Faculty of Biochemical Engineering; 2. Pontifical Catholic University of Valparaíso, Valparaíso, Chile; 3. Nanomedicine and Nanotheranostics Laboratory, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile; 4. Immunotherapy Laboratory, Immunology Program, Institute of Biomedical Sciences, University of Chile, Santiago, Chile; 5. Laboratory of Animal Cell Culture, Faculty of Biochemical Engineering, Pontifical Catholic University of Valparaíso - Regional Center for Studies on Healthy Eating, Curauma-Placilla, Valparaíso, Chile. - IMPACT, Interventional Medicine Center for Precision and Advanced Cell Therapy, Santiago, Chile

Abstract

Gold-based nanostructures have attracted significant interest in cancer therapy due to their ability to induce localized cell death through controlled temperature increase—known as photothermal therapy—under irradiation with light at specific wavelengths, whose efficiency depends on their shape (sphere, rod, etc.). Gold nanorods (GNRs), when irradiated with light in the near-infrared (NIR) range, a biocompatible wavelength, absorb energy and convert it into heat via surface plasmon resonance (SPR), selectively damaging tumor cells. This study incorporates active targeting to improve tumor specificity by using monoclonal antibodies to direct nanoconjugates toward cells expressing specific tumor biomarkers.

To ensure the pharmacological safety of GNRs regarding bioaccumulation and reactivity, their surfaces are modified with polyethylene glycol (PEG). This modification enhances biocompatibility, prevents nanoparticle aggregation, reduces protein corona formation, and minimizes immune system clearance. PEGylation significantly improves nanoparticle behavior in biological systems by prolonging circulation time and reducing nonspecific interactions, with PEGylated products such as antibodies and other proteins approved by agencies like the FDA. Additionally, the presence of carboxyl groups in PEG allows a specific antibody conjugation strategy through orthogonal EDC/NHS coupling chemistry. The terminal carboxyl groups of PEG-COOH are activated with EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), forming unstable O-acylisourea intermediates stabilized with NHS (N-hydroxysuccinimide) to generate reactive NHS esters. These esters readily react with primary amines on lysine residues of antibodies, forming stable amide covalent bonds. This orthogonal method ensures specific conjugation under mild aqueous conditions while preserving antibody functionality.

In this study, a commercial anti-IgG monoclonal antibody, Rituximab (IgG1), was used to functionalize the GNRs via EDC/NHS chemistry, serving as a model antibody for the conjugation process before targeting anti-MICA in cancer cells expressing MICA. The conjugation was verified through changes in hydrodynamic diameter (measured by dynamic light scattering, DLS), surface charge (zeta potential), and shifts in UV-Vis absorbance spectra.

Furthermore, antibody conjugation was confirmed by a dot blot assay, and additional optical characterization techniques will be applied for more comprehensive validation. The development of antibody-functionalized gold nanorods represents a promising strategy to improve the specificity and efficacy of photothermal cancer therapy. The efficient conjugation achieved via EDC/NHS chemistry, combined with rigorous verification through DLS, zeta potential, UV-Vis spectroscopy, and dot blot, ensures high-quality nanoconjugates. This approach paves the way for more targeted and less invasive therapies with the potential to minimize side effects and improve clinical outcomes in patients with tumors expressing specific biomarkers.

Financial support and acknowledgments

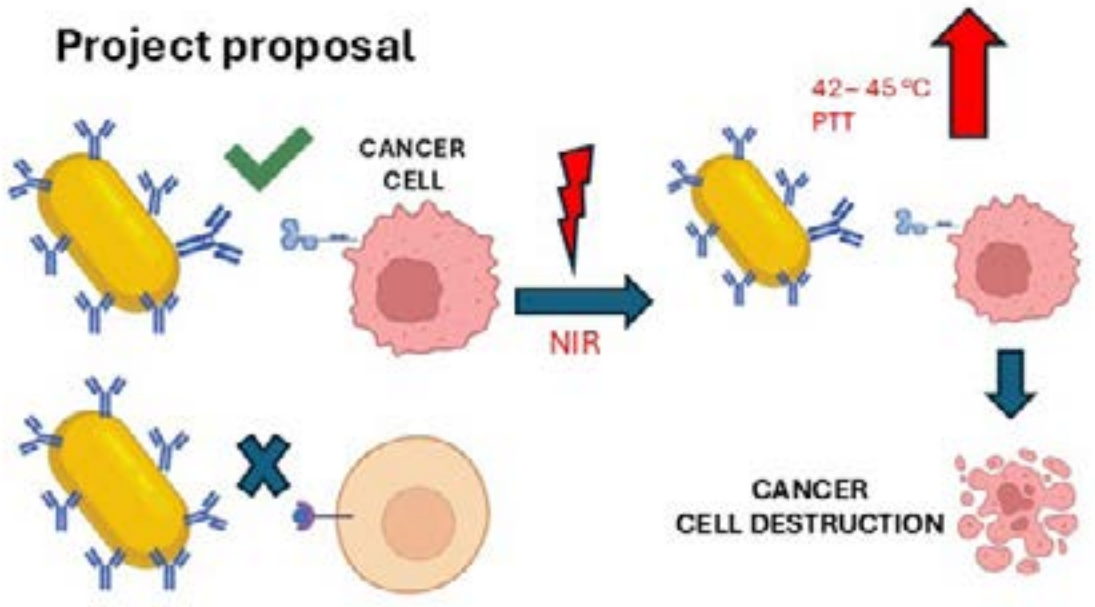
This work was supported by the 2023 National Doctoral Fellowship (2123202), the ANILLO Project in Science and/or Technology 2021 (ACT210068), and the Fondecyt projects (Nos. 1241453 and 1251140).

Keywords

Oncology • Infrared Laser Phototermal Phototherapy • Tumor-biomarkers Antibodies • Nanomedicine • Nanorods

continue –>

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona



July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BS9 • Abstract ID 150

DIMATE TARGETS ALDH1A3: ELUCIDATING THE BINDING MECHANISM THROUGH BIOCHEMICAL AND BIOPHYSICAL APPROACHES

Daniela Covaleda Cortés[1]; Marina Corbella Morató[2]; Marc Serveto Castaño[1]; Juan Artuch Pérez de Ciriza[1]; Sergi Montané Bel[3]; Martin Hugo Pereira[4]; Julia Lorenzo Rivera[5]; Carme Rovira Virgili[2]; Xavier Parés Casasampera[1]; Jaume Farrés Vicén[1]; Raquel Pequerul Pavón[1]

1. Departament de Bioquimica i Biologia Molecular, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain; 2. Institut de Química Teòrica i Computacional (IQTC), Universitat de Barcelona, Barcelona, Spain; 3. Departament de Bioquímica i Biologia Molecular, Institut de Biotecnologia i Biomedina (IBB), Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain; 4. Serra Hunter Fellow, Departament de Bioquimica i Biologia Molecular, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain; 5. Departament de Bioquímica i Biologia Molecular, Institut de Biotecnologia i Biomedina (IBB), Universitat Autònoma de Barcelona y Centro de Investigación Biomédica en Red, Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Cerdanyola del Vallès, Spain

Abstract

Human aldehyde dehydrogenase (ALDH) catalyzes the irreversible oxidation of endogenous and exogenous aldehydes into less reactive carboxylic acids in a NAD(P)+-dependent reaction. Among them, ALDH1A isoforms are homotetrameric enzymes that are highly active in cancer stem cells (CSC), being associated with drug resistance, tumor recurrence, and metastasis . Consequently, ALDH inhibitors have been explored as coadjuvants to improve existing cancer therapies . DIMATE (S-methyl 4-(dimethylamino)-4-methylpent-2-ynethioate) is a time-dependent ALDH1A inhibitor. Active-site C314S (targeting the catalytic residue), C313S, and C313A mutants were generated to investigate the binding mechanism of DIMATE to the ALDH1A3 isoform, based on molecular dynamics and QM/MM simulations. We performed inhibition screenings to evaluate time dependence and conducted an IC50 assay at saturating concentrations to determine the apparent inhibitory potency. C314S and C313S mutants exhibited significantly increased IC50 values as compared to the wild-type enzyme. Notably, C314S but not C313S showed a loss of time-dependent inhibition, suggesting that residue 314 is critical for binding. C313A mutation caused a very moderate increase in the IC50 value and abolished time dependence, indicating a potential role of residue 313 in modulating the interaction with DIMATE. Thermal shift assays revealed an increased stability of the ALDH1A3-DIMATE complex in a concentration range of 1-5 µM DIMATE and 1 µM ALDH (up to 1.25 molecules of inhibitor per subunit). In contrast, higher DIMATE concentrations (20 µM; >5 molecules of inhibitor per subunit) induced protein destabilization, suggesting a ligand-mediated biphasic thermal stability. It appears that, at low concentrations, DIMATE may bind preferentially to the native (folded) state of the protein while, at high concentrations, it binds to the unfolded (denatured) state. These findings are consistent with the observation that excess DIMATE promotes ALDH1A3 aggregation in a concentration-dependent manner, as assessed by size exclusion chromatography analysis. Structural studies are being conducted to elucidate the three-dimensional structure of the ALDH1A3-DIMATE complex.

Financial support and acknowledgments

This research was funded by the Spanish Ministerio de Ciencia, Innovación y Universidades (Agencia Estatal de Investigación, grant numbers PID2020-119424RB-I00/AEI/10.13039/501100011033 and PID2023-150696NB-I00 / MCIU/ AEI / 10.13039/501100011033 /FEDER, UE). This research was also supported by the Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR, grup consolidat N-TETRASCAN 2021SGR00135). R. Pequerul obtained financial support from the company Advanced BioDesign through a research contract agreement with Universitat Autònoma de Barcelona. We thank Dr. Mileidys Pérez-Alea for her helpful support and discussions.

Keywords

Aldehyde dehydrogenase • enzyme inhibition • molecular dynamics simulations • site-directed mutagenesis| • thermal shift assay

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA1 • Abstract ID 30

THE REMARKABLE POTENTIAL OF PSEUDOMONAS CHLORORAPHIS: ADVANCING SUSTAINABLE AGRICULTURE THROUGH BIOSTIMULATION AND BIOLOGICAL CONTROL

Marina Pareja Valero; Hanan El Bakkali; Maria Camila Espinosa Araque; Gloria Andrea Silva Castro
Dept. Microbiology MAFA Bioscience

Abstract

The excessive use of conventional chemical fertilizers and pesticides has resulted major environmental issues. On the other hand, diseases caused by pathogens affect crop quality, causing significant yield and agricultural production losses. To address these challenges, the utilization of Plant Growth-Promoting Rhizobacteria (PGPR), specifically Pseudomonas chlororaphis, is a promising alternative to traditional fertilizers and pesticides. Among the multiple benefits, P.chlororaphis has the ability to solubilise phosphorus, optimising plant nutrition. This characteristic was evaluated by inoculating the bacteria on NBRIP (National Botanical Research Institute Phosphate) medium, where the formation of clearance zones evidenced. P.chlororaphis obtained an average of 0,12cm radius in these zones.

P. chlororaphis produces pyoverdin, a green fluorescent compound that acts as a siderophore, capable of chelating insoluble iron and reducing it to its soluble form, thus increasing the bioavailability of this essential element for plant growth, vigour and photosynthesis processes. The siderophore production was evaluated by inoculating the bacteria on CAS medium (Chrome Azurol S), where the assay is considered positive when zones of orange colouration and clearing are observed; P.chlororaphis achieved an average of 0,38cm radius in these zones. Moreover, on King B medium, pyoverdin production was verified by observing fluorescence under UV light. Furthermore, the auxin production of P.chlororaphis was also quantified by the Salkowski reagent reaction method, resulting in 50µg/ml auxin; these phytohormones promote plant growth and yield. Additionally, P.chlororaphis has demonstrated remarkable efficacy as a biological control agent. The synthesis of various compounds by P.chlororaphis, like Phenazine 1-carboxylic acid (PCA), Pyrrolnitrin, 2-hexyl-5-propyl resorcinol (HRP) or chitinases, plays a crucial role in disrupting the structure of phytopathogenic fungi. Several studies have confirmed the strong biocontrol effect of P.chlororaphis against plant pathogens isolated from environmental crops such as Aspergillus niger, Magnaporthe grisea, Fusarium graminearum, Botrytis cinerea, Phytium and Penicillium, the latter with a mycelial inhibition of 60%. Finally, in vivo tests have shown the high capacity of P.chlororaphis to significantly reduce the severity of diseases caused by the bacterium Dickeya zeae in rice and by the fungus Uncinula necator in grapevines, in the latter case by up to 53%.

Considering the aforementioned points, P.chlororaphis emerges as a perfect candidate for promoting sustainable agriculture. Its ability to enhance plant growth and inhibit disease proliferation makes it a great tool for environmentally friendly agricultural practices. Therefore, different agricultural products based on this bacterium are being developed at an industrial level, including liquid formulations and others involving drying technologies.

Financial support and acknowledgments

Mafa Bioscience

Keywords

Pseudomonas chlororaphis • PGPR • Biocontrol • Biostimulation

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA2 • Abstract ID 41

REMOVAL OF PESTICIDES FROM RINSE WASTEWATER BY TRAMETES VERSICOLOR IN A PILOT SCALE ROTATORY DRUM BIOREACTOR

Adrià Altayó Sanfeliu; Paqui Blánquez, Montserrat Sarrà
Autonomous University of Barcelona

Abstract

Pesticides are substances intended to prevent, destroy or control any pest. They are essential to sustain global food production. However, sustainable agricultural practices must be ensured to minimize environmental risks, such as adoption of hygiene measures including management of rinse wastewater (RWW) produced when cleaning agricultural equipment and machinery contaminated with pesticides. RWW contain high pesticide concentrations, and they are accumulated in artificial ponds but posing risks to groundwater and, environmental and human health if leakage occurs. Scientists had been investigating RWW treatments, with bioremediation emerging as an ecological and low-cost alternative to chemical treatments. Specifically, ligninolytic fungi, like T. versicolor, have shown potential in removing pesticides thanks to their non-specific oxidative enzymatic systems. However, in non-sterile matrices, fungi compete with bacteria, which have faster metabolisms and can use contaminants as carbon and energy sources, while fungi require substrate addition for activity maintenance. Strategies to promote fungi over bacteria include immobilizing fungi on wood chips (used as substrate) and maintaining acid pH. This study proposes scaling up to 50L a Rotatory Drum Bioreactor (RDB) with T. versicolor immobilized on wood partially submerged to treat 200L RWW. The RDB pilot plot is equipped with pH, oxygen, and temperature control installed inside a container with solar panels in Mas Badia (Baix Empordà, Catalonia, Spain), an area of intensive agriculture. Three different RWW were treated in batch mode. Pesticides mixture varied in type and concentration, with acetamiprid, a neonicotinoid pesticide, being common in all three RWW. No pretreatment was applied before RDB filling. For each RWW, new colonized wood chips were introduced into the drum and maintained even with new batch treatments.

RWW1 was treated in two batches. In the first batch, which lasted 22 days, flonicamid, acetamiprid, and ethimirol reached maximum removals of 66 %, 30 %, and 35 %, respectively. In the second batch with the same duration, lower removals for flonicamid, acetamiprid and ethimirol were achieved, with 53%, 14% and 23% removal respectively.

Two batches for RWW2 treatment where performed. In the first batch, promising removal rates were achieved, with average reductions of 13% for acetamiprid and 21% for metribuzin. Although the second batch showed lower removal efficiencies (5% for acetamiprid and 4% for metribuzin), this was largely due to pH sensor malfunctions that led to prolonged acidic conditions, compromising fungal activity. RWW3 is a high concentrated wastewater with high toxicity and pesticide content surpassing 1mg/L only for acetamiprid. Therefore, a new strategy was implemented for the treatment. It was diluted 1:4 with RWW2, which had lower toxicity, and every two weeks, 50L from the RDB were replaced with 50L of fresh RWW3. Acetamiprid removal was 19%, 17% and 75% for three batches respectively, while thiacloprid had 75% the first two batches and 52% the last one.

In conclusion, this study demonstrates the first use of a pilot plant RDB for bioremediation of RWW with T. versicolor. Results indicate that pesticides can be removed, but further investigation is needed to stabilize fungal biomass and improve removal efficiency.

Financial support and acknowledgments

Acknowledgments:Project funded by MCIN ref TED2021-130639B-I00/AEI/10.13039/501100011033/ Unión Europea NextGenerationEU/PRTR

Keywords

Rotatory Drum Bioreactor • Rinse Wastewater treatment • Bioremediation • Trametes versicolor • White rot-fungi

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA3 • Abstract ID 43

ENGINEERING BIOSENSORS FOR THE DEGRADATION AND VALORIZATION OF PLASTIC-RELATED PHTHALATE ESTERS

Alba Calonge García; Marco Antonio Pereyra Camacho; M. Auxiliadora Prieto; Isabel Pardo Mendoza
Centre for Biological Research Margarita Salas (CIB-CSIC)

Abstract

Plastic pollution is a growing health and environmental threat. This is the case for products based on the three phthalate isomers (i.e. “phthalates”): terephthalate (TPA), one of the two main co-monomers of the polyester PET; isophthalate (IPA), used up to 2% (w/w) as co-monomer in PET; and orthophthalate (OPA), commonly used as diesters in plasticizers (i.e. PAEs). Phthalate-based products are difficult to degrade and manage as waste, leaking into the environment and presenting a risk to global health. Recalcitrant PET can accumulate and break into micro- and nanoplastics, whereas PAEs are known endocrine disruptors. In recent years, there has been a growing interest in applying biotechnological approaches to treat phthalate residues and reduce their environmental impact.

These strategies include the use of microorganisms and their hydrolytic enzymes to break down phthalate-based esters for bacterial assimilation and their conversion into valuable bioproducts. Thus, our main goal is to employ Acinetobacter baylyi ADP1, a soil-bacterium with a versatile aromatic metabolism, to upcycle phthalate residues into value-added wax esters.Implementing biotechnological approaches for plastic degradation typically requires the use of high-throughput screening tools that allow the evaluation of hydrolytic enzymes, such as biosensors. Previously, we used a TPA biosensor to evaluate the activity of different PETase variants heterologously produced by A. baylyi. Our aim is to expand this toolset by engineering IPA and OPA biosensors for the in vivo high-throughput screening of new hydrolases.Here, we followed a combinatorial approach using Golden Gate assembly to evaluate different architectures of IPA and OPA biosensing genetic circuits based on the cognate transcription factors from Comamonas sp. E6, a bacterium that natively utilizes phthalates[6], and a fluorescent reporter. However, these first versions of IPA and OPA biosensors had low sensitivity to its inducers (10 and 1 mM, respectively). As it was previously observed that phthalate uptake is a limiting step for detection, we employed a library of A. baylyi transport mutants that unspecifically transport TPA to identify the best transporters for IPA and OPA. This library was additionally expanded by engineering the native OPA transporter from Comamonas sp. E6, OphK. This strategy allowed us to identify more efficient transport mutants to evaluate the detection range and specificity of the IPA and OPA biosensors.In all, this new generation of biosensors for IPA and OPA brings us closer to engineering A. baylyi ADP1 as a platform for the upcycling of phthalate-based waste into high value-added products.

Financial support and acknowledgments

This work is funded by Spanish National Research Council (CSIC), Reina Sofia Foundation and Primafrío Foundation under agreement no. 20210510; MCIN/AEI/10.13039/50110001103 and the European Union “NextGenerationEU/PRTR” under project no. TED2021-130850A-I00; and Comunidad de Madrid (CAM) under contract no. 2022-T1/BIO-23939. IP thanks CAM for an “Atracción de Talento” contract. ACG thanks CSIC for a JAE Intro fellowship and the Spanish Ministry of Science, Innovation and Universities for an Aid for University Teacher Training (FPU).

Keywords

Biosensor • Isophthalic acid • Orthophthalic acid • Plastics upcycling • Golden Gate assembly • Acinetobacter baylyi ADP1

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA4 • Abstract ID 49

FROM COFFEE WASTE TO VALUABLE BIOACTIVES: SUPERCRITICAL CO2 EXTRACTION, KINETICS, AND ANTIOXIDANT POTENTIAL OF SPENT COFFEE GROUNDS OIL

Naira Linhares Sabino[1]; Jose A. Mendiola[2]; Cristiano Soleo Funari[3]; Elena Ibáñez[2]; Kelly J. Dussán[4]

1. São Paulo State University (Unesp), Institute of Chemical, Department of Chemical Engineering, Brazil; Bioenergy Research Institute (IPBEN), Institute of Chemistry Araraquara, Brazil; Foodomics Laboratory, Instituto de Investigación en Ciencias de la Alimentación (CIAL, CSIC-UAM). Madrid, Spain; 2. Foodomics Laboratory, Instituto de Investigación en Ciencias de la Alimentación (CIAL, CSIC-UAM), Madrid, Spain; 3. São Paulo State University (Unesp), School of Agricultural Sciences, Brazil; 4. São Paulo State University (Unesp), Institute of Chemical, Department of Chemical Engineering, Brazil. Bioenergy Research Institute (IPBEN), Institute of Chemistry Araraquara, Brazil

Abstract

The global generation of solid and liquid waste now exceeds 10 billion tons, threatening environmental sustainability and food security, and hindering progress towards the SDGs on pollution control, climate change mitigation, and resource conservation. In 2023/24, Brazil produced 3.97 million tons of coffee, generating approximately 650 kg of spent coffee grounds (SCG) per ton. SCG are a rich but underutilized source of valuable bioactive compounds, including polyphenols, lipids, and proteins. This study addresses this challenge by comprehensively investigating the supercritical CO2 (SC-CO2) extraction of oil from SCG, focusing on extraction kinetics, chemical characterization, and antioxidant activity. Oil was extracted from SCG using a home-built supercritical CO_2 system at 100, 225, and 350 bar and 40, 50, and 60 °C. Extraction yields were evaluated by ANOVA (95% confidence), Pareto analysis, and Tukey's test. Kinetic data at 100 bar/40 °C, 225 bar/50 °C, and 350 bar/60 °C were fitted with Peleg, second-order, and Weibull models. Antioxidant activity was measured by DPPH assay (% inhibition). Extracts were characterized by GC-MS (Shimadzu GCMS-QP2010 Plus, ZB-5plus column); compounds were identified using the NIST MS 2.0 library. Supercritical CO_2 extraction effectively recovered valuable oil from spent coffee grounds. Extraction yields ranged from 0.12% at 100 bar/60 °C to 6.87% at 225 bar/50 °C. ANOVA showed a 53.19% model fit, with pressure having a significant effect, as confirmed by the Pareto chart. The optimal condition selected was 350 bar at 40 °C, based on both statistical analysis and literature 6, and validated in a scale-up (20 g SCG) with a yield of 7.49% ± 0.55%. Tukey's test indicated no significant difference between 225 bar/50 °C and 350 bar/40 °C or 60 °C. Kinetic data fitted best to the Weibull model, with R^2 values of 0.9949, 0.9997, and 0.9903 for 100 bar/40 °C, 225 bar/50 °C, and 350 bar/60 °C, respectively. Antioxidant activity across extraction time ranged from 34.93% to 24.09% inhibition (10–90 min). GC-MS analysis identified caffeine, caryophyllene, DHA methyl ester (all-Z), and fatty acids including palmitic, linoleic, stearic, and oleic acids. The results highlight the potential of spent coffee grounds as a sustainable, inexpensive, and abundant source of bioactive compounds for the food, pharmaceutical, and cosmetic industries. This work provides a viable, green approach to coffee waste valorization that directly contributes to a circular economy. This work highlights the value of reusing residues from coffee production and consumption and advances our understanding of supercritical fluid extraction, particularly with regard to extraction kinetics and the key parameters that most influence the process.

Financial support and acknowledgments

The authors acknowledge the financial support of FAPESP (São Paulo Research Foundation), project numbers 2022/03000-0, 2023/09840-3 and 2024/21780-9, and CNPq (National Council for Scientific and Technological Development), project number 316230/2023-5.

Keywords

Circular economy • Coffee waste valorization • Green extraction technologies

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA5 • Abstract ID 51

AUTOMATED SYSTEM FOR EXPERIMENTAL STUDY, MONITORING AND CONTROL OF TWO-STAGE ANAEROBIC DIGESTION PROCESS OF AGRICULTURAL WASTE

Elena Chorukova
The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences

Abstract

This study presents the automated system of a two-stage anaerobic digestion process employed for the bioconversion of agricultural wastes into hydrogen and methane. The high organic matter content of such wastes positions them as valuable substrates for biotechnological applications. The two-stage anaerobic digestion process was compartmentalized into a hydrogen-producing bioreactor and a methane-producing bioreactor, each harboring distinct microbial consortia. This research showed that using a two-stage process – separating the hydrogen-producing and methane-producing phases – can improve energy recovery from the co-digestion of agricultural waste under controlled conditions. The results enhance our understanding of anaerobic digestion and suggest promising opportunities for wider use in converting waste into energy.

Financial support and acknowledgments

This research was funded by Bulgarian National Science Fund, grant number KII-06-H46/4 “Experimental studies, modeling and optimal technologies for biodegradation of agricultural waste with hydrogen and methane production”.

Keywords

automated system • two-stage anaerobic digestion • renewable energy production • agricultural waste

Panel ID BA6 • Abstract ID 59

ASSESSMENT OF THE BIODEGRADATION POTENTIAL OF THE LINDANE-POLLUTED AQUIFER IN SARDAS (SPAIN) FOR ENHANCED IN SITU BIOSTIMULATION

Dani Salom[1]; Jesica Maiara Soder-Walz[2]; David Juan Fernández-Verdejo[1]; Elena Granados-Rigol[3]; Teresa Vicent[1]; Ernest Marco-Urrea[1]; Paqui Blánquez[1]

1. Departament d'Enginyeria Química, Biològica i Ambiental, Universitat Autònoma de Barcelona (UAB); 2. Department Environmental Biotechnology, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany; 3. EMGRISA, Empresa para la Gestión de Residuos Industriales, S.A., S.M.P., M.P., Madrid, Spain

Abstract

The alluvial groundwater in Sardas, located in Sabiñánigo (Aragón, Spain), is polluted with benzene, chlorobenzenes and hexachlorocyclohexane (HCH) isomers due to the improper disposal of wastes produced during the manufacture of lindane in the 1970s and 1980s.Enhanced in situ bioremediation is a robust, low-cost, and environmentally friendly technology for treating contaminated groundwater. Field-derived microcosms were established using groundwater from three wells to assess the intrinsic bioremediation potential of this site. Four different conditions were tested: (i) natural attenuation, (ii) aerobic biostimulation, (iii) anaerobic biostimulation, and (iv) anaerobic–aerobic treatment train biostimulation.

Results showed that aerobic biostimulation could transform benzene, chlorobenzenes and HCH isomers, although anaerobic biostimulation only transformed the HCH isomers, resulting in the accumulation of benzene and monochlorobenzene. When conditions were switched to aerobic, these low-chlorinated compounds were immediately mineralized. The effect of adding nutrients was also tested, and the addition of phosphorus greatly increased the reaction rate of the pollutants, suggesting that this was a limiting factor in the groundwater.

Given the promising results from the aerobic biostimulation in microcosms, an enhanced in situ aerobic biostimulation was performed in two wells injecting a $CaCO_2$ solution (an oxygen-releasing compound) with phosphorus. Three downgradient wells were used to study the influence of the injection, and another one located outside of the influence area was used as a control. Results mirrored those from the laboratory microcosm tests, transforming chlorobenzenes and HCH isomers (>80%) within four weeks in all five wells.Bacterial population analysis based on 16S rRNA gene amplification revealed an increase in the relative abundance of the Pseudomonas genus in both microcosm tests and the in situ pilot test, suggesting its active role in the transformation of the chlorinated pollutants present in the alluvial groundwater. Groundwater samples were subjected to dilution-to-extinction series, and a novel strain, Pseudomonas veronii SBNG, was isolated. Its genome was sequenced, and the culture was physiologically characterized, demonstrating its ability to aerobically mineralize monochlorobenzene and benzene.This study exemplifies the use of biostimulation as a viable strategy for remediating groundwater polluted with benzene, chlorobenzenes and HCH isomers, and offers evidence supporting the intrinsic biodegradation potential of this site, which enabled the implementation of the enhanced aerobic in situ biodegradation pilot test. Moreover, the isolation of this novel Pseudomonas strain may provide useful biomarkers for monitoring the biodegradation of these pollutants in the field.

Financial support and acknowledgments

This work was supported in part by EMGRISA (project CF618976), the Spanish Ministry of Science and Innovation (project PID2022- 138929OB-I00) and the Generalitat de Catalunya (Consolidated Research Group 2021-SGR-01008). Dani Salom acknowledges Generalitat de Catalunya (AGAUR) for his predoctoral scholarship FI-SDUR 2022.

Keywords

Aerobic Bioremediation • Lindane • In situ biostimulation • Pseudomonas

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA7 • Abstract ID 65

SUSTAINABLE BIORECYCLING OF PET VIA FUNGAL ENZYMES EXPRESSED IN YARROWIA LIPOLYTICA

Alejandro Garcia Miro[1]; Ines Herrera[2]; Francisco Javier Molpeceres-Garcia[1]; David Sanz[1]; Alicia Prieto[1]; Jorge Barriuso[1]
1. CIB-CSIC; 2. EMBL-EBI

Abstract

Plastics are polymers used worldwide due to their properties and broad range of applications, with polyethylene terephthalate (PET) being one of the most widely used. Biological approaches have been explored to develop cleaner and more efficient processes for PET recycling. These processes typically involve two main steps: the production of MHET from PET depolymerization, followed by the conversion of MHET into terephthalic acid (TPA) and ethylene glycol (EG)[1].

Although the bacterial enzymes PETase and MHETase from *Ideonella sakaiensis*, which are respectively involved in the two steps of PET degradation, have been extensively studied to enhance their catalytic performance, several fungal enzymes have also been identified as highly effective in breaking down this polymer[1]. Notably, HiC, a cutinase from the fungus *Humicola insolens*, and CalB, lipase B from the yeast *Candida antarctica* (currently known as *Moesziomyces antarcticus*), have demonstrated strong activity. HiC is particularly efficient in the initial depolymerization step, while CalB excels at converting the resulting intermediates into TPA and EG. Furthermore, the combination of these two enzymes has been shown to significantly improve the overall efficiency of PET biodegradation[2].

Yarrowia lipolytica is a non-conventional yeast that has attracted considerable interest due to its remarkable ability to biosynthesize a wide range of value-added compounds, such as lipids for biofuel production, organic acids, or enzymes. These biosynthetic processes can be carried out even using industrial residues such as volatile fatty acids (VFAs) or glycerol. Moreover, *Y. lipolytica* has proven to be an efficient host for the production and secretion of heterologous proteins, making it a promising platform for various biotechnological applications[3].

This work explores the biorecycling of PET through the use of an engineered *Y. lipolytica* strain heterologously expressing the two fungal enzymes, HiC and CalB, to degrade PET into its monomers - TPA and EG-, which can subsequently be polymerised into new PET or upcycled into other value-added products. This approach enables the valorization of PET waste as a substrate for the sustainable production of high-value biochemicals, offering a non-polluting and energy-efficient alternative to conventional recycling methods.

Financial support and acknowledgments

MICODE (MCIN/AEI, PID2020-114210RB-I00), DEMO (MCIN/AEI/"NextGenerationEU"/PRTR, TED2021-130096B-I00) and BioPlastOmics Project (Biodiversa2023-248) AEI, PCI 2025 call/Biodiversa+.

Keywords

Yarrowia • PET degradation • Biorecycling

1. Molpeceres-García, F. J., Sanz-Mata, D., García-Miro, A., Prieto, A., & Barriuso, J. (2025). Towards polyethylene terephthalate valorisation into PHB using an engineered *Comamonas testosteroni strain. New Biotechnology*, 85, 75-83.
2. Carniel, A., Valoni, É., Junior, J. N., da Conceição Gomes, A., & De Castro, A. M. (2017). Lipase from *Candida antarctica* (CALB) and cutinase from *Humicola insolens* act synergistically for PET hydrolysis to terephthalic acid. *Process Biochemistry*, 59, 84-90.
3. Madzak, C. (2018). Engineering *Yarrowia* lipolytica for use in biotechnological applications: a review of major achievements and rece nt innovations. *Molecular Biotechnology*, 60(8), 621-635.

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA8 • Abstract ID 74

ENGINEERING RECOMBINANT BIOCATALYSTS FOR PLASTICIZERS DEGRADATION: MOLECULAR CHARACTERIZATION OF A SECRETED PHTHALATE ESTERS HYDROLASE

Sofía de Francisco-de Polanco; Eduardo Díaz; Gonzalo Durante Rodríguez
Centro de Investigaciones Biológicas

Abstract

Phthalate esters (PAEs) are esterified derivatives of o-phthalic acid (PA) and are currently one of the most widely used plasticizers. Since they are non-covalently bound to plastics, they easily leach into the environment and are among the most common emerging organic pollutants due to their various toxic effects on living organisms. Bacterial degradation of PAEs is considered one of the most promising strategies for their removal. The catabolic pathways of PAEs involve a peripheral metabolism that transforms the PAE into PA (and the corresponding alcohol), by the action of esterases, and a central metabolism for the mineralization of PA. Previous studies characterized a new PA degradation pathway (transport and degradation of PA via benzoyl-CoA) and demonstrated the potential to redirect this carbon flow toward the production of bioplastics such as polyhydroxybutyrate (PHB). However, a major bottleneck in PAEs degradation is the esterase-mediated hydrolysis to generate PA. In this work, we report the characterization of a novel PAEs esterase, EstB, from Halomonas sp. ATBC28. The estB gene was cloned and expressed in recombinant Escherichia coli cells. Biochemical assays of the overproduced enzyme revealed that it is an active diesterase capable of generating mono-n-butyl phthalate (MBP) as the final product. Amino acid sequence analyses indicate that EstB belongs to family VIII of esterases. Interestingly, the protein contains a predicted N-terminal signal sequence suggesting its secretion to the periplasm or extracellular medium. To test this hypothesis, enzymatic assays were performed using both full-length EstB and a truncated version of the protein lacking the signal peptide. Enzymatic activity was detected in the extracellular fraction only for the full-length protein. N-terminal sequencing of the extracellular enzyme and studies using E. coli protein secretion mutants expressing estB confirmed that this PAEs esterase is the first one whose secretion to the extracellular medium has been experimentally validated. Since PA-degrading biocatalysts are usually unable to uptake PAEs, the estB gene becomes a promising genetic tool for engineering recombinant biocatalysts capable to attack PAEs in the extracellular medium for their removal and bioconversion into value-added products.

Financial support and acknowledgments

This work was funded by Project PID2022-142540OB-I00 and a PRE2020-092563 grant from the Spanish Ministry of Science, Innovation and Universities MICIU/AEI/10.13039/501100011033.

Keywords

phthalate esters • plasticizer • bacterial degradation • esterases

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA9 • Abstract ID 91

STRUCTURAL ALTERATION OF LOW DENSITY POLYETHYLENE BY LIGNINOLYTIC FUNGI

Roberto Sevilla; Rodrigo Ángel Rincón Sanz; Susana Camarero Fernández; Francisco Javier Ruiz Dueñas
Margarita Salas Center for Biological Research, CSIC

Abstract

Plastics are essential in the global economy due to their durability and versatility in a wide range of applications. Global plastic production exceeded 413 million tons in 2023 (Plastics Europe, 2024). As a result, plastic wastes have become ubiquitous and have accumulated in the environment impacting ecosystems and wildlife. There is therefore an urgent need to explore alternative approaches to effectively reduce plastic waste without affecting negatively the environment.

Since 2016, significant progress has been made in the biodegradation of polyesters such as PET from plastic bottles, through bacteria that produce esterase-type enzymes (Yoshida et al., 2016). However, other olefin-type plastic polymers are non-hydrolysable or have not yet been shown to undergo microbial degradation. Several studies suggest the potential of saprotrophic fungi for degradation of synthetic polymers due to their prominent role in the decomposition of organic matter in soil and their metabolic versatility (Zeghal et al., 2021).

The LIG2PLAST project focuses on studying the degradative potential of wood-decaying basidiomycete fungi and other lignocellulose-degrading species to address this challenge. This study leverages knowledge generated by our research group on lignocellulose biodegradation to assess the potential of these fungi (and their enzymes) to transform non-hydrolysable plastics. We selected a fungal species showing robust colonization on low-density polyethylene (LDPE) films in Petri-dish cultures. It was subsequently grown in liquid culture under induction conditions, and LDPE films were added to analyze the secreted enzymes and their effects on the plastic. Through direct visualization and scanning electron microscopy (SEM), fungal colonization and alterations in the surface of the film were observed. Additionally, FTIR-ATR analysis and Raman spectroscopy revealed significant changes in the chemical structure of the plastic. Finally, we analysed the exoproteome of the fungal liquid culture, and identified an enhancement of CAZymes, which might be responsible for the modifications observed in the treated plastic.

Financial support and acknowledgments

LIG2PLAST project (PID2021-126384OB-I00) funded by MICIU/AEI/ 10.13039/501100011033/ and by ERDF/EU.

Keywords

Ligninolytic fungi • LDPE • Degradation • Exoproteome • Enzymes • Polymer characterization

Panel ID BA10 • Abstract ID 95

EFFECT OF RHAMNOLIPID ADDITION OF THE PHENOLIC EXTRACTION OF ASTROCARYUM VULGARE MAT POST-PRESSING CAKE AND ITS ANTIFUNGAL ACTIVITY

Maria Fernanda Santos Mota; Regiane Ribeiro dos Santos; Elisa D Cavalcanti; Daniel Perroni; Denise M G Freire
Federal University of Rio de Janeiro

Abstract

Tucuma (Astrocaryum vulgare Mat.) is a native Amazon palm tree that grows well on poor soils and, thus, can be employed in degraded areas recovery. Tucuma exploration is carried out by small local producers organized in cooperatives, following agroforestry management. Tucuma fruit presents high amounts of oil in both pulp and kernel. Nowadays, its oil extraction is one of the major fruit utilizations, which generates a post-pressing cake as residue. This post-pressing cake still presents a potential industrial utilization including the bioactive compounds extraction. The present work aims to evaluate the phenolic extraction of the tucuma pulp oil post-pressing cake with and without rhamnolipid (RML) addition and the extracts antifungal activity against common strawberry (Botrytis cinerea and Rhizopus stolonifera) and citrus (Penicillium digitatum, Penicillium crustosum, Penicillium critrinum and Fusarium fujikuroi) post-harvest pathogens. For this, the phenolic extraction composition was performed at 62°C using solid: liquid ratio of 1:13 and ethanol concentration of 54% with and without the RML addition. The addition of two RML type surfactants in the phenolic composition was evaluated, one with majority of mono-RML (88% mono-RML/12% di-RML) with hydrophilic-lipophilic balance (HLB) of 7.0, and other with majority of di-RML (27% mono-RML/73% di-RML) with HLB of 9.0. The extract without RML presented three different phenolic compounds (catechin, epigallocatechin and epicatechin). Whereas the mono-RML extract presented catechin and more three phenolic compounds with higher hydrophobicity (p-hydroxybenzoic acid, epigallocatechin gallate, and 3,4-dihydroxybenzoic acid) when compared to the ones extract without RML. The addition of di-RML enables the extraction of a higher number of phenolic compounds (catechin, epicatechin, epigallocatechin, epigallocatechin gallate and p-hydroxybenzoic acid) possibly due the mono/di-RML proportion. This effect in the phenolic extraction is probably due to the relationship between the HLB of the RMLs and the phenolic compound polarity (Log P). Both mono-RML and di-RML extract presented higher inhibitory activity than the sole RML against common orange and strawberries fungi pathogens, which suggests a synergism effect between the phenolic compounds and RML. The sole hydroethanolic extract did not presented any inhibitory activity.

Financial support and acknowledgments

CNPq and INCT - HOI-B and FAPERJ - Brazilian financial agencies

Keywords

Surfactant assisted extraction • Biochemical pesticide • Amazon palm tree • Bioactive compounds • Bioeconomy

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA11 • Abstract ID 100

STUDY OF POLYETHYLENE TRANSFORMATION BY A LIGNOCELLULOLYTIC FUNGUS

Rodrigo Ángel Rincón Sanz; Roberto Sevilla Ortega; Susana Camarero Fernández; Francisco Javier Ruiz Dueñas
Centro de Investigaciones Biológicas Margarita Salas

Abstract

Plastic pollution has emerged as one of the most important environmental problems in recent decades. Managing plastic waste represents a major challenge for governments, industry and society as a whole, particularly as plastic production continues to rise in response to a growing global demand. This surge in production vastly exceeds the current technological capacity for degradation and recycling, mainly due to the high chemical stability and persistence of plastic materials.In nature, the decomposition of polymers such as lignin, cellulose and hemicellulose occurs through the action of complex microbial enzymatic systems. Inspired by these biological processes, biocatalytic depolymerization has recently emerged as a promising option for plastic degradation and recycling. In this regard, significant advances have been made in the enzymatic depolymerization of hydrolyzable plastics such as polyethylene terephthalate.

However, most commercial plastics, including polyethylene, polypropylene, polystyrene, or polyvinyl chloride, are fossil-derived polymers highly recalcitrant to hydrolytic enzymes. In light of this limitation, lignocellulolytic fungi have been proposed as potential agents for transforming these materials. These fungi possess an enzymatic arsenal specialized in lignin degradation, based on oxidoreductases, which has proven effective in oxidizing highly recalcitrant compounds. Therefore, it has been hypothesized that this enzymatic system could also be active in the degradation of non-hydrolyzable plastics.

To explore this hypothesis, we selected a lignocellulolytic fungal species, from a group of analyzed fungi able to grow on lignocellulose, based on its ability to induce changes in the FTIR spectra of low-density polyethylene (LDPE) when grown in the presence of this plastic. To confirm its ability to transform LDPE and identify the enzymes potentially involved in these changes, the fungus was cultivated for 60 days in the presence of this plastic under different experimental conditions, and its secretome was obtained and analyzed over time. The effects of the fungus on LDPE were evaluated using various characterization techniques. Thus, SEM revealed alterations in the plastic surface, while its oxidation was confirmed using micro-ATR/FTIR. Additionally, changes in its chemical structure and crystallinity were determined using Raman spectroscopy.

The results obtained suggest that an in-depth study of the enzymatic system of this fungus could lead to the identification of promising biotechnological tools for the transformation of non-hydrolyzable plastics such as polyethylene, thereby contributing to the development of new strategies for mitigating plastic pollution.

Financial support and acknowledgments

Funding: LIG2PLAST project (PID2021-126384OB-I00) funded by MICIU/AEI/ 10.13039/501100011033/ and by ERDF/EU.

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA12 • Abstract ID 101

VALORIZATION OF COCOA BEAN SHELL AS ADSORBENT FOR THE REMOVAL OF EMERGING CONTAMINANTS FROM WASTEWATER

Amanda Laca; Alba Rodríguez, Yolanda Patiño
Department of Chemical and Environmental Engineering, University of Oviedo, Oviedo, Spain

Abstract

Emerging pollutants, including pharmaceutical compounds, entail many environmental concerns, which are mainly related to water pollution. Among the technologies that have been employed to remove these contaminants from wastewater, adsorption stands out because of its simple and low-cost process. Additionally, the interest on the search for new eco-sustainable materials for wastewater treatment has been increasing in recent years. In particular, adsorbents based on lignocellulosic biomass have been reported as an interesting option due to their availability and attractive chemical composition and structure. In this context, agri-food waste has been widely investigated as novel adsorbents (Xiao et al., 2023).

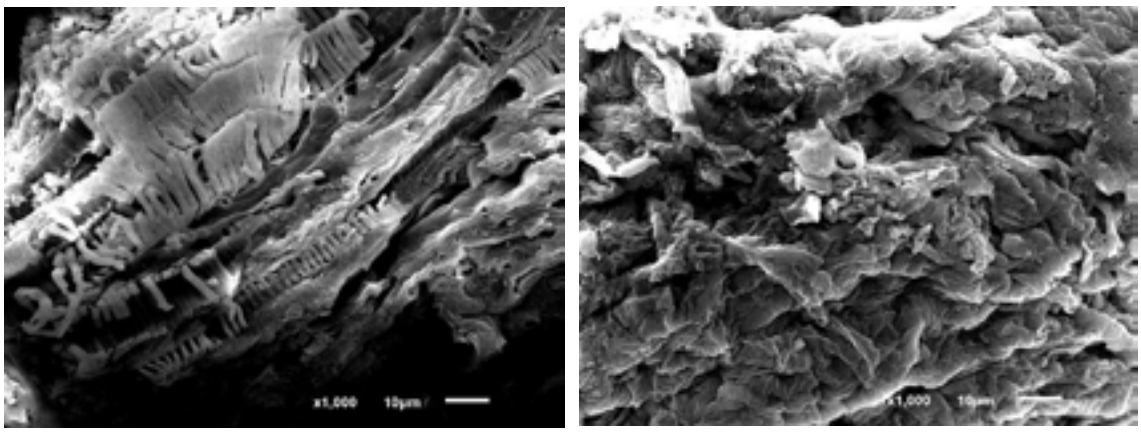
Cocoa bean shell (CBS), the outer part that cover the cocoa bean, is a by-product, which is usually discarded as waste, generated during the cocoa roasting process (Sánchez et al., 2023). In this work, this material has been evaluated in adsorption processes to treat wastewater, employing diclofenac, a nonsteroidal anti-inflammatory drug with well-demonstrated environmental toxicity, as model of emerging contaminant. Specifically, two different materials have been assayed, raw CBS and the waste solid obtained from the hydrothermal hydrolysis of CBS treated at 135ºC for 10 min, process which was carried out to obtain a hydrolysate enriched in polyphenolic compounds (Sánchez et al., 2024). The materials were thoroughly characterized by different techniques (FTIR, N2 adsorption-desorption, SEM (Fig. 1), TPD-MS and TGA) to understand the interaction between the adsorbents and diclofenac and the adsorbent morphology and stability. Equilibrium studies were performed in batch at different temperatures to obtain isothermal and thermodynamic behavior, modeling the results by using traditional models.
Figure 1. SEM micrographs of CBS powder: untreated (left) and hydrolysis waste (right)

Financial support and acknowledgments

The authors are grateful to Chocolates Lacasa for providing the cocoa bean shell employed in this work.

Keywords

cocoa bean shell • adsorbent • emerging contaminants • wastewater • diclofenac



July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

Panel ID BA13 • Abstract ID 102

PROTEIN ECO-FRIENDLY EXTRACTION FROM AGAR INDUSTRY WASTE USING DEEP EUTECTIC LIQUIDS

María Carpintero; Ismael Marcet, Manuel Rendueles, Mario Díaz
University of Oviedo

Abstract

Agar is a hydrocolloid widely used in the food and cosmetic industries, with an annual global production that exceeds the 14,500 tonnes (Trigueros et al., 2021). Its industrial agar extraction process involves an alkaline extraction, generates substantial solid waste. The amount of waste produce during the process also exceeds the thousands of tonnes and, although, part of the solid residue, rich in proteins and polysaccharides, is usually used as animals fodder and fertilizer most of residues are discarded (Ferrera-Lorenzo et al., 2014). However, these residues are rich in value added products that can be recover within the circular economy concept. In this sense, this study explores the recovery of bioactive proteins from agar industry waste deep eutectic solvents (DES), a safe and eco-friendly alternative to conventional organic solvents.

For this purpose, a series of extractions were carried out using different DES based on chlorine chloride (ChCl) in combination with lactic acid, ethylene glycol or glycerol in a 1:2 ratio. To perform the extraction, 20 g of ChCl (C1) were mixed with 40 g of the second component (C2) at 80 ºC until a transparent and homogeneous liquid was obtained. Then, 2 g of dry agar industry waste were added, and the mixtures were stirred at 80 ºC for 2 hours. Carbohydrates and protein extraction and recovery yields were analysed to select the best-performing DES. Once selected, the extraction process was optimized by varying three parameters: reaction temperature, C1/C2 ratio and DES-to-solid residue ratio. Finally, the antioxidant activity of the extracted proteins was evaluated.

The results showed that combination of ChCl and lactic acid was the most efficient DES system for proteins extraction and recovery. Optimal extraction was achieved at 50 ºC with a 1:1 ChCl:lactic acid ratio and a solid-to-liquid ratio of 3.33 g of dry agar waste for 100 mL of DES, recovering the 99% of the total protein content. The extracted proteins exhibited significant antioxidant activity, with inhibition values of 58.04% for DPPH and 99.64% for ABTS assays.

Financial support and acknowledgments

The authors are grateful for the financial support from the Spanish Ministry of Science and Innovation through project MCIU-22-PID2021-125942OB-I00 and from the Principality of Asturias through project Sek-25-grugic-24-010. The author, María Carpintero, also acknowledges the Spanish Ministry of Universities for the financial support received through the FPU pre-doctoral aid program (FPU21/01528).

Keywords

Agar industry waste • Deep eutectic solvents • Proteins • Extraction • Antioxidant

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA14 • Abstract ID 108

"ISOLATION, CONSERVATION, AND OPTIMIZATION OF BACTERIAL CELLULOSE (BC)-PRODUCING STRAINS: BIOSYNTHESIS OF BACTERIAL CELLULOSE, EVALUATION OF CULTURE MEDIA AND SCALING-UP STRATEGIES FOR YIELD INCREASE. - ELDRIN PROJECT"

Anita Kmetova[1]; Ana Torrejón[1]; Ramón Morcillo[2]; Concha Bosch[2]; Aldana Nazareth[1]; Elena Usala[2]
1. Department of Industrial Biotechnology, AINIA, Paterna, Spain; 2. Department of Materials and Packaging Technology, AINIA, Paterna, Spain

Abstract

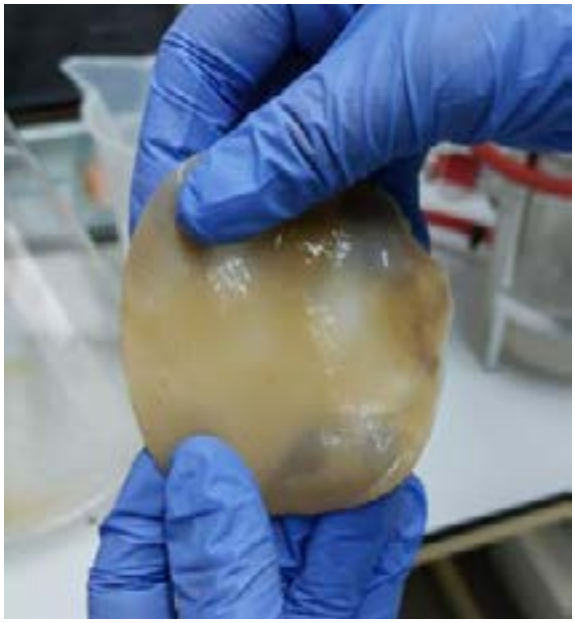
In the proyect ELDRIN, the researchers of AINIA are aiming to develop innovative and sustainable methodologies for the production and functionalization of micro/nanocellulose fibers (MNFC), bacterial cellulose (BC), and nanolignin.A key component of the project involves the isolation and characterization of BC-producing bacterial strains from natural sources such as whey, fermented fruits, fruit waste residue, and kefir. Selected strains underwent partial genome sequencing for taxonomic identification and were stored by cryopreservation. Various culture media were assessed to optimize BC yield, with parameters such as nutrient composition, temperature, agitation and incubation time systematically varied. Promising strains were scaled up and the resulting cellulose was harvested, purified, lyophilized, and analyzed via FTIR and SEM spectroscopy. The findings provide a foundation for the industrial-scale production of BC and its integration into sustainable material solutions.

Financial support and acknowledgments

The ELDRIN project is funded by the Valencian Institute of Business Competitiveness (IVACE) with co-financing from the European Union through the European Regional Development Fund (ERDF) under the program for R&D PROJECTS IN COLLABORATION WITH COMPANIES.

Keywords

Bacterial cellulose biosynthesis • Cellulose-producing bacteria • fermentation|new strains isolation



Panel ID BA15 • Abstract ID 132

BIOCATALYTIC STRATEGIES FOR THE DEGRADATION OF EMERGING MICROPOLLUTANTS: FROM NANOPLASTICS TO PHARMACEUTICALS

Fadia Victoria Cervantes Dominguez[1]; David Almendral[1]; Tatyana N. Chernikova[2]; Rafael Bargiela[1]; Peter N. Golyshin[2]; Manuel Ferrer[1]

1. Instituto de Catalisis y Petroleoquimica (ICP), CSIC, Madrid, Spain; 2. Centre for Environmental Biotechnology, School of Environmental and Natural Sciences, Bangor University, Bangor, UK

Abstract

In recent decades, the increasing occurrence of micropollutants, including nanoplastics, microplastics, pesticides, hydrocarbons, chlorinated solvents, and pharmaceuticals, among others, has raised growing concern. Innovative enzymatic systems targeting nanoplastic degradation have been reported and are under active development, though extending these approaches to plastics beyond PET remains a significant challenge. In the case of pharmaceuticals, the biodegradation of some compounds is possible; however, for others, the degradation pathways remain unresolved. In this work, we focus on some such pharmaceuticals: paracetamol, enalapril, ibuprofen, and atenolol micropollutants that have attracted significant attention due to their widespread use as an antipyretic and analgesic, and their potential to be released into surface waters, groundwater, wastewater and seawater at concentrations ranging from 0.1 to 200 µg/L. Microorganisms with a strong natural capacity to degrade for example paracetamol, often characterized by diverse metabolic capabilities and unique physiological traits, can be exploited as natural biocatalysts in wastewater treatment, while their degradation mechanisms can also be elucidated.

In this work, we present results on the efficacy of novel natural and engineered biologics for the degradation of two types of micropollutants: nanoplastics/microplastics and the aforementioned pharmaceuticals. For the latter, we provide evidence of the degradation capacity of bacteria from the genera Alcanivorax, Paraburkholderia, and Pseudomonas. We also describe an alginate-based bacterial immobilization process that enables complete paracetamol degradation, enhances cell stability and reusability, and reduces downstream processing costs[5].

Financial support and acknowledgments

The authors acknowledge funding from the EU's Horizon Europe programme (GA101060625), MICIU/AEI/10.13039/501100011033, NextGenerationEU/PRTR (TED2021-130544B-I00), and the CAM "César Nombela" Research Talent Attraction Program (2024-T1/ECO-31227).

Keywords

Bioremediation • pharmaceuticals • Micropollutants • Biocatalysis

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA16 • Abstract ID 135

ARSENIC REMOVAL BY IRON-OXIDIZING MICROORGANISMS: A SYSTEMATIC STUDY OF THE INFLUENCE OF TEMPERATURE AND IRON CONCENTRATION

Fernando Vera-Espíndola[1]; David Jeison[1], Ernesto González[2]
1. Pontificia Universidad Católica de Valparaíso; 2. Universidad Complutense de Madrid

Abstract

Bioprocesses are established methodologies for remediation of diverse contaminants. Currently, arsenic removal by iron-oxidizing microorganisms has garnered attention due to their operational simplicity, cost-effectiveness, and ecofriendly characteristics. However, a predominant focus of existing research has been on bioscorodite ($FeAsO_4 \cdot 2H_2O$) synthesis under thermophilic conditions (above 70 °C), which restricts its application to high-temperature processes. To date, systematic investigations addressing the combined influence of temperature and initial iron concentration in these biobased systems have not been conducted. This study evaluates arsenic removal efficiency mediated by an iron-oxidizing microbial consortium across an extended temperature range (from 35 to 70 °C) and varying the iron concentration within an acidic arsenic-rich solution.

The results demonstrate that the initial arsenic species significantly influences arsenic removal efficiency. Maximum removal efficiencies of 25 and 93 % were achieved with initial As(III) and As(V), respectively. Statistical analysis demonstrates that iron concentration exerts a positive effect in arsenic removal in both initial arsenic species, As(III) or As(V). In contrast, temperature only exerts a positive effect only when As(V) is the initial arsenic species. Profiles of arsenic and total iron concentration also demonstrate a congruent precipitation of arsenic and iron. The latter suggests arsenic precipitates like a kind of ferric arsenate. These findings could contribute to develop an innovative biobased method of arsenic removal from acidic arsenic-rich industrial wastewaters.

Financial support and acknowledgments

This work wass upported by ANID Doctoral Scholarship (ANID2021/21211483) and Corporación Nacional del Cobre de Chile, CODELCO.

Keywords

arsenic • iron-oxidizing microorganism • wastewater treatment • industrial biotechnology

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA17 • Abstract ID 138

ENGINEERING CYANOBACTERIA FOR ENVIRONMENTAL MONITORING

Inés Federío Zalaya; Emma Sevilla Miguel
Department of Biochemistry and Molecular and Cell Biology, University of Zaragoza

Abstract

Lindane is a broad-spectrum organochlorine insecticide whose production and indiscriminate use have caused severe global environmental issues. It is produced by isolating the active γ-HCH isomer through an inefficient process that generates large amounts of toxic and bioaccumulative persistent organic pollutants (POPs). Inquinosa, a lindane factory established in Sabiñánigo (Aragón), operated for nearly 20 years without environmental safeguards, dumping massive amounts of organochlorine waste into the Sardás and Bailín landfills, near the Gállego River. Leachates from these sites have contaminated inland waters with DNAPL, an immiscible dense phase of high toxicity and difficult to manage. Groundwater and surface water contamination is currently monitored using sensitive but complex and costly chromatographic methods.

As a more practical alternative, bioanalytical techniques are proposed for in situ monitoring. Existing biosensors for lindane, based on lin pathway enzymes in Sphingomonas, face limitations such as low specificity and sensitivity. This study presents a whole-cell biosensor for lindane detection, based on the fusion of the green fluorescent protein (GFP) to the promoter of the putative linC gene from Anabaena sp. PCC 7120, inducible by HCH. A triparental conjugation protocol was optimized for DNA transformation in Anabaena, along with a cryopreservation method to improve the cyanobacteria's long-term viability.

Financial support and acknowledgments

PROY_E05_24 (DGA) to Emma Sevilla and grant CPE_23_24 (DGA) to Inés Federío

Keywords

Lindane • Biosensor • Cyanobacteria • Monitoring

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BA18 • Abstract ID 156

ENGINEERING AZOHYDROMONAS LATA DSM 1123 TO OPTIMIZE THE PRODUCTION OF POLY (3-HYDROXYBUTYRATE-CO-4-HYDROXYBUTYRATE)

Beatriz Galán; Paula Chacón; Juan Ibero; José Luis García; María Auxiliadora Prieto Prieto
Centro de Investigaciones Biológicas Margarita Salas

Abstract

Polyhydroxyalkanoates (PHAs) are polymers synthesized by a wide variety of microorganisms under conditions of nutritional imbalance. A broad range of PHAs with varying monomer compositions leads to different physical and chemical properties. Poly-β-hydroxybutyrate (PHB) is one of the best-characterized and most widely studied PHAs. However, this material is difficult to use and process due to its thermal and mechanical properties. The incorporation of other monomers, such as 4-hydroxybutyrate (4HB), into the polymer leads to the production of commercially more attractive copolymers. For example, poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HB-4HB)] exhibits interesting properties due to its high flexibility and lower crystallinity. This copolymer is notable for its excellent biocompatibility, biodegradability, and mechanical properties, which make it suitable for use in medical devices such as sutures, tissue engineering scaffolds, and drug delivery systems. In this sense, P(3HB-co-4HB) is currently the only PHA that has received approval from the U.S. Food and Drug Administration (FDA) for specific biomedical applications. In this work, the PHA-producing strain Azohydromonas lata (formerly Alcaligenes latus) was used as microbial cell factory. This bacterium is a facultative autotroph capable of utilizing sucrose as a carbon source and it has been reported to have the highest PHB accumulation productivity to date. Moreover, its production is growth-associated and does not require nutrient limitation.

First, we have applied genetic engineering to A. lata to optimize the production of P(3HB-4HB). Golden Standard MoClo toolkit3, specifically designed for non-model bacteria, was used to perform the constructions. The phaC, phaP1 genes encoding the PHB synthase and phasin, respectively, from Cupriavidus necator and cat2 encoding a 4-hydroxybutyrate coenzyme A transferase from Clostridium kluyvery were cloned and expressed in A. lata. The molar fractions of 4HB and 3HB obtained from the recombinant strains were analyzed, showing improvements in copolymer production compared with the wild type strain. Our results confirm that A. lata is an excellent candidate for the efficient production of P(3HB-4HB). The successful genetic manipulation of A. lata paves the way for its use as a model organism aimed at the efficient production of various types of PHAs in the future.

Financial support and acknowledgments

SynEco Project (TED2021-130689B-C32) funded by MCIN/AEI/10.13039/501100011033 and the European Union NextGenerationEU/PRTRFrontest Project (PID2021-125370OB-I00)

Keywords

Azohydromonas lata • biopolymers • poly (3-hydroxybutyrate-co-4-hydroxybutyrate)

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

Panel ID BI1 • Abstract ID 14

FERMENTACIÓN DE RESIDUOS ORGÁNICOS: PLATAFORMA BIOTECNOLÓGICA PARA LA OBTENCIÓN DE MONÓMEROS Y BIOPLÁSTICOS

Rafael Jiménez
AIMPLAS

Abstract

La fermentación de residuos orgánicos se posiciona como una solución innovadora para la producción sostenible de monómeros y bioplásticos, al tiempo que contribuye a la valorización de desechos y a la mitigación del impacto ambiental de los plásticos convencionales. A través de procesos biotecnológicos, como la fermentación anaeróbica, es posible transformar residuos alimentarios, agrícolas e industriales en compuestos de alto valor añadido, como ácidos orgánicos y alcoholes, que pueden ser utilizados como precursores en la síntesis de bioplásticos biodegradables, entre ellos el polihidroxialcanoato (PHA) y el ácido poliláctico (PLA).Un ejemplo destacado de esta estrategia es el proyecto europeo PROMOFER, coordinado por AIMPLAS, en el que se desarrolla una plataforma biotecnológica integrada para la conversión de residuos orgánicos en monómeros y su posterior uso en la fabricación de bioplásticos. PROMOFER combina tecnologías de fermentación avanzada con la ingeniería genética de microorganismos para optimizar la eficiencia del proceso, desde la transformación inicial del residuo hasta la obtención y purificación de los compuestos deseados. Este enfoque promueve la economía circular y busca alternativas rentables y escalables para reemplazar los plásticos derivados del petróleo.

Financial support and acknowledgments

Este proyecto ha sido financiado por la Unión Europea a través de la iniciativa Circular Bio-based Europe Joint Undertaking (CBE JU) dentro del tipoc HORIZON-JU-CBE-2023-IA-03. Proposal ID: 101157239.

Keywords

Biotecnología • Fermentación • Valorización de residuos • Bioplásticos

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BI2 • Abstract ID 40

ALKALINE PH TUNABLE EXPRESSION OF RECOMBINANT PHYTASE IN KOMAGATAELLA PHAFFII: A SAFER ALTERNATIVE TO METHANOL-INDUCIBLE SYSTEMS

Marcel Albacar[1]; Núria Bernat[2]; Xavier Garcia-Ortega[2]; Francisco Valero[2]; Joaquín Ariño[1]; Antonio Casamayor[1]
1. Institut de Biotecnologia i Biomedicina, Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Cerdanyola del Vallès; 2. Dept. d'Enginyeria Química, Biológica i Ambiental. Escola d'Enginyeria. Universitat Autònoma de Barcelona, Cerdanyola del Vallès

Abstract

Komagataella phaffii (formerly known as Pichia pastoris) is a yeast widely used for heterologous protein expression, typically utilizing the methanol-inducible AOX1 promoter. However, industrial-scale protein production using methanol as an inducer and carbon source presents challenges due to its toxicity and flammability, as well as higher oxygen consumption and heat generation derived from its metabolism.

To develop more sustainable expression systems, we investigated the transcriptional response to alkalinization of the culture medium. Among other genes, PHO89 was identified as being potently regulated under alkaline conditions. Using a PHO89 promoter-GFP reporter, we characterized its pH-dependent transcriptional response and identified two CDRE (Calcineurin Dependent Response Elements) sequences in the promoter crucial for induction at alkaline pH.

As proof of concept, we employed the pPHO89 to drive the expression of a secreted phytase, an enzyme of industrial interest. In shake-flask cultures, shifting the medium to pH 8.0 resulted in strong and tightly regulated phytase expression. In contrast, the production was negligible at acidic pH. Expression levels were further enhanced under phosphate-limited conditions, achieving phytase yields comparable to or even exceeding those obtained with the AOX1 promoter. These promising results prompted us to scale up and optimize conditions for phytase expression at the benchtop bioreactor scale. Current work is focused on identifying the influence of carbon addition and time-dependent pH changes to ensure optimal enzyme production.This methanol-free system offers a safer and more economical alternative for industrial enzyme production, with potential applications in various biotechnological processes.

Financial support and acknowledgments

Supported by grants PID2020-113319RB-I00 and PID2023-150535OB-I00 (Ministerio de Ciencia, Innovación y Universidades & Agencia Estatal de Investigación) to JA and AC; grant 2023 PROD 00006 (AGAUR, Generalitat de Catalunya) to JA; grant SGR 00143 to NB, XG and FV (Dept of Research and Universities of Generalitat de Catalunya).

Keywords

Komagataella phaffii • Pichia pastoris • Heterologous protein expression • PHO89 promoter • Alkaline pH-inducible promoter

Panel ID BI3 • Abstract ID 60

EVALUATION OF SYNTHETIC AGRO-INDUSTRIAL WASTE FOR CAROTENOID PRODUCTION USING RHODOTORULA TAIWANENSIS RTM13A

Paul Teran[1]; Sergio Romero[2]; Javier Viña[2]; Ginés Martínez[2]; Victoria Santos[3]
1. Cultiply SL. - Universidad Complutense de Madrid; 2. Cultiply SL.; 3, Universidad Complutense de Madrid

Abstract

The global carotenoid market, widely driven by their use as food colorants, reached an estimated value of USD 1.4 billion in 2014 and continues to grow. Traditionally extracted from plants, carotenoid production is often limited by issues related to crop cultivation and extraction processes. This has prompted the search for alternative sources, with red yeasts emerging as promising candidates due to their ability to produce a variety of carotenoids (astaxanthin, torulene, β-carotene, γ-carotene, lycopene, among others) using low-cost substrates. Rhodotorula taiwanensis RTM13A is an oleaginous red yeast capable of metabolizing a broad range of carbohydrates and tolerating toxic compounds typically found in agro-industrial residues, making it a suitable microorganism for carotenoid production.In this study, 14 carbon sources (including carbohydrates, organic acids, and alcohols) and 9 nitrogen sources were evaluated to identify the most suitable substrates for R. taiwanensis RTM13A growth. Based on the best-performing compounds, agro-industrial residues were selected from the literature, and seven synthetic media were formulated to replicate the composition of molasses, crude glycerol, and hydrolysates of potato, bagasse, apple, and orange residues.

Experiments were conducted in microplates and shake flasks to assess optical density, dry weight, cell count, and total carotenoids. Growth data were fitted to a logistic model to estimate kinetic parameters and compare the performance of the media. Six carbohydrates—glucose, mannose, sucrose, fructose, arabinose, and glycerol—supported robust growth. Among nitrogen sources, organic compounds such as yeast extract and corn steep liquor outperformed inorganic sources. Within the inorganic group, a clear preference was observed for ammonium salts over nitrates. Analysis of the synthetic hydrolysate media revealed two distinct patterns: media rich in easily metabolizable carbohydrates (e.g., sucrose, glucose, and fructose) led to high biomass productivity but relatively low carotenoid concentrations. In contrast, xylose- and glycerol-based media, which supported lower biomass accumulation, yielded higher carotenoid concentrations. These results suggest that stress-related metabolic responses may be involved in the enhanced carotenoid synthesis under suboptimal growth conditions.

Based on these findings, molasses, crude glycerol, and potato and apple residues were selected for subsequent stages of the project, with the aim of optimizing the culture medium and scaling up β-carotene production by R. taiwanensis RTM13A. These results provide a strong foundation for future studies using real agro-industrial waste to develop sustainable and cost-effective strategies for next-generation food additive production.

Financial support and acknowledgments

This presentation is part of the DIN2022-012383 project funded by MCIN/AEI/10.13039/501100011033.

Keywords

Rhodotorula taiwanensis • Carotenoids • Agro-industrial residues • Synthetic media

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BI4 • Abstract ID 73

BIOPROCESS PERFORMANCE OF NOVEL PROMOTERS FOR RECOMBINANT PROTEIN PRODUCTION IN PICHIA PASTORIS UNDER CONTROLLED PHYSIOLOGICAL STRESS CONDITIONS

Albert Sales Vallverdú; Arnau Gasset; Francisco Valero; Xavier Garcia-Ortega; José Luis Montesions-Seguí
Universitat Autònoma de Barcelona

Abstract

Komagataella phaffii (Pichia pastoris) is a well-established host for recombinant protein production (RPP), valued for its robustness and scalability in industrial bioprocesses. Recent advances in promoter engineering, combined with bioprocess strategies such as oxygen limitation, have shown significant potential to enhance protein yields. In this study, the effect of hypoxic conditions on the bioprocess efficiency under the regulation of six different promoters—five novel (pDF, pUPP, pPGK, pCAT, pDH) and one conventional (pGAP)—in carbon-limited fed-batch cultures using Candida antarctica lipase B (CalB) as a model protein has been investigated. All cultivations were conducted at a constant specific growth rate of 0.065 h-1 under both normoxic (pO2 > 30%) and hypoxic conditions, the latter implemented by using an automated respiratory quotient (RQ) control system with a RQ of 1.4.

The results obtained show a robust process control and reproducible cultivation performance across conditions applied. Comparative analysis reveals that only promoters associated with glycolytic pathways (pPGK and pGAP) exhibit improved production efficiency under oxygen-limiting conditions. CalB specific production rate (qP) increased about 50% and 160% respectively. However, notably, the novel promoters pDF and pUPP showed the highest CalB production levels, representing up to 3-fold increase compared with the conventional pGAP in hypoxic conditions, positioning them as promising tools for efficient RPP in K. phaffii.As conclusion, this systematic promoter evaluation under physiologically controlled stress conditions provides valuable insights for optimizing RPP process in K. phaffii. Furthermore, the implementation of precise and accurate RQ control demonstrates the potential of integrating physiological parameters into next-generation bioprocess designs for improved performance and scalability.

Financial support and acknowledgments

This work was funded by the Project PID2022-136936OB-I00 of the Spanish Ministry of Science and Innovation. The authors receive support from the Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) of the Catalan Government (grant 2021-SGR-00143). Albert Sales acknowledges the award of a PIF scholarship from the Universitat Autònoma de Barcelona.

Keywords

P. pastoris • Recombinant protein production • Bioprocess • Novel promoters

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BI5 • Abstract ID 75

INDUSTRIAL DESIGN OF AN ENYMATIC EXTRACTION PLANT OF CRUDE HEPARIN IN CONTINUOUS OPERATION

Ricard Gené Torrandell; Francisco Valero; Glòria González Anadón
Department of Chemical, Biological and Environmental Engineering, Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain

Abstract

Industrial Design of an enzymatic extraction plant of crude heparin in continuous operation Gené Torrandell, R.; Valero, F.; González Anadón G.Department of Chemical, Biological and Environmental Engineering, Universitat Autònoma de Barcelona, Bellaterra (Barcelona), 08193, Spain. Heparin is a glycosamino-glycan which principal use is as an anticoagulant medication preventing blood clots. It is an Active Pharmaceutical Ingredient (API) mainly extracted from porcine intestinal mucosa by means of an enzymatic digestion under harsh conditions of temperature and pH control including enzyme concentration. The usual process is carried out under batch mode and as the concentration of heparin in the mucosa is quite low (~ 150 mg heparin/kg of mucosa), large bioreactors are required in industrial facilities to get economically interesting yields. As the reaction is performed in liquid conditions, the enzyme (hydrolase type) is dissolved in the reaction crude and is deactivated after the digestion step by heat and acidification, being lost after every batch. Due to the conditions required during the reaction (temperatures between 60 to 90ºC as almost a 10% of animal fat is freed during the digestion step and must be separated), pH control with strong acids and bases and oxidizing products (hydrogen peroxyde) to reduce the sodium bisulphite added in the slaughterhouses for product conservation during transportation, the energy required, reagents used and waste generated becomes a big issue. A continuous operated plant for such process is seen in the near future as the best approach to increase yield and productivity, reduce equipment volume and layout footprint, improving product quality while reducing waste products produced from reagent consumption thus lowering the CAPEX and OPEX of the plant. The use of immobilized enzymes should be also an option to reduce the major product cost in the process, apart from the mucosa itself and will be explored to be used instead of enzyme in solution that is lost after every batch.

Keywords

heparin • crude heparin • enzyme • batch • continuous operation

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BI6 • Abstract ID 81

BYPASSING INSOLUBILITY: CHOLINE-BINDING MODULES AS SOLUBLE MOLECULAR SWITCHES FOR THE FUNCTIONALIZATION OF HYDROPHOBIC SUPPORTS WITH RECOMBINANT PROTEINS

Maria Isabel Torices[1]; Helena Soto[2]; Celia Atalaya[3]; María Pilar Lillo[4]; Ana Victoria Villar[5]; Beatriz Maestro[6]; Jesús Miguel Sanz[7]

1. Centro de Investigaciones Biológicas Margarita Salas. Consejo Superior de Investigaciones Científicas, Madrid, España; 2. Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC); 2. Consejo Superior de Investigaciones Científicas (CSIC)-Universidad de Cantabria (UC), Santander, Spain; Valdecilla Biomedical Research Institute (IDIVAL), Santander, Spain; 3. Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC), Consejo Superior de Investigaciones Científicas (CSIC)-Universidad de Cantabria (UC), Santander, Spain; 4. Departamento Química Física Biológica. Grupo de Fluorescencia y Biofísica Molecular Instituto Química Física "Blas Cabrera" CSIC Serrano 119. Madrid; 5. Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC), Consejo Superior de Investigaciones Científicas (CSIC)-Universidad de Cantabria (UC), Santander, Spain; Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Cantabria, Santander, Spain; 6. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas. Universidad Complutense de Madrid, España; 7. Centro de Investigaciones Biológicas Margarita Salas. Consejo Superior de Investigaciones Científicas, Madrid, España; CIBER De Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain

Abstract

The design of hydrophobic supports amenable to be functionalised with proteins is a subject of increasing interest in the biomedical and biotechnological fields, since it would allow the specific targeting of a particle to a target cell and deliver a drug in situ or even catalyse reactions as enzymatic bioreactors. Lipid vesicles as liposomes are considered one of the most effective drug delivery systems, with potential therapeutic applications due its low toxicity. Moreover, magnetic nanoparticles can be manipulated by an external magnetic field, favouring its manipulation. Normally, to immobilise proteins in those hydrophobic supports, these need to contain solvent-exposed hydrophobic zones, which lead to solubility problems. Nevertheless, beta-beta solenoid proteins are polypeptides which are soluble in aqueous solutions, and that change their conformation in presence of hydrophobic compounds. In this work, we present a novel protein immobilization procedure by demonstrating that fusion proteins containing a choline binding module (CBM) tag from *Streptococcus pneumoniae*, which displays a soluble, beta-beta solenoid structure in aqueous buffer. Thanks to an unprecedented beta-hairpin to an amphiphilic alpha-helix transition it can be immobilised in different hydrophobic supports, such as hydrophobic magnetic nanoparticles and extracellular vesicles extracted from human cardiac fibroblasts from patients which confer a proof of concept in a natural vesicle source. This method leads to a remarkable binding stability while overcoming the use of less soluble, hydrophobic tags.

Financial support and acknowledgments

PID2022-139209OB-C21/AEI/10.13039/501100011033/FEDER/UE, PID2021-125702OB-I00/AEI/MCI/10.13039/501100011033/FEDER/UE, PID2021-125024NB-C2/AEI/MCI/10.13039/501100011033/FEDER/UE

Panel ID BI7 • Abstract ID 106

CONTINUOUS PRODUCTION OF CRL1 IN PICHIA PASTORIS UNDER PGAP PROMOTER USING SACCHARIFIED FRUIT BIO-WASTE AS THE MAIN CARBON SOURCE: EVALUATION OF OPERATIONAL STRATEGIES

Ruth Ordóñez; Juan Moreno-Cid; Ana Del Hierro; Fuensanta Verdú Navarro
Bionet

Abstract

This study evaluates the feasibility of producing the model enzyme CRL1 in Pichia pastoris under the constitutive pGAP promoter using continuous cultivation strategies. Trials were conducted at bench-top scale using a Bionet F1 bioreactor, where different continuous operation modes were tested to optimize lipase production. The process was implemented using saccharified sugar-rich substrates derived from enzymatically hydrolyzed fruit bio-waste, as developed in the Horizon Europe MixMatters project. Due to the high costs associated with the saccharification and subsequent concentration of these substrates, factors that limit the economic viability of fed-batch operation, continuous fermentation was selected as a cost-effective and sustainable alternative for the use of these substrates, aligned with the principles of the circular bioeconomy

Financial support and acknowledgments

The project is supported by the Circular Bio-based Europe Joint Undertaking and its members under grant agreement Nº 101112409. Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or CBE JU. Neither the European Union nor the CBE JU can be held responsible for them.

Keywords

Pichia pastoris • Continuous process • Circular bio-economy • Fruit Bio-waste

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BI8 • Abstract ID 110

RECOMBINANT EXPRESSION OF ANTI-HSA NANOBODIES IN PICHIA PASTORIS

Gina Liarte Castillo; Pau Ferrer Alegre; Francisco Valero Barranco; Xavier García Ortega; Guillem López-Grado Salinas
UAB

Abstract

Nanobodies, also known as single-domain antibodies (VHH fragments), have revolutionized the field of biotechnology and biopharmaceuticals due to their small size, high specificity, and stability. Their discovery in camelids opened the door to new diagnostic and therapeutic applications, offering similar properties to conventional antibodies but with simpler expression requirements. However, large-scale production of nanobodies still faces challenges, primarily due to the complexity of achieving proper folding and solubility, factors that significantly impact production costs. In this context, microbial hosts such as Pichia pastoris have emerged as an attractive cell factory for recombinant nanobody expression thanks to their ability to perform post-translational modifications and their scalability for industrial processes.

In this study, we focused on optimizing nanobody (VHH anti-HSA) production in P. pastoris using the De-repressed Formaldehide dehydrogneas promoter (PDF). Initial cultivations used as production tests in lab-scale bioreactors revealed substantial protein precipitation, indicating suboptimal expression conditions that could negatively affect yields and product quality. To address this, the impact of different operational parameters, particularly pH and osmolarity, on nanobody solubility were systematically evaluated.

Through a series of screenings, a specific pH range was identified in which protein precipitation was minimized, leading to an improvement in nanobody solubility and stability. Biomass and nanobody time evolution profiles have been monitored by offline methods (optical density, ELISA assays and SDS-PAGE). Furthermore, early stages of downstream processing were evaluated, including His-tag purification via nickel-affinity FPLC and a novel HPLC-based application for nanobody detection, all of which yielded highly promising preliminary results.In conclusion, this combined approach enabled robust bioprocess characterization and confirmed the advantages of pH control in preventing protein aggregation. This study reinforces the importance of integrating bioprocess optimization with analytical monitoring to ensure high yield, functionality, and quality of recombinant products, paving the way for more cost-effective manufacturing of nanobodies.

Financial support and acknowledgments

This work was funded by project MCIN/AEI/10.13039/501100011033/FEDER, EU (project PID2022-136936OB-I00) by the Spanish Ministry of Science and Innovation. The research group is part of the 2021 SGR 00143 program of the Department of Research and Universities of the Generalitat de Catalunya. Gina Liarte-Castillo acknowledges support from a FPU (University Teaching and Research Training Fellowship) scholarship granted by the Ministry of Science, Innovation and Universities of the Government of Spain. Furthermore, we thank Bisy Gmbh for kindly providing the strain for our research purposes.

Keywords

Pichia pastoris • Yeast • Nanobody • PDF • Bioprocess • Fermentation

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BI9 • Abstract ID 115

DEVELOPMENT OF A METHOD FOR HIGH CELL DENSITY TRANSFECTION

Paula Pérez Mateos; Pol Pérez Rubio; Laura Cervera Gracia; Francesc Gòdia Casablancas
UAB

Abstract

Transient Gene Expression -TGE- is a rapid and flexible method widely used in R&D and preclinical stages to obtain the necessary amount of a drug candidate. It is performed by introducing the DNA to be expressed in the cells via its complexation with polymers like polyethylenimine. Recently, its potential has been increased by the rise of personalized therapies and viral outbreak responses. Although some TGE-based biologics, like recombinant adeno-associated virus therapies, have reached commercial application, scaling up TGE remains challenging. One main limitation is the Cell Density Effect -CDE-, which leads to drastic reduction in transfection efficiency and cell-specific productivity at cell densities higher than 3-4 · 10e6 cells/mL. Recent research has demonstrated that DNA:PEI polyplex interaction with Extracellular Vesicles -EVs- prevents polyplex uptake and trafficking. Therefore, transfection efficiency can be partially restored at high cell densities -HCD, 8-12 · 10e6 cells/mL-, by depleting EVs or performing a complete media replacement, but the decrease in cell-specific productivity remains impaired.

This study aims to maximize transfection efficiency and enhance production in HCD-transfected cultures using HEK293 cells producing HIV-1 Gag virus-like particles -VLP- as a TGE-model at shake flasks scale. First, a Face-Centered Central Composite Design -varying shaking speed and percentage of liquid volume- was applied to identify optimal conditions for HCD growth. Second, a previously developed strategy, combining retransfection and continuous feeding -named as Extended Gene Expression, EGE-, was adapted to HCD. Third, cell-specific productivity reduction at HCD was mitigated by a cell engineering strategy involving shRNA-mediated silencing of the ataxia telangiectasia mutated -ATM- protein.

The optimal growing condition combined to EGE completely restored transfection efficiency at HCD reaching 80–90% GFP+ cells and improved VLP production achieving 75% of cell-specific productivity yield compared to low cell density -LCD, 2 · 10e6 cells/mL-. Additionally, ATM knock-down successfully increased cell-specific productivity 4-fold at HCD. Notably, TGE at HCD showed significantly higher plasmid efficiency, with multi-fold improvements in the ratio of produced VLPs per plasmid copy, suggesting a promising way to reduce the impact in costs of GMP-grade plasmid at production stages. Overall, this work offers an effective strategy to overcome the CDE and advance TGE-based processes.

Keywords

High cell density • Transient Gene Expression • HEK293 • Virus Like Particles • Cell Density Effect

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BI10 • Abstract ID 123

BIOPROCESS DEVELOPMENT TO PRODUCE FUNCTIONAL CHITOOLIGOSACCHARIDES

Marc Carnicer Heras[1]**;** Paula Morer[1]; Chiara Guidi[2]; Wouter Demeester[2]; Marjan de Mey[2]; Antoni Planas[1]
1. Laboratory of Biochemistry, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain; 2. Center for Synthetic Biology, Department of Biotechnology, University of Ghent, Belgium

Abstract

Chitooligosaccharides (COS) are a family of functional oligosaccharides with immunostimulatory activity and applications in agriculture for crop protection, as well as with potential applications in the food and feed industry, cosmetics and health care. They are currently produced by chemical processing of chitin extracted from different sources (shells of crabs and shrimps, cell wall of fungi, ..) under harsh acidic and basic treatments that render complex mixtures with regard to the degree of polymerization, degree of acetylation and pattern of acetylation. Most of the bioassays are performed with mixtures of products with inconsistent results due to batch to batch variations. There is a strong demand to develop more sustainable and environmentally-friendly processes to produce pure compounds with defined structures to expand their functionalities and applications. We are developing a biotechnological approach where COS are produced by engineered E. coli strains expressing a recombinant chitin oligosaccharide synthase NodC (wt and mutants) for the production of defined COS. Here we report the bioprocess development at pilot plant scale for the production of a target COS. The process involved the design and optimization of culture media (with particular attention to trace elements) and fed-batch operation to enhance volumetric production at 100 L scale up to several grams per liter. Additionally, preliminary downstream processing was performed to isolate the target compounds through centrifugation, ultrafiltration, and desalting steps.The IQS Bioprocess Pilot Plant is a service facility dedicated to scaling up microbial bioprocesses (upstream and downstream operations) up to a 100 L bioreactor. We develop and optimize bioprocesses in collaboration with companies in the agrifood, pharma, cosmetics and environmental sectors.

Financial support and acknowledgments

This work was supported by GLYCOENGIN (PID2022-138252OB-I00) and paCOSVALOR (PDC2022-133580-I00) to M.C and A.P. funded by MICIU, Spain. and 2021 SGR 00535 (A.P. and M.C) by Generalitat de Catalunya.

Keywords

Chitooligosaccharides • Bioprocess • Upstream and Downstream operations

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

Panel ID BI11 • Abstract ID 125

COMBINATION OF IN SILICO AND IN VIVO METABOLIC ENGINEERING TOOLS FOR ENHANCED RECOMBINANT PROTEIN PRODUCTION IN THE YEAST KOMAGATAELLA PHAFFII

Eduard Adorna-Sangüesa; Eric Antón-García, Pau Ferrer, Joan Albiol
Departament d'Enginyeria Química, Biològica, i Ambiental

Abstract

Over the years, many studies have explored the genetic and metabolic optimization of the methylotrophic yeast Komagataella phaffii to enhance its performance as a microbial cell factory. An aspect identified in these studies is the interconnection between redox balance and the production of recombinant proteins. Specifically, an increased NADPH availability has been associated with improved yields of the target protein. Building upon this knowledge, metabolic engineering strategies based on the overexpression or deletion of key genes involved in NADPH metabolism have become attractive approaches.In this study, we focused on the gene isocitrate dehydrogenase 1 (IDH1, also referred to as IDP1), which catalyzes the oxidative decarboxylation of isocitrate to ketoglutarate in the tricarboxylic acid (TCA) cycle, thereby generating NADPH from NADP. The host strain used is K. phaffii X-33 ROL (X33_ROL), producing the recombinant lipase from Rhizopus oryzae (Rol) as a model protein. Following a Design-Build-Test-Learn (DBTL) cycle, in silico simulations were conducted to predict cell and product generation under various metabolic (i.e. flux through the Idh1 reaction) and environmental conditions were performed enabling the selection of the most suitable genetic engineering strategy. Once the strategy was determined, a gene expression cassette was constructed using Golden Gate assembly, inserting a copy of the IDH1 gene under the control of the constitutive GAP promoter to drive overexpression.

Enzymatic activity assays of Rol and Idh1 [PF1] were used to characterize strain performance. Three strains were compared: the reference strain (X33_ROL), a strain overexpressing IDH1 (X33_ROL::IDH1_OE), and a double mutant with IDH1 overexpression and IDH2 knockdown (X33_ROL::IDH1_OE/IDH2_KD). Overall, this design aims to evaluate whether these combined genetic modifications can synergistically enhance mitochondrial NADPH availability and thereby increase recombinant protein yield under methanolic growth conditions.

Financial support and acknowledgments

This work was funded by Project MCIN/AEI/10.13039/501100011033/FEDER, UE (Project PID2022-136936OB-I00) of the Spanish Ministry of Science and Innovation The group is a member of the 2021 SGR 00143 Department of Research and Universities of Generalitat de Catalunya. Eric Antón García acknowledges an FI-SDUR scholarship from the AGAUR (University and Research Grants Management Agency).

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BI12 • Abstract ID 128

DESIGN OF A BACTERIAL EXPRESSION PLATFORM TO PRODUCE AN INDUSTRIAL ENZYME FOR FOOD APPLICATIONS

Clàudia Lliso Pascual[1]; Sergi Abad[2]; Marc Carnicer[1]; Antoni Planas[1]
1. Laboratory of Biochemistry, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain; 2. CYGYC BIOCON S.L., Les Franqueses del Vallès, Barcelona, Spain

Abstract

Enzymes are key catalysts in food processing, enabling efficient and selective biochemical transformations that impact physicochemical and functional properties. Their application also contributes to cleaner-label formulations by reducing reliance on artificial additives, while supporting cost-effective and sustainable processes. However, industrial use of enzymes is often limited by low production yields in native hosts, where expression levels are insufficient to meet process demands. To address this limitation, heterologous expression systems are required to enable high-level enzyme production under scalable conditions.

Among bacterial hosts, Bacillus subtilis is a strong candidate widely employed in industrial biotechnology due to its GRAS status, high secretion capacity, rapid growth, and genetic tractability. In this study, we developed a modular expression platform in Bacillus subtilis aimed at improving the extracellular production of enzymes through the combinatorial optimization of key regulatory elements.

Our standardized platform is based on the one-pot Golden Gate assembly method, which allows rapid and modular construction of expression plasmids from interchangeable genetic parts. Part libraries were designed and assembled combinatorially, including a set of promoters (inducible, constitutive, and growth-phase dependent), signal peptides (from Sec and Tat secretion pathways), and coding sequences of the protein of interest (POI). The resulting constructs were transformed into B. subtilis 168 and the protease-deficient derivative KO7-S, as commonly done in studies optimizing extracellular protein production.

To evaluate protein expression and secretion efficiency, the extracellular fraction of the culture was analyzed. Promoter-signal peptide (P-SP) combinations were initially screened using mCherry as a fluorescent reporter protein. Fluorescence measurements and SDS-PAGE confirmed successful secretion. The same P-SP pairs were tested in combination with the enzyme of interest, and extracellular activity was then quantified. In strain 168 clones, proteolytic activation of the enzyme of interest occurred extracellularly via native host proteases.Among all tested combinations, Pgrac100-amyQ* yielded the highest expression level and this construct was then selected for subsequent scale-up and bioprocess optimization.

Overall, our results highlight the potential of a standardized modular expression platform for optimizing heterologous protein expression and secretion in B. subtilis. This approach may offer a robust and scalable solution for the production of lowly expressed enzymes relevant to industrial applications.

Financial support and acknowledgments

This work was supported by the Pla de Doctorats Industrials awarded to C.L., with grant number 2022DI00059, AGAUR, Departament de Recerca i Universitats de la Generalitat de Catalunya

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

Panel ID BI13 • Abstract ID 131

KINETIC MODELLING OF GALLIC ACID OXIDATION BY LACCASE

Jorge García-Montalvo; Ernesto González; Sara Preciado; Miguel Ladero; Juan Manuel Bolívar
Universidad Complutense de Madrid

Abstract

Laccases are multi-copper oxidases found in numerous organisms: fungi, higher plants and bacteria. They catalyze direct reduction of molecular oxygen to water through one electron oxidation of a broad range of chemical substances without production of hydrogen peroxide. Despite their natural substrate is lignin, it can also oxidize substituted phenols (e.g. methyl syringate), aromatic amines (e.g. diphenyl amine) and some inorganic compounds (e.g. Mn2+). For this reason, several authors have proposed and tested their application in food and pharmaceutical industry, paper and pulp industry, and wastewater treatment.

Developing a kinetic model requires experimentation measuring and controlling the concentration of reactants (and products). Despite dissolved oxygen can be measured using polarographic and optochemical electrodes, controlling its concentration is a difficult task. Hence, pseudo single substrate kinetics have been frequently used to model the reaction rate of laccases as a function of reducing substrate, while other authors report a Ping-pong mechanism where both reducing substrate and oxygen concentration are relevant. Since oxygen is a required substrate, its concentration should be considered to obtain a strong a useful model. Hence, in this work we used gallic acid as model substrate to apply a methodology and obtain kinetic constants of gallic acid oxidation by laccase from Myceliopthora thermophila.

A set of experiments was first performed in non-sparged vials using a single concentration of enzyme and different concentrations of gallic acid. Oxygen was continuously monitored using an optochemical sensor. These results were used to obtain values of constants related to substrates affinity using the software Aspen Custom Modeler. Secondly, a set of experiments using different concentrations of enzyme and substrate was performed into an air-sparged bioreactor. Samples were periodically withdrawn to measure the concentration of substrate by HPLC. These results allowed us to obtain the catalytic constant and validate the model by applying a leave-one-out cross validation.

Financial support and acknowledgments

Acknowledgments to the Grant Reference CNS2022-135541 funded by MICIU/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR, and to the Research Project PID2020-114365RB-C21 funded by MICIU/AEI/10.13039/501100011033

Keywords

laccase • dissolved oxygen • gallic acid|kinetic modeling

Panel ID BI14 • Abstract ID 134

PRODUCTION OF POLYHYDROXYALKANOATES FROM DAIRY WHEY USING CUPRIAVIDUS NECATOR

Ismael Marcet; Florencia Ridella; Adrián Fernández-Menéndez; Manuel Rendueles de la Vega; Mario Díaz
Universidad de Oviedo

Abstract

The problem with conventional plastics includes their resistance to biodegradation, leading to accumulation in landfills and oceans, and contributing to climate change due to their production based on fossil resources. A sustainable solution is the production of polyhydroxyalkanoates (PHA) from whey, a by-product of the cheese industry that is rich in lactose. The bacterium Cupriavidus necator (C. necator) can ferment this lactose to produce PHA, a biodegradable bioplastic. PHAs have a wide range of applications, from biodegradable packaging to medical uses, offering a sustainable alternative to traditional plastics.

This study focused on the production of PHA from whey sourced from the dairy industries Monteverde and Reny Picot, using different strains of C. necator. In this study, the lactose concentration in the whey was measured in order to hydrolyze it into glucose, as well as the protein concentration to obtain the Carbon/Nitrogen ratio in the whey, to determine whether the bacteria were under stress. The environmental burden of conventional plastics, largely due to their resistance to biodegradation and dependence on fossil resources, has motivated the search for sustainable alternatives. Polyhydroxyalkanoates (PHAs) are biodegradable bioplastics synthesized by various microorganisms under nutrient-limited conditions. This work explores the use of cheese whey, a lactose-rich byproduct of the dairy industry, as a carbon source for PHA production using Cupriavidus necator.

Two whey streams from Monteverde and Reny Picot dairy plants were evaluated. Their compositions were characterized in terms of reducing sugar and nitrogen content using DNS, HPLC, and protein assays (Bradford and Lowry), to estimate the C/N ratio and assess its effect on microbial stress and PHA synthesis. Lactose in whey was hydrolyzed enzymatically into glucose using β-galactosidase from Kluyveromyces lactis, with reaction conditions optimized to balance hydrolysis efficiency and enzyme consumption. Fermentations were conducted using two strains: C. necator DSM 545 and CECT 4635. While CECT 4635 showed no PHA accumulation from the glucose obtained from the lactose hydrolysis, DSM 545 successfully produced intracellular PHA. Fermentation media were prepared by supplementing hydrolyzed whey with a mineral salt solution (DSMZ 81 formulation). Biomass was quantified by dry weight and plate counting, while PHA was extracted using a sodium hypochlorite-chloroform method and characterized by NMR spectroscopy. Results showed a PHA yields up to 5.8 g/L by using these industrial whey streams. NMR analysis confirmed that the polymer produced was mainly poly(3-hydroxybutyrate) (PHB).

This study demonstrates the viability of using dairy industry waste, specifically whey, as a cost-effective and sustainable substrate for PHA production. It highlights the critical influence of substrate composition—particularly the C/N ratio—on microbial stress and biopolymer yield, and confirms C. necator DSM 545 as a suitable strain for valorizing whey into PHAs. These findings support the integration of waste valorization and bioplastic production as part of a circular bioeconomy.

Financial support and acknowledgments

Principado de Asturias, Consejería de Medio Rural y Política Agraria. Programa Grupos Operativos Autonómicos (GOP/07/2023 AYUD/2023/6965).

Keywords

polyhydroxyalkanoates • Dairy whey • fermentation

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BI15 • Abstract ID 136

POTENTIAL OF DAIRY INDUSTRY WASTEWATER RESIDUE FROM DISSOLVED AIR FLOTATION AS A PROMISING CARBON SOURCE FOR BIOPLASTIC (PHA) PRODUCTION

Ismael Marcet; Florencia Ridella; Pablo González-García; Manuel Rendules; Mario Díaz
Universidad de Oviedo

Abstract

The cleaning of pipelines and tanks used to store milk in the dairy industry generates wastewater with a high organic load. The treatment of this wastewater through anaerobic fermentation is challenging, as the long-chain fatty acids it contains act as inhibitors of microbial growth. These lipids can be separated on an industrial scale using dissolved air flotation (DAF); however, the separated lipids remain a difficult-to-manage waste product.

In this study, the potential of this lipid-rich residue obtained by DAF as a carbon source to produce polyhydroxyalkanoates (PHAs), biopolymer used for preparing biodegradable plastics, was evaluated. A comprehensive characterization of the DAF residue was carried out using various analytical techniques, quantifying its content of carbon, nitrogen, hydrogen, and sulfur, as well as salt concentration, protein and carbohydrate levels, moisture content, and fatty acid composition. Furthermore, the bacterial strain Cupriavidus necator H16 was chosen for its high efficiency in PHA production usings fats. The fermentation was done with a saline medium with limited nitrogen, using carbon/nitrogen ratios between 20 and 30, and comparing the effectiveness of the DAF lipid-rich residue with sunflower oil.

The analysis of the DAF lipid-rich residue showed a high percentage of lipids in its composition (76.71 ± 11.93), as well as a relatively low ammonium and protein content (845,53 ± 80,34 g/L and 870 ± 196 g/L, respectively). This high ratio of carbon/nitrogen was perfectly suited for PHA production. Furthermore, the salt composition of the DAF residue was comprised of all the necessary microelements needed for the growth of C. necator. A four-fold increase in PHA production was observed with the DAF residue, over sunflower oil, with a value of 3.91 ± 0.37 g/L after 72 hours. This result highlights the potential of this residue as a valuable carbon source for sustainable production of PHAs.

Financial support and acknowledgments

Principado de Asturias, Consejería de Medio Rural y Política Agraria. Programa Grupos Operativos Autonómicos (GOP/07/2023 AYUD/2023/6965).

Keywords

bioplastics • fermentation • polyhydroxyalkanoates • Dairy Industry Wastewater

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

Panel ID BI16 • Abstract ID 141

ON THE CARBON DIOXIDE TRASPORT RATE IN A STIRRED AND SPARGED TANK: STIRRING SPEED AND GAS FLOW RATE INFLUENCES

Victoria E. Santos; Emilio Gómez Castro; Felix García-Ochoa; Itziar A. Escanciano
Universidad Complutense de Madrid

Abstract

The oxygen transfer rate has received considerable attention, with hundreds of papers published on this subject in various contactors and for a wide range of systems. However, the same is not true for CO_2 transfer. Most studies on CO_2 absorption have been conducted in gas–liquid contactors such as bubble and packed columns, hollow fiber membrane contactors, and more recently, in microreactors and microchannel reactors. Nevertheless, few studies address carbon dioxide transfer in pure water and/or saline solutions, in the absence of chemical reactions or cultures of microorganisms, plant, or animal cells—systems in which CO_2 transfer is still relevant and often necessary to consider.

Currently, suspension cell cultures have garnered significant interest for carrying out bioprocesses, due to their ability to enable the production of high value-added products and their potential to replace conventional chemical processes. In many of these bioprocesses, carbon dioxide transfer plays a crucial role, either as an essential substrate for cellular metabolism—as in the case of microalgae production—or as a co-substrate. In other cases, CO_2 is a metabolic by-product whose removal or control is necessary for the proper functioning of the process, such as in bacterial fermentations and mammalian cell cultures.

The aim of this work is to perform a comprehensive study of the CO_2 transfer rate in water using a stirred and sparged tank reactor (STR). Superficial gas velocities and stirring speeds were varied from 0.1 to 1.0 $L \cdot min^{-1}$ and from 100 to 700 rpm, respectively. Experimental values of the CO_2 volumetric mass transfer coefficient (kLa) were obtained using a dynamic absorption/desorption method in water. To obtain accurate results, the acid–base equilibria of several species generated in water must be considered. The most reliable measurement method involves CO_2 desorption while allowing the pH to evolve freely.

As expected, the values of CO_2 kLa increased with increasing superficial gas velocity and stirring speed, showing trends like those described in the literature for oxygen transfer. The effect of stirring speed was significantly greater than that of gas flow rate. The CO_2 kLa values were empirically correlated with superficial gas velocity (Vs) and stirring speed (N), yielding an equation fitted to the experimental data with deviations within ±10%.

Financial support and acknowledgments

This research was funded by the Spanish Science and Innovation Ministry through the project: PID2020-114365RB-C21

Keywords

Carbon dioxide mass transfer coefficient • Stirred Tank Reactor • Empirical equation

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BI17 • Abstract ID 148

ENZYMATIC REVALORIZATION OF POLLUTED STREAM: FROM INDUSTRIAL COTTON WASTEWATER TO COTTON BIOBLEACHING AGENT

Yerko Fredes; Gerard Guerra; Marina Guillén; Oscar Romero
UAB

Abstract

The cotton textile industry presents a significant environmental challenge, consuming vast amounts of water and chemicals. This leads to substantial wastewater streams, particularly from desizing and pre-wash stages, which are rich in carbohydrates and contribute nearly 20% of global water pollution. Furthermore, conventional cotton bleaching is an energy-intensive process, demanding high temperatures and alkaline pH, as well as high concentration hydrogen peroxide (H2O2). The high presence of these carbohydrate-rich wastewaters opens the possibility to utilize them as feedstock for the enzymatic production of H2O2, a crucial component in cotton bleaching. Previous studies have already demonstrated the effectiveness of N-acetylglucosamine oxidase (NagOX) as a biocatalyst for H2O2 generation from textile industry effluents, suggesting its strong potential for developing a novel and more sustainable biobleaching process.

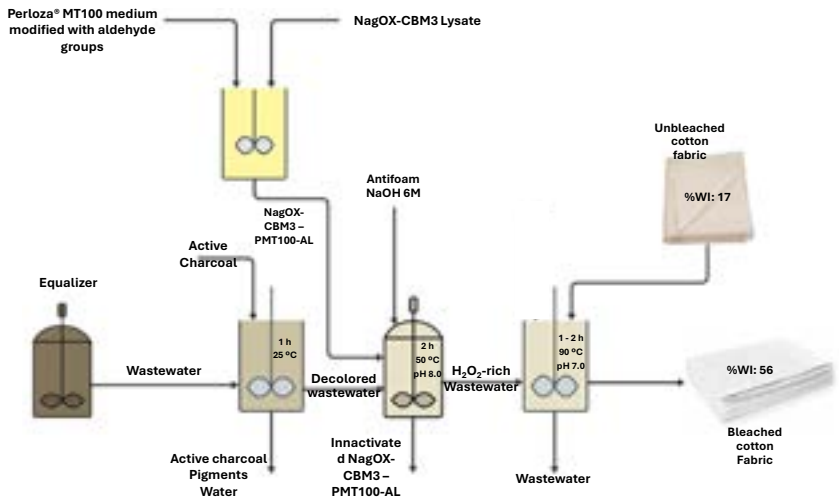
Here in this work, is explored the optimized production of H2O2 using NagOX immobilized on cellulosic support via a fused affinity tag. A comparative analysis of different reactor modalities was executed, specifically contrasting a continuous bed-packed reactor discontinuous stirred tank reactor (DSTR). Evaluation was performed considering key parameters such as oxygen and substrate consumption, as well as overall productivity. DSTR emerged as significantly more effective, successfully achieving a concentration of 56.5 mM of H2O2 after two hours of process. Subsequently, the H2O2-rich wastewater generated from this process was successfully applied in a bio-bleaching treatment of cotton fabric. By employing milder temperature and pH conditions, facilitated by the use of a hydrogen peroxide activator, significant degree of whiteness was achieved, highlighting the potential for reduced energy and chemical usage in industrial cotton processing.

Financial support and acknowledgments

• OXIPRO funded by European Union's Horizon 2020 research and innovation program under Grant Agreement 101000607
• Generalitat de Catalunya (2021 SGR 00143).
• Personal investigador predoctoral en formació (PIF-UAB) scholarship.

Keywords

H2O2 • Cotton biobleaching • Wastewater revalorization • Carbohydrate oxidase



July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BI18 • Abstract ID 154

SUSTAINABLE BIOFUEL PRODUCTION FROM CO2: INTEGRATING BY-PRODUCTS EFFICIENTLY

Pamela Torres Salas[1]; Enrique Medina Martos[2]; Francisco Gabriel Acien Fernández[3]; Daniel Murphy[1]; Vicente Bernal[1]; Mar Gonzalez Barroso[1]; Javier Gil[2]
1. Repsol; 2. Centro Nacional de Energias Renovables; 3. Universidad de Almería

Abstract

Repsol, a global multi-energy provider, is committed to driving the transition towards a decarbonized energy model, aiming to achieve net zero emissions by 2050. To support this vision, Repsol is undergoing a transformation process to establish multi-energy hubs capable of processing alternative feedstocks for renewable fuels and circular materials production.In line with this strategy, Repsol focuses on developing sustainable methods for producing biofuels from CO_2. The approach shown here leverages the photosynthetic capabilities of genetically engineered cyanobacteria to convert CO_2 and sunlight into biofuels. By integrating CO_2 capture technologies with cyanobacteria cultivation systems, this innovative research seeks to reduce greenhouse gas emissions and promote cleaner energy sources. This research aims to utilize cyanobacteria to produce isobutanol with net-zero emissions, providing an eco-friendly alternative to fossil fuels. Growth conditions and genetic modifications were optimized to enhance CO_2 conversion efficiency, using a real biogenic gas composition that meets future emissions regulations and reduces greenhouse gases. Once the concept was demonstrated at the laboratory scale, process design was initiated, focusing on creating a circular system where all byproducts are valorized or utilized within the same facility. The work presents a preliminary outline of the possible industrial process, from product purification to byproduct valorization. This research advances the field of biotechnology and supports efforts to mitigate climate change and transition towards sustainable energy sources.

Keywords

biofuels • circular economy • cianobacteria • Process design • CO_2 utilization

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BI19 • Abstract ID 22

SYNTHESIS OF POLYHYDROXYALKANOATES BY E. COLI DH5A USING SODIUM ACETATE AS A SOLE CARBON SOURCE

William Mawuko Siegu[1]; Vignesh Kumaravel[1], Olga Marchut-Mikołajczyk[2]
1. International Centre for Research on Innovative Biobased Materials (ICRI-BioM), Lodz University of Technology;
2. Institute of Molecular and Industrial Biotechnology, Faculty of Biotechnology and Food Sciences, Lodz University of Technology

Abstract

Polyhydroxyalkanoates (PHAs) are biodegradable polymers that have gained significant attention in various industries due to their comparable properties to conventional polymers. However, the microbial synthesis of PHAs faces major challenges, including low yields and generally high operating costs. To address these challenges, the HELVA project (https://helva.eu/), uses a novel and potentially cost-effective approach in the biosynthesis of PHAs. This approach involves using acetate synthesized from anthropogenic carbon dioxide (CO_2) along with genetically modified microbial strains and green extraction techniques to achieve higher yields. By converting CO_2 into valuable biopolymers, the project supports the principles of a circular economy and significantly lowers feedstock costs.

In this present study, engineered Escherichia coli DH5α was employed for the biosynthesis of PHAs using sodium acetate as the sole carbon source. Cultivations across a concentration range of 1–10 g/L acetate yielded consistent biomass production, with an average optical density (OD_{600}) of 1.200 after 72h bioreaction. Preliminary structural analysis of the extracted polymer using FT-IR and 1H NMR spectra indicate the polymer synthesized is poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). A comparative analysis with other polymer structures within the FT-IR database revealed an ~60% similarity to conventional PET, highlighting its potential as a functional bio-based alternative. Additionally, employing the Taguchi method to establish optimized operating conditions, biomass yield improved producing dry biomass to PHA yield of 29.5% from a laboratory scale of 450ml total media volume. Further physicochemical and mechanical characterizations are underway to evaluate the PHA\'s suitability for scalable, sustainable applications.

Financial support and acknowledgments

WMS, OMM, and VK would like to thank National Science Centre (NCN), Poland for the HELVA project (UMO-2022/04/Y/ST5/00161). Project HELVA was selected in the Joint Transnational Call 2022 of M-ERA.NET 3, which is an EU-funded network of about 49 funding organisations (Horizon 2020 grant agreement No 958174). The project is funded by the Agencia Estatal de Investigación (AEI, Spain); National Science Centre (NCN, Poland); São Paulo Research Foundation (FAPESP, Brazil). This paper was completed while the first author was a Doctoral Candidate in the Interdisciplinary Doctoral School at the Lodz University of Technology, Poland.

Keywords

Polyhydroxyalkanoates (PHAs), Escherichia Coli DH5 • Sodium acetate, Biosynthesis, Optimization

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BV1 • Abstract ID 86

ESTABLISHMENT OF CALLUS AND CELL SUSPENSION OF OCIMUM BASILICUM

Miquel Vilafranca Tello[1]; Martí Lecina[2]; Pablo Leivar[2]; Ruth Margalef[3]; Mikel Artola[3]
1. Biogründl, S.L.; Departament de Bioenginyeria, Institut Químic de Sarrià, Universitat Ramon Llull; 2. Departament de Bioenginyeria, Institut Químic de Sarrià, Universitat Ramon Llull; 3. Biogründl, S.L.

Abstract

The production of metabolites of interest resulting from plant in vitro cell cultures has been gaining ground over the last years. Ocimum basilicum presents an interesting content of flavonoids, alkaloids, terpenoids and phenolics. However, the adaptation and culturing of plant cells in a suspension commonly appears to be a bottleneck and is key to ensure a robust scale-up to a bioreactor necessary for industrial applications. The aim of this study is, firstly, to establish a protocol for the induction of O. basilicum callus and the following adaptation to a liquid culture and subsequently, to adjust the optimal subculturing time and the concentration of inoculum of the cell suspension. In order to generate friable callus for a proper disaggregation in a further suspension, callus induction was studied in Murashige and Skoog (MS) and Gamborg (B5) media, under both continuous light exposure and dark conditions. To select the optimal concentrations of plant growth regulators (PGRs), a Design of Experiment (DoE) was formulated testing specific concentrations of cytokines and auxins. Generated calluses were evaluated in terms of size (callus growth), friability, coloring and rooting. Selected calluses were transferred and adapted to cell suspensions cultures. Main substrates of the media were monitored to determine the physiological parameters of cultures and the stablish the optimal subculturing time based on the depletion of the carbon sources. A window time of subculturing in between 7 and 11 days significantly reduces the lag phase, and allows reducing the culturing time to the half without affecting the final biomass concentration reached compared to published data. Finally, a range from 20g/l to 80 g/l of biomass (fresh weight) was also evaluated to establish the minimum concentration of the inoculum required. It was concluded that below 40 g/l the lag phase was prolonged and the final biomass values subsided.

Financial support and acknowledgments

This work was supported by Nuclis R+D, ACCIÓ Catalonia trade&Investment (ACE088/23/000059)and AGAUR DI program.

Keywords

Callus induction • Cell suspension • Plant growth regulator • Cytokine • Auxin • Friable • Subculturing interval • |Secondary metabolite • Ocimum basilicum

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

Panel ID BV2 • Abstract ID 119

DEVELOPMENT OF A ONE-STEP MODULAR CLONING VECTOR FOR CHLOROPLAST ENGINEERING IN GREEN MICROALGAE

Xavier Melero-Cobo[1]; Marçal Gallemí[1]; Marc Carnicer[1]; Antoni Planas[1]; Elena Monte[2]; Pablo Leivar[1]
1. Laboratory of Biochemistry, Institut Químic de Sarrià, Universitat Ramon Llull; 2. Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB - and - Consejo Superior de Investigaciones Científicas (CSIC, Spanish National Research Council)

Abstract

Synthetic biology tools are reshaping the landscape of genetic and metabolic engineering. Design-Build-Test-Learn (DBTL) pipelines have been developed in several organisms thanks to the construction of standardized genetic parts (biobricks) that can be easily assembled into multi-cassette plasmids using Modular Cloning (MoClo) toolkits. We have recently developed MoCloro, an extension of the green microalgae Chlamydomonas reinhardtii (Chlamydomonas) MoClo toolkit to facilitate chloroplast genetic and metabolic engineering. The MoCloro cloning pipeline consists of a two-step procedure: first, genetic parts (level 0 biobricks) are assembled into single transcriptional units (TUs, level 1 vectors); second, up to seven TUs can be cloned into a destination vector containing homology arms for chloroplast genome (plastome) transformation (level 2 vectors). Although this system represents a significant advancement for multigenic designs, the two-step cloning workflow may be simplified for monogenic designs in a single reaction using adapted destination vectors. Here, we present a novel MoCloro-compatible chloroplast transformation vector, pLM20-022, which carries two BsaI recognition sites and a spectinomycin selectable marker, flanked by plastome homology regions for integration at the pLM20 locus. The BsaI restriction sites in pLM20-022 allow direct cloning of genetic parts (level 0 biobricks) into a destination vector ready for transformation into Chlamydomonas chloroplast (level 2), therefore skipping one cloning step of the standard MoCloro workflow. We demonstrate the functionality of the pLM20-022 vector in Chlamydomonas chloroplast by expressing a fluorescent protein reporter and the T7 RNAP polymerase as proof of principle.

Financial support and acknowledgments

This work was supported by MICIU/AEI, Spain. M.G. acknowledges a Beatriu de Pinòs postdoctoral fellowship from AGAUR, Generalitat de Catalunya and XM-C acknowledges a predoctoral fellowship from Institut Químic de Sarrià.

Keywords

MoClo • Chlamydomonas • Chloroplast genome engineering

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

ENGINEERING PHA DEPOLYMERASES TO MODULATE STRUCTURE–FUNCTION RELATIONSHIPS

Laura Isabel de Eugenio; José Daniel Jiménez; Elena Ramos; M. Auxiliadora Prieto
Polymer Biotechnology Group, Centro de Investigaciones Biológicas Margarita Salas (CIB-MS), CSIC. Madrid, Spain

Abstract

Plastic materials are widely used across modern industries due to their durability, versatility, and low production cost. However, their extensive use has led to a growing environmental problem, as they are difficult to biodegrade and tend to accumulate in landfills and aquatic ecosystems. Bioplastics have gained increasing attention as a sustainable alternative, as they can be derived from renewable resources, exhibit biodegradability, or combine both features. Among them, polyhydroxyalkanoates (PHAs) constitute a diverse family of microbial polyesters whose monomer composition varies, giving rise to distinct material properties. Short-chain-length PHAs (sclPHA) generally yield more rigid, brittle materials, whereas medium-chain-length PHAs (mclPHA) exhibit greater flexibility and elastomeric behavior. Notably, PHAs are the only bioplastics entirely synthesized and degraded by microorganisms, making them particularly well suited to circular material design. This dual biological control over synthesis and degradation positions PHAs as a valuable model for studying microbial metabolic cycles, offering insights for industrial application. Understanding their biodegradation is especially relevant when designing end-of-life strategies, as biodegradability alone is not enough for bioplastics to replace conventional plastics. Effective management strategies are needed to prevent PHA waste from being treated as inherently harmless and to recover valuable monomers. One promising approach to valorize these monomers is their enzymatic recycling, which relies on hydrolase activity to break down the polymer into reusable oligomers or monomers under mild, selective conditions. In this context, the enzymes responsible for PHA degradation are known as depolymerases, which can be classified as extracellular or intracellular, each playing distinct roles in the PHA cycle. Extracellular depolymerases act on polymer released into the environment from lysed producer cells, while intracellular ones are associated with cytoplasmic PHA granules, mobilizing the polymer as a carbon and energy source depending on the bacterium's nutritional status and environmental conditions. Despite functional differences, both enzymes share a conserved α/β-hydrolase structure, but intracellular depolymerases also feature a lid domain partially covering the catalytic site, likely involved in interfacial activation mechanisms that regulate substrate access and activity. Although PHA is the natural substrate of both enzymes, extracellular mclPHA depolymerases often show greater efficiency in hydrolyzing soluble substrates, such as p-nitrophenyl esters. In this study, we sought to enhance the esterase-like activity of the intracellular depolymerase from P. putida KT2440 and explore how this functional shift relates to structural changes in the lid domain and its activation mechanism. To achieve this, both directed and random mutagenesis approaches were employed. Two mutants, Z88 and Z32, were selected and characterized as the most promising catalysts. Z88 showed significantly increased activity on p-nitrophenyl esters, while Z32 outperformed the wild-type enzyme in the degradation of PHA nanoparticles, suggesting distinct substrate preferences. In Z88, the S184F mutation is located near the hinge between the lid and catalytic core, pointing to a structural origin of the functional enhancement. Molecular dynamics simulations of Z88 revealed reduced lid flexibility, suggesting a more stable conformation that may favor substrate positioning and catalytic efficiency. These results highlight the role of the lid-core interface in tuning enzymatic specificity and activity.

Financial support and acknowledgments

This research received funding from the ReBioCycle project (HORIZON-JU-CBE-2023-IA-04, 101156032) and the Spanish Ministry of Science, Innovation and Universities MCIU/AEI/10.13039/501100011033 under the research grant SMARTERIAL PID2023-146557OB-C21. This work was supported by the CSIC Interdisciplinary Thematic Platform (PTI+) Sustainable Plastics towards a Circular Economy (PTI-Susplast+).

Keywords

PHA depolymerase • random mutagenesis • bioplastic recycling • enzyme engineering

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

A NOVEL HYBRID BIOCATALYST APPROACH FOR PLASTIC WASTE RECYCLING AND UPCYCLING INTO HIGH ADDED VALUE COMPOUNDS

Maialen Iturralde
CIC biomaGUNE

Abstract

Depolymerization of synthetic plastics is a subject undergoing intense study at the moment since the window for action is approaching rapidly. Poly(ethylene terephthalate) (PET) is one of the most commonly used plastic around the world, yet its recycling and upcycling is still challenging and demands high energy waste. A change towards more sustainable methodologies is needed and that's where biocatalysis emerges as a green alternative opposed to chemical depolymerization. Inspired in nature, where cells evolve into spores to shield themselves from the extracellular environment, we created metal chelated artificial spores to mimic this behaviour, and, more interestingly, to allow enzyme immobilization on the surface of the spores. The combination of heterologous intracellular protein expression and extracellular His-tagged multi-enzyme immobilization yields in a novel hybrid biocatalyst, with the aim of performing and enhancing the multi-step PET waste upcycling. We explored a 3-enzyme system, where a glycerol dehydrogenase from Bacillus stearothermophilus was endogenously expressed in Escherichia coli (E.coli) and employed as a resting cell, in addition to the fastPETase from Ideonella sakaiensis and a transaminase from Halomonas elongata immobilized on the surface of the spores. From the hydrolysis of the BHET monomer, terephthalic acid (TPA) and 2 molecules of ethylene glycol (EG) are obtained. Our work proposes the upcycling of the diol, EG, by an oxidative amination yielding in glycolaldehyde (GA) and the industrially relevant ethanolamine (EA) subsequently. Moreover, ethanolamine can also be a carbon and nitrogen source to the cells, and be assimilated into their metabolism, further leveraging PET waste.

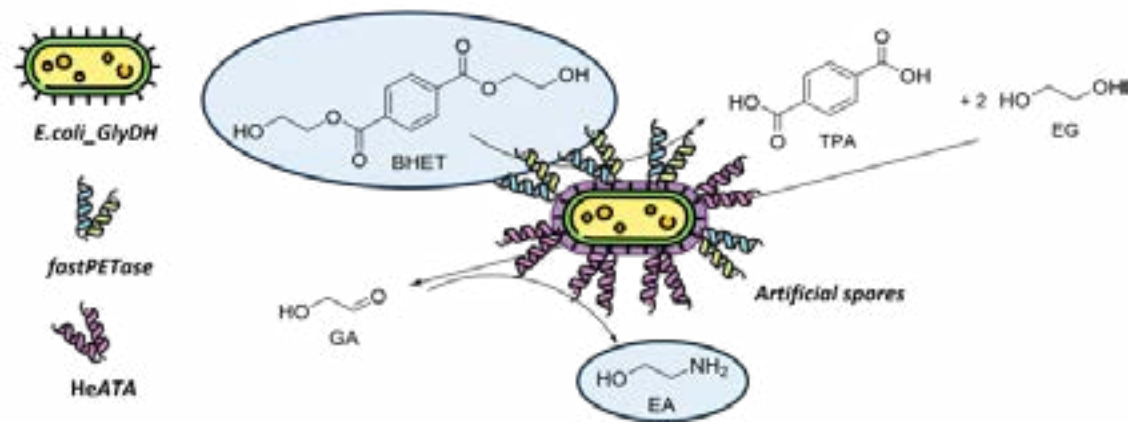
These new generation hybrid biocatalysts allow us to experiment with different spatial distributions, where the immobilization of the PETase and the soluble transaminase yielded in 51% of conversion to EA, lasting up to 3 reaction cycles of 72h. In this work we present a versatile biocatalyst, that allows us to reuse the system, as well as the extrapolation of the methodology to any kind of desired enzyme or reaction.

Financial support and acknowledgments

This work was supported by the Ministerio de Ciencia e Innovación with a FPI fellowship (PRE2022-101787).

Keywords

Hybrid biocatalyst • Enzyme immobilization • PET degradation • Artificial spores



July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

CO-IMMOBILIZATION OF A MULTIENZYME CASCADE IN SILK FIBROIN

Juan Cruz Almada; Susana Velasco-Lozano; Miguel Marco-Martin; Uriel A. Ruiz
Instituto de Síntesis Química y Catálisis Homogénea (ISQCH), CSIC-Universidad de Zaragoza, Zaragoza, Spain

Abstract

The adoption of biocatalytic cascades is transforming industrial chemical synthesis by offering efficient and sustainable alternatives to traditional multi-step processes. By customizing these cell-free reactions into a single pot, these systems minimize operational overhead, reduce purification needs, and enhance reaction efficiency.

However, a key challenge lies in the simultaneous immobilization of multiple enzymes, which often require distinct attachment strategies and exhibit divergent stability profiles. Overcoming these limitations is crucial for advancing sustainable and efficient industrial biocatalysis.In this study, we employ silk fibroin (SF) —a versatile biomaterial derived from silkworm cocoons— as a platform to immobilize fragile multienzyme systems under mild conditions. SF's intrinsic properties enable the formation of tunable structures that enhance the integration of multienzyme assemblies, expanding its applicability in complex biocatalytic cascades. While SF has been widely used for immobilizing single enzymes and whole cells, its potential for stabilizing cell-free multienzyme systems remains underexplored.

We investigated the previously reported dual-enzyme system composed of an alanine dehydrogenase (AlaDH) and formate dehydrogenase (FDH) for synthesizing non-canonical amino acids where the heterogeneous system immobilized on glyoxyl-activated agarose microparticles show low estability. By co-immobilizating these enzymes in SF microspheres under mild conditions, we achieved an efficient performance, significantly enhancing the functional stability of the entire biocatalytic cascade.

Financial support and acknowledgments

All authors thank PIEZOZYMES ERC-St project (Nº 101041192) for the funding.

Keywords

Enzyme immobilization • Silk Fibroin • Biocatalytic cascades|co-immobilization

ENHANCED PRODUCTION, OPTIMIZATION, AND IMMOBILIZATION OF PECTINASES FROM PENICILLIUM CRUSTOSUM ON MAGNETIC CORE-SHELL NANOSTRUCTURES FOR EFFICIENT JUICE CLARIFICATION

Alcione García-González; Arely Sugey Núñez-Serrano; Refugio Bernardo García-Reyes
Universidad Autónoma de Nuevo León, FCQ

Abstract

The global market of food enzymes is held by pectinases, mostly sourced from filamentous fungi via submerged fermentation. Given the one-time use of enzymes to clarify juices and wines, exploring new fungi alternatives for enzyme production and optimization is crucial. Additionally, enzyme immobilization enables their reuse in food applications. This research evaluated an isolated fungal strain (*Penicillium crustosum* OR889307) as a new potential pectinase producer in submerged fermentation, focusing on production optimization. Furthermore, the enzyme was immobilized in magnetic core-shell nanostructures for juice clarification, facilitating the separation of the enzyme from the fruit juice and allowing the reuse of the enzymatic material in many use cycles. Findings revealed that *Penicillium crustosum* exhibited enzymatic activities higher than other *Penicillium* species, and pectinase production was enhanced with lemon peel as a co-substrate in submerged fermentation. The enzyme production (548.93 U/mL) was optimized by response surface methodology, determining the optimal conditions at 35 °C and pH 6.0. Subsequently, the enzyme was covalently immobilized on synthesized magnetic core-shell nanoparticles. The immobilized enzyme exhibited superior stability at higher temperatures (50 °C) and acidic conditions (pH 4.5). Finally, the immobilized pectinases decreased 30 % the orange juice turbidity and maintained 84 % of the enzymatic activity after five consecutive cycles.

In conclusion, *Penicillium crustosum* is a confirmed producer of pectinase, and the immobilization of pectinase on nanoparticles enhances its stability and reusability for juice clarification, maintaining efficiency for at least five reuse cycles.

Financial support and acknowledgments

Arely Núñez-Serrano thanks CONAHCyT for the scholarship received during graduate studies. In addition, all authors acknowledge the School of Chemical Sciences for the infrastructure to perform this research, as well as the Universidad Autónoma de Nuevo León (PAICYT-72-CAT-2022) and CONAHCyT (CB-2016/285462) for financial support. Also, the authors acknowledge the Laboratorio Nacional de Nano y Biomateriales, Cinvestav-IPN Mérida, financed by CONAHCYT, and the invaluable help of Dra. Patricia Quintana-Owen for the characterization of the materials synthesized in this research.

Keywords

Penicillium crustosum • Pectinase production • Covalent immobilization

Bibliography

W. Xiong, J.K. Yang, F. Yuan Chen, Z. Gang Han, The catalytic domain of *Penicillium crustosum* endoglucanase EGL1 has cellulose-binding capacity and cellulolytic activity, Enzyme Microb. Technol. 97 (2017) 71–81.

J. da Câmara Rocha, et al., Yellow mombin pulp residue valorization for pectinases production by Aspergillus niger IOC 4003 and its application in juice clarification, Biocatal Agric Biotechnol 30 (2020).

Sojitra, U. V., Nadar, S. S., & Rathod, V. K. . Immobilization of pectinase onto chitosan magnetic nanoparticles by macromolecular cross-linker. Carbohydrate Polymers, 157, (2017), 677–685.

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

DIGITIZING THE BLUE LIGHT-ACTIVATED T7 RNA POLYMERASE SYSTEM WITH A TET-CONTROLLED RIBOREGULATOR

Sara Baldanta; Guillermo Rodrigo
Instituto de Biología Integrativa de Sistemas (I2SysBio, CSIC-UV)

Abstract

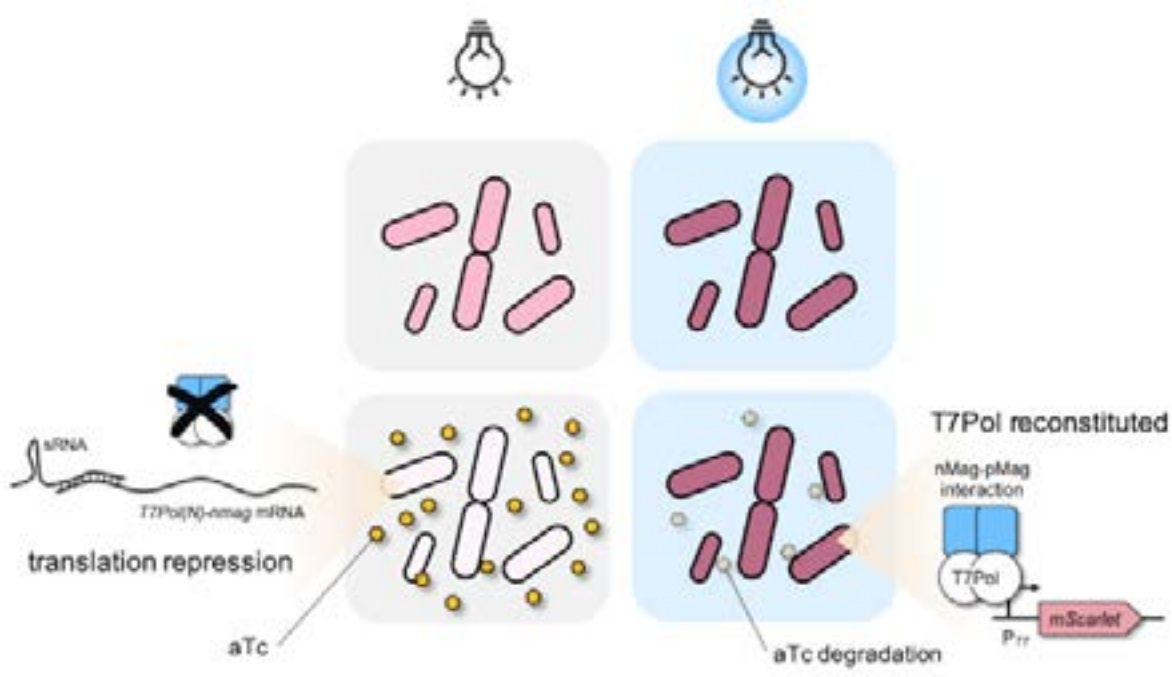
Optogenetic systems offer precise control over gene expression, but leaky activity in the dark limits their dynamic range and, consequently, their applicability. Here, we enhanced an optogenetic system based on a split T7 RNA polymerase fused to blue light-inducible Magnets by incorporating a tet-controlled riboregulatory module. This module exploits the photosensitivity of anhydrotetracycline and the designability of synthetic small RNAs to digitize light-controlled gene expression, implementing a repressive action over the translation of a polymerase fragment gene that is relieved with blue light. Our engineered system exhibited 13-fold improvement in dynamic range upon blue light exposure, which even raised to 23-fold improvement when using cells preadapted to chemical induction. As a functional demonstration, we implemented light-controlled antibiotic resistance in bacteria. Such integration of regulatory layers represents a suitable strategy for engineering better circuits for light-based biotechnological applications.

Financial support and acknowledgments

This work was recently published in ACS Synthetic Biology (https://doi.org/10.1021/acssynbio.5c00142). This work was supported by the Spanish Ministry of Science, Innovation, and Universities and AEI/10.13039/501100011033 (PID2021-127671NB-I00, co-financed by the European Regional Development Fund). SB acknowledges a Juan de la Cierva contract from that Ministry (JDC2023-052427-I).

Keywords

Optogenetics • Small RNA • Antibiotic resistance



July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

TOWARD MORE EFFICIENT HETEROLOGOUS PROTEIN EXPRESSION IN YEAST: INVESTIGATING TRANSLATION CONTROL UNDER ALKALINE PH STRESS

Asier González[1]; Meric Ataman[2]; Marcel Albacar[1]; Alex Schmidt[2]; Antonio Casamayor[1]; Mihaela Zavolan[2]; Joaquin Ariño[1]
1. Universitat Autònoma de Barcelona; 2. Biozentrum. University of Basel

Abstract
Protein synthesis is driven by the translation of genetic information from mRNA. However, mRNA levels often do not reliably predict protein abundance, indicating that translation is tightly regulated. This regulation becomes especially important under conditions like cellular stress, where rapid adjustments in protein production are needed. Recent advances in improving heterologous protein production in the yeast Komagataella phaffii (formerly Pichia pastoris) have focused on enhancing secretion pathways and on developing methanol-free promoters. Our lab is contributing to this effort by engineering novel promoters that are activated by alkaline pH, enabling protein expression when the growth medium becomes alkaline. These novel pH-responsive promoters show competitive performance compared to traditional methanol-inducible systems. Despite these improvements, translation efficiency is often overlooked in designing protein production strategies. To address this, we are using an omics-based approach, including ribosome profiling (ribo-seq), RNA sequencing (RNA-seq), and proteomics, to investigate how the K. phaffii translatome changes under alkaline conditions. Specifically, we aim to identify codon usage preferences in genes that are differentially translated upon alkalinization. This insight will guide codon optimization strategies to re-engineer heterologous genes, ultimately enhancing protein yield.

Keywords
Translation control • Protein production • Yeast

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

EXPLORING COA-TRANSFERASES FOR A NEW IN VITRO PHA PRODUCTION SYSTEM

Santiago Roque de Miguel Sanz[1]**;** María Auxiliadora Prieto[1]; Fernando López Gallego[2]; Idania López López[2]
1. Polymer Biotechnology Group (POLYBIO). Department of Biotechnology, Centro de Investigaciones Biológicas Margarita Salas-Spanish National Research Council (CIB-CSIC), Madrid, Spain; 2. Basque Research and Technology Alliance (BRTA), Cooperative Research in Biomaterials Center (CIC biomaGUNE), Heterogeneous Biocatalysis Laboratory, Donostia-San Sebastián (Spain)

Abstract
Petroleum-derived plastics are materials with excellent properties, essential for sustaining our modern technological society. However, their waste is causing a negative impact on ecosystems worldwide. In this context, bioplastics such as polyhydroxyalkanoates (PHAs) emerge as a sustainable alternative. These are biodegradable and biocompatible polymers produced by bacteria from different hydroxy alkanoic acids. Their thermal and mechanical properties can be tuned by varying their monomer composition, with over 150 different monomers identified to date, allowing the production of materials that could replace their petroleum-based counterparts. PHA production has been extensively studied, with bacterial fermentation established as the predominant method. However, there is growing interest in the development of cell-free systems for synthesizing these polymers. Although still less efficient, these approaches bypass cellular metabolic complexity, avoid harmful chemical polymer extraction, and allow for precise control over the resulting PHA composition. In this work, we describe a new in vitro process for PHA production using a coenzyme A (CoA) recycling system that does not rely on ATP consumption. In this two-protein strategy, acetyl-CoA is produced by the spontaneous reaction between isopropenyl acetate and free CoA when they are mixed in a 100:1 ratio. Then, the CoA groups from the acetyl-CoA are transferred by CoA-transferases to activate 3-hydroxybutyrate monomers. This reaction generates 3-hydroxyacyl-CoAs, which are the active substrates used by PHA synthases for polymer production, and this last step releases CoA molecules, closing the system. The resulting polymer was then characterized by Fourier-transform infrared spectroscopy (FTIR). Finally, we discuss about how our CoA-transferase-based activation approach could enable incorporation of monomers that are otherwise toxic, membrane-impermeable, or metabolically diverted when used in bacteria, thereby providing a direct route to control bioplastic monomer composition.

Financial support and acknowledgments
This work has been supported by the \'Ayudas para contratos predoctorales para la formación de doctores\' fellowship (PRE2021-098281), granted by the Spanish Ministry of Science and Innovation, and by the BIOCIR (PID2020-112766RB-C21) and OPENVIRO (PID2023-146557OB-C21) projects funded by AEI/10.13039/501100011033.

Keywords
Polyhydroxyalkanoates (PHAs) • Cell-free PHA synthesis • Bioplastics • CoA-transferase activation

FadDI FUNCTION IN THE SUSTAINABLE PRODUCTION OF THE ANTIMICROBIAL BIOPOLYMER PHACO

Marina Rodríguez Carreiro; Celia Parrado Castaño; Mª Auxiliadora Prieto Jiménez
Centro de Investigaciones Biológicas - Margarita Salas (CSIC)

Abstract

Over the past few decades, antibiotic resistance has emerged a critical global threat to medicine and public health, endangering our ability to effectively treat infections. This growing menace has intensified the urgency for novel antimicrobial strategies and new antibacterial materials. Polyhydroxyalkanoates (PHAs), natural bio-based bacterial polyesters, have attracted significant attention for biomedical use due to their biodegradability, biocompatibility, and promising mechanical properties, along with advances in sustainable production methods. However, PHAs lack intrinsic antimicrobial activity, though they can be functionalized to gain such properties. A notable example is PHACOS, a second-generation PHA with antimicrobial activity against Staphylococcus aureus, attributed to thioester groups in its monomer structure. This biopolymer is synthesized by Pseudomonas putida KT2440 using fatty acids and the precursor 6-acetylthiohexanoic acid (6-ATH). In this work, we explored sugar-based carbon sources (glucose, fructose and sucrose) to enhance PHACOS production, aiming to improve sustainability and economic feasibility within a circular economy framework.In the course of our research, we observed that co-feeding fructose and 6-ATH supressed bacterial growth. After 72 hours of cultivation, a spontaneously adapted strain emerged, later named P. putida A1. This strain exhibited a 5.5-fold reduction in PHA accumulation compared to the KT2440 wild-type strain under the same growth conditions. Whole-genome sequencing of A1 revealed a prevalent single-nucleotide deletion in the fadDI gene (PP_4549), which encodes an essential enzyme for fatty acid activation in β-oxidation and PHA biosynthesis pathways. To investigate the gene's role, a ΔfadDI knockout strain was generated via homologous recombination. When grown with fructose and 6-ATH, the mutant mirrored A1's phenotype. Further experiments with octanoic and decanoic acids revealed that the ΔfadDI mutant produced significantly less PHA than KT2440, particularly when exposed to 6-ATH. In decanoate-supplemented media, both growth and PHA synthesis were drastically reduced in the mutant, highlighting the critical role of FadDI protein in fatty acid metabolism and resistance to 6-ATH.This study offers new perspectives on the role of the FadDI fatty acid acyl-CoA synthetase in P. putida KT2440 and it suggests that 6-ATH may inhibit PHA biosynthesis.

Financial support and acknowledgments

This research received funding from the Spanish Ministry of Science, Innovation and Universities MCIN/AEI/10.13039/501100011033 under the research grant SMARTERIAL (PID2023-146557OB-C21) and CEX2021-001226-S-20-5, the European Union Project PROMICON #101000733 and the CSIC Interdisciplinary Thematic Platform (PTI+) Sustainable Plastics towards a Circular Economy (PTI-Susplast+).

Keywords

Polyhydroxyalkanoates • Pseudomonas putida KT2440 • Bioprocess • FadDI • 6-ATH • Staphylococcus aureus

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

STRUCTURAL AND FUNCTIONAL STUDIES OF THE ENZYBIOTIC VL-1L, THE LYSOZYME OF BACTERIOPHAGE VB_PAES_VL1 OF PSEUDOMONAS AERUGINOSA

Inés Pareja-Cerbán[1]; Mario Martínez[2]; Rafael Molina[2]; Beatriz Maestro[3]; Jesús Miguel Sanz[4]
1. CIBER de Enfermedades Respiratorias (CIBERES), Madrid, España; Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, España; Centro de Investigaciones Biológicas Margarita Salas. Consejo Superior de Investigaciones Científicas, Madrid, España; 2. Departamento de Cristalografía y Biología Estructural, Instituto de Química-Física Blas Cabrera, Consejo Superior de Investigaciones Científicas, Madrid, España; 3. Departamento de Bioquímica y Biología Molecular. Facultad de Ciencias Biológicas. Universidad Complutense de Madrid, España; 4. Centro de Investigaciones Biológicas Margarita Salas. Consejo Superior de Investigaciones Científicas, Madrid, España; CIBER de Enfermedades Respiratorias (CIBERES), Madrid, España

Abstract
The emergence of multi-resistant strains to antibiotics has encouraged the search for new antimicrobials as an alternative to treat infections. Phage lysins are a promising technological strategy to introduce a more precise approach to antimicrobial therapy. In this work, we have identified and characterized the antimicrobial activity of the endolysin encoded by the bacteriophage vB_PaeS_VL1 (VL1) from Pseudomonas aeruginosa, which we have named VL-1L. The enzyme, when added exogenously, has high bactericidal activity at relatively low concentrations (2 micromolar) against P. aeruginosa. In addition, viability assays confirmed that VL-1L has bactericidal activity against other Gram-negative pathogens (Escherichia coli) but not against Gram-positive bacteria. Experiments have also been initiated to crystallise the protein in order to elucidate its three-dimensional structure. In any case, experiments with different mutants of the protein based on a theoretical structural model have identified amino acids essential for its peptidoglycan hydrolytic activity and suggest that the bactericidal activity of the enzyme is based primarily on its ability to interact with and alter the bacterial surface. In conclusion, VL-1L endolysin has biological characteristics that make it a potential antimicrobial candidate for treating infections against P. aeruginosa and other Gram-negative pathogens.

Financial support and acknowledgments
PID2022-139209OB-C21/AEI/10.13039/501100011033/FEDER/UE

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

MYCOBROW: TRANSFORMING WASTE INTO A BIOSTIMULANT SOLUTION

Maria Camila Espinosa Araque; Marina Pareja Valero; Inmaculada García Romero; Gloria Andrea Silva Castro

Abstract

Agriculture is key to society, so it is essential to adopt sustainable practices. One of these is the use of organic amendments, which improve soil quality and reduce reliance on chemical fertilizers. In Spain, large volumesof dry olive residues, known as “alperujo”, are generated during olive oil production. Although rich in organic matter, alperujo contains high levels of phenolic compounds, which make it phytotoxic and unsuitable for direct application to the soil. The study focused on transforming alperujo into a biostimulant through biotechnological processes using saprobic fungi, microorganisms capable of degrading these toxic compounds, to reduce its phytotoxicity and generate bioactive compounds. Initially, eight saprobic fungal strains were selected based on their potential for degrade phenolic compounds. The fungi were grown in malt extract broth for six days, with copper sulfate added on the fourth day to stimulate enzyme activity, followed by the addition of alperujo extract on the seventh day for biotransformation. Results identified four strains: Penicillium spp. (EEZ-22), Fusarium spp. (EEZ-28), Pycnoporus spp. (EEZ-55), and Bjerkandera spp. (EEZ-120), as particularly effectiveThese strains significantly reduced phenolic content by over 65% and increased the germination index (GI) up to 80% compared to controls.Subsequently, greenhouse trials were conducted on wheat, carrot, corn and tomato to evaluate the effect of biostimulants on plant physiology and soil quality. Soil enzyme activity generally increased with all treatments. Products derived from EEZ-55 and EEZ-120 strains notably increasing wheat biomass by 3 and 4 times, respectively, and stimulated vegetative development in tomato by around 60%. In corn and carrot, fruit development occurred only in plants treated with the products.

Based on its biotransformation efficiency and positive agronomic effects, Pycnoporus cinnabarinus (EEZ-55) was selected as the most promising candidate for further development. The resulting product, MycoBrow, exemplifies how fungal biotransformation can convert phytotoxic agro-industrial waste into high-value biostimulants, advancing sustainable agriculture through improved plant performance, enhanced soil health, and circular residue management in the olive oil industry.

Financial support and acknowledgments

This study was supported by grants from the Spanish National Research Agency (AEI) for the formation of doctors in industry 2024. The research was also co-funded by MAFA Bioscience and the European Regional Development Fund (FEDER).(Id. project: MYCO-BROW: IDI-20210451).

Keywords

Alperujo • Saprobic fungi • Biotransformation • Biostimulants • Sustainable agriculture

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

MICROBIAL EVALUATION OF THE HEALTH OF AQUATIC ECOSYSTEMS ALONG THE SOUTHWEST ANDALUSIAN COAST

Marina Barbudo-Lunar[1]; Chiara Trombini[2]; Laura Pérez-González[1]; Verónica Luna-Guerrero[1]; Carmen Michán[1]; Julián Blasco[2]; José Alhama[1]

1. Dpt. Biochemistry and Molecular Biology, ceiA3, Campus Rabanales, Universidad de Córdoba, Córdoba, Spain; 2. Institute of Marine Sciences of Andalusia (CSIC). Department of Ecology and Coastal Management. Puerto Real, Cádiz, Spain

Abstract

Pollution constitutes a major global concern, which threatens the health of ecosystems worldwide. Aquatic ecosystems are particularly vulnerable, as they often are the ultimate sink for pollutants. Anthropogenic activities such as intensive agriculture and urban development have emerged as a pressing menace to biodiversity and human health. Microorganisms are being increasingly recognised as promising bioindicators for assessing ecosystem health due to their ubiquitous distribution, direct contact with pollutants, and rapid responses. Changes in the composition, diversity and functionality of the microbiome can serve as effective biomarkers to assess the impact of stressors. In this regard, we have studied sediment samples of ten different sampling points along the southwest of the Andalusian coast (Spain). These points are distributed across four different areas: San Pedro River (S1), Sancti Petri (S2, S3, S4), the Guadalquivir Estuary (S5, S6, S7) and Huelva (S8, S9, S10). Our objective is to characterise the microbial communities present at these locations, assess their variability between sampling points, and explore associations between metagenomic profiles and pollution. Bacterial identification in the sediment samples was carried out through 16S rDNA sequencing. A total of 16 different bacterial phyla were detected. The predominant phylum across all sampling points was Proteobacteria, followed by Cyanobacteria and Bacteroidetes. At a lower taxonomic level, 161 bacterial families were identified, and the most represented families were Ectothiorhodospiraceae, Nostocaceae and Flavobacteriaceae. The microgAMBI index was calculated, and Sancti Petri and Guadalquivir Estuary were found to have a good water quality, whereas San Pedro River and Huelva showed moderate levels. Alpha-diversity indexes were calculated, and the Shannon-Wiener index was notably higher in Sancti Petri and Huelva. Beta-diversity was evaluated calculating Aitchison distances, which were represented by multidimensional scaling (MDS) analysis. A PERMANOVA test was performed to assess the differences among the four areas, but no significant differences were observed, although Guadalquivir Estuary showed the greatest differentiation. Differential abundance analysis, considering the compositional nature of data, was conducted using the R package ALDEx2. Comparisons were made against S1, which has been previously described as a clean site. The Guadalquivir Estuary showed the largest number of significant changes and Huelva the lowest number. The use of Metagenomics has proven to be a powerful tool in ecotoxicological studies as microorganisms are sensitive biomarkers of pollution. Field studies in combination with controlled exposure experiments will allow us to relate changes in microbiome composition and functionality with specific sources of pollution.

Financial support and acknowledgments

MICINN PID2019-110049RB-I00 and Junta de Andalucía PCM_00118. Fundings to BIO187 and RNM306 groups (PAIDI, UCO/CSIC). Research Support Services (SCAI) of the University of Córdoba. M. Barbudo-Lunar received a predoctoral contract from the University of Cordoba (“Plan Propio”) and the “Ministerio de Ciencia e Innovación” (FPU).

Keywords

aquatic ecosystem • pollution • metagenomics • biomarkers

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

UNVEILING A. BAYLYI ADP1 METABOLISM TO ENHANCE WAX ESTER PRODUCTION

Susana Capel; M. Auxiliadora Prieto, Isabel Pardo
Centro de Investigaciones Biológicas Margarita Salas (CSIC)

Abstract

Cetyl palmitates are a specific type of wax ester derived from hexadecanoic acid and hexadecanol, widely used in the cosmetic industry. In the past, they were extracted from sperm whales (Physeter macrocephalus), as these animals can accumulate it in large quantities. However, due to the endangerment of this species, this source was banned. Today, cetyl palmitates are produced through chemical synthesis from fossil-derived resources using energy-intensive processes, prompting growing interest in their sustainable biological production.Acinetobacter baylyi ADP1 naturally accumulates wax esters (WEs), mainly cetyl palmitates, as carbon storage compounds. However, to exploit bacterial WEs industrially, it is necessary to increase their natural production rates. Since fatty acids are the precursors for WE biosynthesis, we performed transcriptomic analyses under different conditions to identify genes involved in fatty acid degradation. Specifically, we aimed to identify fadE orthologues (acyl-CoA dehydrogenase that catalyse the first step of β-oxidation) in A. baylyi ADP1, as their deletion could increase the pool of fatty acids available for wax ester synthesis.

We identified a group of four genes annotated as acyl-CoA dehydrogenases that were highly transcribed under all tested conditions, especially in those where cells were consuming previously accumulated wax esters. Single and combined deletion mutants of these genes were constructed in order to assess their growth on palmitic acid, revealing that certain mutants exhibited impaired growth, thereby indicating a role for these genes in β-oxidation. To assess the impact of these deletions on wax ester production, we used a luminescent biosensor that enables real-time monitoring of wax ester synthesi. Among the tested strains, the quadruple fadE deletion mutant showed the highest performance, with a six-fold increase in cumulative luminescence relative to the wild-type strain. According to the literature, the highest wax ester-producing strain reported to date combines the overexpression of acr1 (encoding acyl-CoA reductase, involved in the first step of WE synthesis) with the deletion of aceA (encoding isocitrate lyase). Applying the same strategy to our quadruple fadE deletion mutant, we observed a 13-fold increase in luminescence compared to the wild type, and a 5.5-fold increase compared to the best previously-reported mutant. Interestingly, the addition of aceA deletion to the quadruple mutant did not greatly improve luminescence.

These findings suggest that we have identified four potential fadE genes whose deletion significantly enhances wax ester production in A. baylyi ADP1, providing a significant step forward toward the improved microbial production of value-added cetyl palmitates.

Financial support and acknowledgments

This work is funded by the Spanish National Research Council (CSIC), the Reina Sofia Foundation, and the Primafrío Foundation under agreement no. 20210510; Comunidad de Madrid (CAM) under contract no. 2022-T1/BIO-23939; and MCIN/AEI/10.13039/50110001103 under project no. CNS2024-154152. IP thanks CAM for an “Atracción de Talento” contract. SC thanks CAM for a PIPF predoctoral contract.

Keywords

Cetyl palmitate • Wax esters • Metabolic engineering • Transcriptomics • Fatty acid metabolism • Acinetobacter baylyi ADP1

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

ENGINEERING RECOMBINANT BIOCATALYSTS FOR PLASTICIZERS DEGRADATION: MOLECULAR CHARACTERIZATION OF A SECRETED PHTHALATE ESTERS HYDROLASE

Sofía de Francisco-de Polanco; Gonzalo Durante-Rodríguez; Eduardo Díaz

Abstract

Phthalate esters (PAEs) are esterified derivatives of *o*-phthalic acid (PA) and are currently one of the most widely used plasticizers. Since they are non-covalently bound to plastics, they easily leach into the environment and are among the most common emerging organic pollutants due to their various toxic effects on living organisms. Bacterial degradation of PAEs is considered one of the most promising strategies for their removal. The catabolic pathways of PAEs involve a peripheral metabolism that transforms the PAE into PA (and the corresponding alcohol), by the action of esterases, and a central metabolism for the mineralization of PA[1].

Previous studies characterized a new PA degradation pathway (transport and degradation of PA via benzoyl-CoA) and demonstrated the potential to redirect this carbon flow toward the production of bioplastics such as polyhydroxybutyrate (PHB)[2]. However, a major bottleneck in PAEs degradation is the esterase-mediated hydrolysis to generate PA.

In this work, we report the characterization of a novel PAEs esterase, EstB, from *Halomonas* sp. ATBC28[3]. The *est*B gene was cloned and expressed in recombinant *Escherichia coli* cells. Biochemical assays of the overproduced enzyme revealed that it is an active diesterase capable of generating mono-*n*-butyl phthalate (MBP) as the final product. Amino acid sequence analyses indicate that EstB belongs to family VIII of esterases. Interestingly, the protein contains a predicted N-terminal signal sequence suggesting its secretion to the periplasm or extracellular medium.

To test this hypothesis, enzymatic assays were performed using both full-length EstB and a truncated version of the protein lacking the signal peptide. Enzymatic activity was detected in the extracellular fraction only for the full-length protein. N-terminal sequencing of the extracellular enzyme confirmed that this PAEs esterase is the first one whose secretion to the extracellular medium has been experimentally validated.
Since PA-degrading biocatalysts are usually unable to uptake PAEs, the estB gene becomes a promising genetic tool for engineering recombinant biocatalysts capable to attack PAEs in the extracellular medium for their removal and bioconversion into value-added products.

Funding

This work was funded by Project PID2022-142540OB-I00 and a PRE2020-092563 grant from the Spanish Ministry of Science, Innovation and Universities MICIU/AEI/10.13039/501100011033.

1. Durante-Rodríguez G, de Francisco-Polanco S, Fernández-Arévalo U, Díaz E. (2024). Engineering bacterial biocatalysts for the degradation of phthalic acid esters. *Microb Biotechnol.*;17(10):e70024.

2. Sanz, D., García, J.L. and Díaz, E. (2020). Expanding the current knowledge and biotechnological applications of the oxygen-independent ortho-phthalate degradation pathway. *Environ Microbiol*, 22: 3478-3493.

3. Wright, R. J., Bosch, R., Gibson, M. I., & Christie-Oleza, J. A. (2020). Plasticizer Degradation by Marine Bacterial Isolates: A Proteogenomic and Metabolomic Characterization. *Environmental Science and Technology*, 54(4), 2244–2256.

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

FUNCTIONALIZED HIV-1 GAG VLPS FOR TARGETED DELIVERY TO CXCR4-POSITIVE CANCER CELLS

Elianet Lorenzo[1]; Marc García[1]; Eric Voltà[2]; Ugutz Unzueta[2]; Francesc Gòdia[1]; Laura Cervera[1]
1. Universitat Autònoma de Barcelona; 2. Institut de Biotecnologia i de Biomedicina

Abstract

The use of virus-like particles (VLPs) as nanocarriers for biomolecules is a promising approach in drug delivery research. CXCR4 is a cell surface receptor associated with several types of cancer. The T22 peptide is a known antagonist of this receptor that efficiently binds to and penetrates CXCR4-positive cells. In this work, we produced Gag-GFP VLPs in HEK293 cells via transient transfection. We then optimized a four-step downstream process for these VLPs, including clarification via depth and standard filtration, intermediate purification using tangential flow filtration or multimodal chromatography, capture through ion exchange, hydrophobic interaction, and heparin affinity chromatography, and final polishing with size exclusion chromatography. A complete purification run using the best conditions for each step resulted in an overall recovery of 38% and a purity of 64%, with host cell DNA and protein levels complying with regulatory standards. These VLPs were then functionalized with the T22 peptide via click chemistry. The ability of Gag-GFP VLPs-T22 to penetrate CXCR4-positive cells was dose- and time-dependent. CXCR4-positive cells exposed to 8.0×10^9 functionalized Gag-GFP VLPs/mL exhibited 30-fold higher fluorescence than those exposed to non-functionalized Gag-GFP VLPs after 24 hours of incubation. The absence of Gag-GFP VLPs-T22 internalization in CXCR4-negative cells confirmed specificity via the CXCR4 receptor. The functionalization of VLPs with the T22 ligand for targeting CXCR4-positive cells is significant given the receptor's role in cancer progression and metastasis. By specifically targeting CXCR4, these VLPs can selectively deliver therapeutic biomolecules to cancer cells, potentially enhancing treatment efficacy while minimizing off-target effects and reducing systemic toxicity.

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

MICROBIAL VALORIZATION OF HYDROTHERMAL LIQUEFACTION WASTEWATER

Beatriz Pasero[1]; Marcos Mendieta-Fernández[1]; Carlos Sainz[2]; Luis Fernando Bautista[2]; Gemma Vicente[2]; Beatriz Maestro[3]; Jesús Miguel Sanz[4]; José Luis García[1]

1. Centro de Investigaciones Biológicas Margarita Salas, Consejo Superior de Investigaciones Científica Madrid, España; 2. Department of Chemical and Environmental Technology, Universidad Rey Juan Carlos, Móstoles, Spain; 3. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad Complutense de Madrid, España; 4. Centro de Investigaciones Biológicas Margarita Salas, Consejo Superior de Investigaciones Científica Madrid, España; CIBER de Enfermedades Respiratorias (CIBERES), Madrid,España

Abstract

Within the concept of chasing for renewable sources for the production of biofuel and other industrial, high value-added chemicals, the use of anthropogenic waste as feedstock represents a solution to the problem of sustainable production and reduces the environmental and economic impact of waste disposal. Hydrothermal liquefaction (HTL) is an emerging physico-chemical procedure which involves the conversion, at high temperatures and pressures, of waste material into a bio-oil phase which may be further processed to be used as biofuel. Several by-products are obtained in this process: a gas fraction, mainly CO_2, a solid fraction or biochar and an aqueous fraction, which is often too toxic to be released to the environment. The main objective of this work is to valorise the hydrothermal liquefaction wastewater (HTWW) generated as by-product of HTL carried out on different raw materials, such as Arthrospira platensis biomass, cellulose, lignin, oils and conventional plastics. Characterization studies were performed on different HTWW samples, whose composition depends on the feedstock and HTL reaction conditions and indicates their potential for biological valorization. HTWW samples were subjected to enzymatic treatments with bacterial and fungal oxidoreductases reducing their toxicity and enabling the growth of microorganisms such as Escherichia coli, Pseudomonas putida, and Yarrowia lipolytica in media containing up to 55% HTWW. This lays the foundation for converting wastewater into valuable products such as polyhydroxyalkanoates (PHA), lipids, or other compounds of industrial interest.

Financial support and acknowledgments

TED2021-129747B-22/AEI/10.13039/501100011033/NextGenerationEU/PRTR; PID2022-139209OB-C21 AEI/10.13039/501100011033/FEDER/UE

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

CHITOSAN MODIFICATION WITH OCTENYL SUCCINIC ANHYDRIDE (OSA): MATERIAL CHARACTERIZATION AND APPLICATIONS

María Carpintero; Ismael Marcet, Manuel Rendueles, Mario Díaz
University of Oviedo

Abstract

Petroleum-based plastics are the most used synthetic polymers in everyday life all over the world. Their non-biodegradable character has made their disposal an environmental challenge that has intensified the search for biodegradable alternatives based on natural polymers. Among these, chitosan has attracted considerable attention due to its biocompatibility, biodegradability and film-forming properties. Nonetheless, its limited mechanical, barrier and functional properties restricts its industrial applications. In this sense, research on chemical modification of biopolymers—particularly esterification with octenyl succinic anhydride (OSA)—has been considered a promising strategy to enhance the properties of biopolymers. This study explores the structural modification of chitosan with OSA and evaluates its impact on the functional and film-forming properties of the biopolymer.

For this purpose, chitosan (1 g) was dissolved in 100 mL of 1% acetic acid for 30 minutes and diluted with 100 mL of ethanol. OSA was then added dropwise at varying ratios (0.3, 0.6, and 0.9 g OSA/g chitosan) under constant stirring. After 4 hours at 40 °C, the reaction was stopped by adjusting the pH to 7–8, precipitating the modified chitosan. The product was characterized using FTIR, NMR, XRD, and DSC, and its film-forming and emulsifying properties were evaluated. For film preparation, both modified and native chitosan (0.25% w/v) were dissolved in 1% acetic acid, stirred until clear, poured into Petri dishes, and dried at 37 °C for 12 hours. The films were then conditioned and analyzed for mechanical, structural, thermal, and barrier properties.

As expected, it was observed that increasing the concentration of OSA used in the reaction resulted in an increase in the number of OSA molecules attached to the biopolymer backbone and, therefore, in the degree of substitution (DS), which ranged from 0.10 to 0.42. NMR and FTIR analyses confirmed the introduction of OSA, as new peaks appeared in the spectra, and XRD revealed that the introduction of these hydrophobic groups altered the crystalline structure of chitosan. In this sense, the introduction of OSA hydrophobic groups in chitosan structure increased its emulsifying properties. Regarding the films prepared with OSA-modified chitosan, they exhibited improved water vapor and light barrier properties, and a significant decrease in water solubility. The mechanical properties of these films were also improved at lower modification values, as higher degrees of modification led to molecular aggregation and matrix instability. A decrease in emulsion droplet size and, therefore, an increase in emulsion stability was observed with increasing DS values. It can be concluded that the modification of chitosan with OSA allows to expand the industrial applications of the biopolymer.

Financial support and acknowledgments

The authors are grateful for the financial support from the Spanish Ministry of Science and Innovation through project MCIU-22-PID2021-125942OB-I00 and from the Principality of Asturias through project Sek-25-gru-gic-24-010. The author, María Carpintero, also acknowledges the Spanish Ministry of Universities for the financial support received through the FPU pre-doctoral aid program (FPU21/01528).

Keywords

Chitosan • Chemical modification • Octenyl succinic anhydride (OSA) • Films • Emulsion

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

A NOVEL PSEUDOMONAS PUTIDA CHASSIS FOR THE UPCYCLING OF ETHYLENE GLYCOL DERIVED FROM PET DEGRADATION

Alejandro Garcia-Miro[1]; Francisco Javier Molpeceres-Garcia[1]; Elvira Mateos-Garcia[2]; David Sanz[1]; Alicia Prieto[1]; Jorge Barriuso[1]
1. CIB-CSIC; 2. UCM

Abstract

Plastic pollution represents one of the most pressing environmental challenges of our time, with polyethylene terephthalate (PET) being among the most commonly used plastics, particularly in packaging applications. PET is a synthetic polymer composed of terephthalic acid (TPA) and ethylene glycol (EG) units linked by ester bonds[1].

Currently, the treatment and recycling of PET waste rely on physicochemical techniques that are often inefficient and environmentally unsustainable. As an alternative, biotechnological approaches based on the use of microorganisms and their enzymes have gained increasing attention[1].

Several microbial enzymes capable of efficiently degrading PET have been identified, including PETase and MHETase from the bacterium Ideonella sakaiensis. PETase initiates the breakdown of PET by depolymerizing it into mono(2-hydroxyethyl) terephthalate (MHET), which is subsequently hydrolyzed by MHETase to yield the monomers TPA and EG[2].

Moreover, the reuse or upcycling of PET-derived monomers is a subject of considerable interest, and various studies have demonstrated their potential conversion into value-added products such as polyhydroxybutyrate (PHB)[2]. Nevertheless, most of these efforts have focused on the valorization of TPA, while ethylene glycol remains largely overlooked. This is partly due to its lower carbon content and the fact that few microorganisms are capable of utilizing EG efficiently as a sole carbon and energy source[3].

To address this limitation, Pseudomonas putida JM37—a strain recently identified for its high efficiency in metabolizing EG and its inherent ability to produce polyhydroxyalkanoates (PHAs)—was genetically modified to express the PETase and MHETase enzymes. These modifications enable the strain to both depolymerize PET and grow on one of its resulting monomers (EG). This work presents a circular economy strategy in which a single microbial host is engineered to degrade PET, utilize its monomers as a substrate, and simultaneously produce biopolymers (PHAs) that serve as sustainable alternatives to conventional plastics.

Financial support and acknowledgments

MICODE (MCIN/AEI, PID2020-114210RB-I00), DEMO (MCIN/AEI/"NextGenerationEU"/PRTR, TED2021-130096B-I00) and BioPlastOmics Project (Biodiversa2023-248) AEI, PCI 2025 call/Biodiversa+.

Keywords

Biodegradation • PET upcycling • Ethylene glycol • PHAs

1. Carr, C. M., Clarke, D. J., & Dobson, A. D. (2020). Microbial polyethylene terephthalate hydrolases: current and future perspectives. *Frontiers in Microbiology*, 11, 571265.
2. Molpeceres-García, F. J., Sanz-Mata, D., García-Miro, A., Prieto, A., & Barriuso, J. (2025). Towards polyethylene terephthalate valorisation into PHB using an engineered *Comamonas testosteroni strain. New Biotechnology*, 85, 75-83.
3. Franden, M. A., Jayakody, L. N., Li, W. J., Wagner, N. J., Cleveland, N. S., Michener, W. E., & Beckham, G. T. (2018). Engineering *Pseudomonas putida* KT2440 for efficient ethylene glycol utilization. *Metabolic engineering*, 48, 197-207.

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

RESTING CELLS OF CUPRIAVIDUS NECATOR FOR SUGARS PURIFICATION AND EFFICIENT PRODUCTION OF POLY-3-HYDROXYBUTYRATE

Ernesto González; Juan Manuel Bolivar; Miguel Ladero; Victoria Santos
Universidad Complutense de Madrid

Abstract

Separating molecules with similar structures, functional groups, and molecular weights is a challenging task. However, the metabolic machinery of living organisms has evolved to catalyze highly specific and selective reactions.

Hence, the ability of certain biocatalysts may be used for purification of complex mixtures via transformation of undesired compounds and facilitate the separation process. The bacterium Cupriavidus necator stands out by its capacity of accumulating large amount of poly-3-hydroxybutyrate (PHB) under conditions of nutrient limitation. Since PHB accumulation occurs non associated with microbial growth, the metabolic activity of non-growing cells (resting cells) is used in this work for purification of sugar mixtures.A series of experiments were performed to study glucose consumption by resting cells of C. necator DSM545 changing the concentration of glucose and PHB-free biomass. These experiments showed that glucose is consumed according to a zero-order kinetic when its concentration is higher than ~4 g/L. Analysis performed to biomass samples showed that PHB-free biomass remains constant while PHB increases linearly. The experiments performed using three concentrations of PHB-free biomass showed that the glucose consumption rate depends on this parameter. Additional experiments were conducted to elucidate the relationship between the concentration of PHB-free biomass and glucose consumption rate. These experiments showed a linear relationship between these parameters when the PHB-free biomass is below ~3.3 g/L. However, above this value the glucose consumption rate was almost constant. Mathematical correlations and subsequent experiments allowed to estimate the volumetric mass transfer coefficient, which was useful to explain the observed behavior.

The purification of binary and ternary mixtures of monosaccharides was tested using resting cells of C. necator. Tests performed using glucose-galactose mixtures showed that the bacterium consumed glucose while the concentration of galactose remained constant. Regarding tests performed with ternary mixtures, the bacteria consumed glucose and fructose present in a mixture that initially contained glucose, fructose and mannose. In both cases the non-consumed sugar represented more than 95% of the sugars present at the end of the process. During this process, consumed sugars were efficiently transformed into PHB, achieving a yield near 0.48 g/g which is the theoretical value reported for PHB accumulation from sugars in C. necator.

Financial support and acknowledgments

We gratefully thank the Spanish Ministry of Universities for the support provided through the grant program for the requalification of the University System (María Zambrano), the PID2023-147776OB-I00 research project funded by MICIU/AEI/10.13039/501100011033 and by FEDER, EU, and the PCI2022-132971 research project funded by MICIU/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR.

Keywords

Cupriavidus necator • resting cells • purification • sugar • poly-3-hydroxybutyrate

CHARACTERIZATION OF SOAR1 AS A TRANSLATIONAL REGULATOR IN THE CHLOROPLAST

Elena Rodríguez San Miguel; M. Mar Castellano; Esther Novo-Uzal
Centro de Biotecnología y Genómica de Plantas (UPM-INIA/CSIC), Pozuelo de Alarcón (Madrid)

Abstract

Translation is one of the most energy-demanding processes in the cell and, therefore, it is finely regulated to coordinate growth and metabolism. Plants primarily produce energy through photosynthesis, providing peaks of energy associated to the light diel cycle. Subsequently, plants have evolved specific mechanisms to regulate mRNA translation into proteins in response to these light fluctuations (Missra et al. 2015). Despite being well established that translation is coupled to light conditions and photosynthesis, experimental evidence elucidating the key molecular players involved in this regulation remains elusive. SOAR1 is a pentatricopeptide repeat protein, recently demonstrated to modulate mRNA translation (Bi et al. 2019). The binding of SOAR1 to the translation initiation factors eIFiso4G1 and eIFiso4G2 inhibits their association with the other partners of the eIF4F complex, hindering factor assembly and inhibiting global translation (Bi et al. 2019). Additionally, SOAR1 is known to negatively regulate abscisic acid (ABA) signalling (Mei et al. 2014). Importantly, SOAR1 directly binds to the mRNA of ABI5, a key ABA-responsive gene, and cooperates with eIFiso4Gs to repress ABI5 translation (Bi et al. 2019). While the collaboration between ABA and light signalling is generally accepted, there is currently no evidence linking SOAR1 and light-dependent translation regulation. In this work, we performed a proteomic analysis revealing that numerous putative SOAR1-interacting proteins are located in the chloroplast, suggesting a potential role of SOAR1 in controlling translation in a light-dependent manner. We then demonstrated by two different approaches the in vivo interaction of SOAR1 with one chloroplast-located protein, the latter playing a role in D1 protein regulation. Moreover, we observed that the accumulation pattern of D1 throughout the diurnal period differs in wild-type and soar1 mutant Arabidopsis plants. We will discuss the implications of SOAR1 in regulating D1 translation in this light-dependent context. This work has a clear biotechnological potential to engineer plants that may show greater productivity when coupling translational rate to photosynthesis in response to light.

Financial support and acknowledgments

This work is supported by Ministerio de Ciencia, Innovación y Universidades through a Consolidación Investigadora project (grant CNS2024-154582) and Comunidad de Madrid Atracción de Talento Investigador programme (grant 2020-T1/BIO-19900).

Keywords

SOAR1 • translation • chloroplast

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

EXPRESSION OF ARABIDOPSIS HOP3 UNDER THE CONTROL OF A STRESS INDUCIBLE PROMOTER ENHANCES PLANT TOLERANCE TO SALT STRESS IN PLANTS

Aurora Navarro[1]; René Toribio[1]; Silvina Mangano[2]; Concepción Gómez-Mena[3]; M. Mar Castellano[1]
1. Centro de Biotecnología y Genómica de Plantas (UPM-INIA/CSIC), Pozuelo de Alarcón (Madrid); 2. INTECH, CONICET-UNSAM, Provincia de Buenos Aires, Argentina; 3. Instituto de Biología Molecular y Celular de Plantas. CSIC-UPV, Algirós, Valencia

Abstract

HOP is a family of TPR containing co-chaperones that assist the folding of specific proteins within the HSP70-HSP90 cycle. In Arabidopsis, this family includes three members, being AtHOP3 highly inducible by some stresses, such as heat stress.In our lab we demonstrated that hop mutants show a defect in hypocotyl elongation at warm conditions, indicating that HOPs play a major role in plant adaptation to moderate increases of temperature. Remarkably, our data reveal that, during this process, HOPs participate in the proper folding and stabilization of the HSFA1a, one master regulator of the Heat Stress Response (HSR). Interestingly, activation of the HSR is a hallmark of the plant response not only to warm temperatures but also to different stresses. Probably, related to the role of HOP in the activation of the HSR, hop mutants show multiple defects in response to different stresses such as heat, ER stress or salinity.

Since HOPs seem to play a main role in plant adaptation to different environmental insults, we wondered whether the overexpression of HOPs could enhance plant tolerance to those stresses. For this, we generated Arabidopsis and tomato plants expressing AtHOP3 under the control of a stress inducible promoter. Remarkably, AtHOP3 inducible plants do not show significant differences in plant performance under control conditions. Nevertheless, these lines show a higher tolerance to heat, ER stress or salt in Arabidopsis. As in the case of Arabidopsis, the expression of HOP3 highly reduces salt toxicity in tomato and fully abolishes the deleterious effects of salt stress in tomato fruit ripening and fruit quality.

Soil salinity is a major thread for agriculture, since it negatively affects crop growth, reproduction and yield, limiting the arable land on Earth. Based on our data, overexpression of HOPs could be relevant biotechnological tool to mitigate the negative effects of high salinity in crop productivity.

Financial support and acknowledgments

This work is supported by the projects PID2021-126956OB-I00 from Ministerio de Ciencia, Innovación y Universidades (MICIU) and the 'Severo Ochoa Programme for Centers of Excellence in R&D' from the Agencia Estatal de Investigación of Spain (grant CEX2020-000999-S to the CBGP).

Keywords

HOP • co-chaperone • HSFA1a • tomato • salt stress

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

PLENARY CONFERENCE

STEPPING ON THE GAS: INNOVATING FOR A CIRCULAR CARBON ECONOMY

Michael Koepke
Lanzatec

Abstract

LanzaTech is the carbon recycling company transforming waste carbon into everyday products. Using its revolutionary bio-recycling technology, LanzaTech captures carbon generated by energy-intensive industries at the source, preventing it from being emitted into the air. The process can handle a diverse range of high-volume, low-cost feedstocks including industrial emissions (e.g., steel mills, processing plants or refineries) or syngas generated from any biomass resource (e.g., unsorted, and non-recyclable municipal solid waste (MSW), agricultural waste, or organic industrial waste), as well as CO_2 with green hydrogen. LanzaTech gives the captured carbon a new life as a clean replacement for virgin fossil carbon in everything from fuels to household cleaners, clothing fibers and packaging. By partnering with companies across the global supply chain like LanzaTech is paving the way for a circular carbon economy.At the heart of the technology are LanzaTech's biocatalysts, highly evolved autotrophic bacteria. Building a technology platform around non-model organisms was always going to be an ambitious project: a theoretical description of the biological system was incomplete, genetic tools did not exist and working with gases added further complexity. Several innovations were necessary to unlock this biology for industrial use. Starting from the basics, LanzaTech has created a state-of-the-art synthetic biology platform with an anaerobic biofoundry, developed scalable bioreactor systems with high gas mass-transfer capabilities, and a range of predictive models based on billions of data points.

Today, LanzaTech's technology is globally licensed with six commercial facilities in operation that have an annual capacity to abate 500,000 tons CO_2 and bio-manufacture 300,000 tons of products. Commercialization started with ethanol, but the company developed solutions for a variety of markets including sustainable aviation fuels (SAF), chemicals and nutritional protein through process and Synthetic Biology innovations.

OPTIMIZING WINE FERMENTATIONS THROUGH INNOVATIVE APPROACHES: YEAST BIODIVERSITY, MULTI-OMICS ANALYSIS, AND MODEL-BASED METABOLIC FLUX DESIGN

Amparo Querol
IATA (CSIC)

Abstract

Although S. cerevisiae is the most widely used yeast in wine fermentation, other species of the Saccharomyces genus, such as S. uvarum or hybrids between S. cerevisiae and S. uvarum or S. kudriavzevii, have attracted special interest in the sector due to their potential to respond to the challenges posed by climate change. These species and their hybrids present clear metabolic differences, producing significantly higher amounts of glycerol, organic acids, 2,3-butanediol, and 2-phenylethanol, and reduced ethanol yield, which are very interesting in the sector. To understand the existing differences, we have used several omics techniques to analyze the dynamics of the (intra- and extracellular) metabolomes and/or transcriptomes of representative strains of S. cerevisiae, S. uvarum, S. kudriavzevii, and hybrids. These data indicate that the NADH/NADH+ cofactor regeneration differs among these species. We also observed that these species produce more erythritol, never described before as a by-product in S. cerevisiae. Using phylogenetic and genetic comparative approaches with Y. lipolytica erythrose reductases, we demonstrated that ΔGRE3 was the single mutant that decreased erythritol production. Related to the ethanol yield, by whole genome comparative analysis, we have detected an ADH2 allele specific to the wine strains, derived from an ADH1-ADH2 gene conversion. This allele results in a lower affinity for ethanol and a higher affinity for acetaldehyde and provides an advantage over other strains in wine fermentation.In recent years, we have also developed kinetic and genomic-scale metabolic models that, together with a flux balance analysis, have allowed us to quantify the fluxes through the carbon and nitrogen metabolism of batch-cultured yeasts. We have also developed digital twins (DTs), monitoring techniques, and predictive control to help winemakers achieve more environmentally responsible production. Finally, we will present examples of different applications to the industry.

Financial support and acknowledgments

PID2021-126380OB-C31 and PID2021-126380OB-C33, AGROALNEXT/2022/021, PLEC2021-007827; MCIN/AEI/10.13039/501100011033, as a 'Severo Ochoa' Center of Excellence (CEX2021-001189-S).

Keywords

wine fermentation • Saccharomyces species and hybrids • metabolic differences • multiomics analysis|digital twins • model-based optimization

METABOLOMICS AND BIOINFORMATICS AS DRIVERS OF FUTURE FOODS THROUGH THE ESTABLISHMENT OF ONE HEALTH SYSTEMS

Pascual García Pérez
Department of Food Technology, Nutrition and Food Science, Veterinary Faculty, University of Murcia, Regional Campus of International Excellence "Campus Mare Nostrum", Murcia, Spain

Abstract
Current agri-food systems face a bleak and ever-changing landscape due to the consequences of the global climate crisis. In response to this paradigm, significant efforts are being made in the field of biotechnology to provide efficient and sustainable solutions that positively impact the agri-food sector by establishing more resilient crops and designing safer food products. However, the increasingly diminished area of fertile soils and the growing need to feed a continuously expanding population limit the resources available for agricultural studies and urge the maximization of results. To overcome these limitations, throughout my research career, I have focused on studying plant biotechnological systems aimed at establishing the One Health paradigm, which seeks to ensure a healthy state at all agri-food levels, from environmental health to plant health and human health. Specifically, my experience has centered on the combined application of metabolomics and bioinformatics studies to investigate the establishment of One Health systems, as a solution that provides, on the one hand, a large-scale perspective of the phenotypic state of biological systems and how they respond to different stimuli, and on the other hand, generates integrative knowledge that maximizes the scope of agri-food research. As an example of this approach, this contribution includes a compendium of publications based on the multi-omic study of Cruciferous microgreens to illustrate the potential of this combined approach. The use of microgreens in these studies is motivated by multiple reasons: ease of cultivation in vertical farming systems, rapid growth, nutritional properties, and plasticity to adapt their metabolism under certain conditions. In short, the combination of biotechnological disciplines is presented as an effective tool to facilitate agri-food research and lay the foundations for productive models that respond to the One Health paradigm and ensure the food for the future.

Financial support and acknowledgments
Pascual García-Pérez thanks the financial support through the Ramón y Cajal program (reference: RYC2023-044123-I) by the Spanish Ministry of Science, Innovation and Universities, the National Research Agency (MCIU/AEI/10.13039/501100011033) and the European Social Fund Plus (FSE+); and the ATRACT-RYC program from the University of Murcia.

Keywords
Metabolomics • Chemometrics • Food Biotechnology • Plant Biotechnology • Microgreens

July 15-18 2025
Biotec 2025
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences.
Universitat Autònoma de Barcelona

PLENARY CONFERENCE

AI AND BIOTECHNOLOGY: CHALLENGES, OPPORTUNITIES IN EUROPE

Alfonso Valencia
BSC-CNS

Abstract

Artificial Intelligence (AI) is rapidly transforming all areas of science and technology, with biotechnology being one of the most directly impacted fields. A striking example is the development of AI-driven methods for protein structure prediction and protein engineering, which have had profound scientific and industrial repercussions—earning recognition with a Nobel Prize just a year ago. Indeed, it would have been difficult to foresee just a few years ago that major tech companies like Google or Meta would become key contributors to protein structure prediction research. While proteins represent one of the most advanced applications of AI in biology, the technology is now expanding to other domains, including genomics, epigenomics, single-cell and spatial transcriptomics, biological and medical imaging, as well as other areas of biomedicine. These advances are influencing preclinical and clinical studies, opening new frontiers in biotechnology and medicine. Despite the tremendous benefits of these rapid developments, there is a growing need for rigorous evaluation frameworks tailored to these emerging technologies—a field still in its infancy. Additionally, the success of AI in biotechnology depends on the availability of large-scale, accessible datasets and sufficient computational power. In today's global landscape, achieving independence (sovereignty) in these resources is crucial. Recent European initiatives, such as the establishment of the "AI factories" and the plans for the development of "Gigafactories", will play a pivotal role in supporting the development of biotechnology and ensuring technological autonomy.

In this talk, I will explore these transformative developments, addressing both the opportunities and challenges at the intersection of AI and biotechnology.

Keywords

IA • Biotechnology

July 15-18 2025
2025 Biotec
XIX Congress of the Spanish Society of Biotechnology
Faculty of Biosciences. Universitat Autònoma de Barcelona

SPONSORS

Protective Partners

ASE BIO Asociación Española de Bioempresas
ADM Biopolis Tailor-made Biotechnology

DIAMOND

ALLBIOTECH
Aplitech BIOLAB
BIOGEN Científica
bionet
Condalab
greiner BIO-ONE
Kuhner shaker
LEVPROT BIOSCIENCE The Yeast Company
werfen

PLATINUM

HIPRA
klinea Biotech & Pharma Engineering
Leanbio
REPSOL
SARTORIUS
Solmeglas
ThermoFisher SCIENTIFIC

GOLD

aizon
bioingenium
CAPSA FOOD
darwin Bioprospecting Excellence
evoenzyme customized biocatalysts
FRIT RAVICH
HAUSMANN
ecugen
vytrus biotech