Yeast in bioeconomy conference

Proceedings

1st International

Yeast in Bioeconomy Conference

(YBC2025)

October 22-24, 2025 • Compiègne, France

Chairs: Dr. Mohamed Koubaa and Dr. Elia Tomás Pejó

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Book of Abstracts

October 22-24, 2025 • Compiègne, France

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Dr. Elia Tomás Pejó

Biotechnological Processes Unit, IMDEA Energy, Avda. Ramón de la Sagra 3, 28935 Móstoles, Madrid, Spain.

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Welcome message

Dear colleagues and participants,

It is our great pleasure, as Chairs of the 1st International Yeast in Bioeconomy Conference, to warmly welcome you to our upcoming event from **October 22nd to 24th, 2025**. We are excited to present an outstanding program dedicated to the latest advances in **strain engineering, fermentation technology, bioprocess scale-up, and techno-economic assessment of bio-based compounds** —all essential for the valorization of industrial wastes and byproducts and the advancement of a global **Bioeconomy**.

Our three-day conference will feature **plenary lectures, oral presentations, and poster sessions**, bringing together leading researchers and industry experts from across the globe: Spain, France, Belgium, Italy, Germany, Turkey, Brazil, the UK, and more. This is a unique opportunity to **share knowledge, foster collaborations, and explore innovative solutions for sustainable bioeconomy applications**.

We are confident that the discussions and exchanges during the programme sessions will **inspire new ideas, collaborations, and strategies for advancing the field**. Please take full advantage of the opportunities to interact with fellow participants, ask questions, and engage in lively discussions.

On behalf of the organizing committee, we wish you an enjoyable and fruitful conference. We look forward to sharing three days of science, innovation, and networking with you.

Best regards,
Mohamed Koubaa & Elia Tomás Pejó
Chairs of the Yeast in Bioeconomy Conference 2025

Scientific Program

October 22-24, 2025 • Compiègne, France

Oral Presentations

October 22nd, 2025

📝 **10:00 AM - 01:30 PM: Registration**

🔔 **01:30 PM - 01:45 PM: Opening Ceremony**

Session 1 – Strain Engineering for Sustainable Bioeconomy Applications

(Session chairs: Verena Siewers 🇸🇪, Patrick Fickers 🇧🇪, Elvira Romero 🇪🇸, Jean-Marc Nicaud 🇫🇷)

🎤 **1:45 PM – 2:30 PM: Plenary Lecture**

- **1:45 PM – Rodrigo Ledesma-Amaro** (Imperial College London, UK): *Engineering biology in yeast cells and communities for a sustainable bioeconomy.*

🎤 **2:30 PM – 3:50 PM: Oral Presentations**

- **2:30 PM – Florence Bordes** (Toulouse Biotechnology Institute, France): *OP1. Easy and efficient tools for multiple gene integrations and deletions in the yeast Y. lipolytica.*

  2:50 PM – Marta López-Rubio (University of Salamanca, Spain): *OP2. Production of vitamin B2 in Ashbya gossypii through precision fermentation.*

  3:10 PM – Ewelina Celińska (Poznan University of Life Sciences, Poland): *OP3. Towards a "sustainable" source of proteins from Yarrowia lipolytica.*

  3:30 PM – Brigitte Gasser (University of Natural Resources and Life Sciences, BOKU, Austria): *OP4. Flo8 – A versatile regulator improving sustainable recombinant protein production in Komagataella phaffii.*

☕ **3:50 PM – 4:15 PM: Coffee break & Poster session**

🎤 **4:15 PM – 6:15 PM: Oral Presentations**

- **4:15 PM – Gennaro Agrimi** (University of Bari Aldo Moro, Italy): *OP5. Biocatalytic depolymerization and upcycling of plastics using engineered Yarrowia lipolytica.*
- **4:35 PM – Inge Van Bogaert** (Ghent University, Belgium): *OP6. Turning non-pathogenic yeasts into powerful long-chain dicarboxylic acid production hosts.*
- **4:55 PM – Putu Virgina Partha Devanthi** (Constructor University, Germany): *OP7. Can the re-introduction of a functional GUT1 allele (GUT1JL1) improve succinic acid production from glycerol in S. cerevisiae?*
- **5:15 PM – Hulya Karaca Atsaros** (Anadolu University, Turkey): *OP8. Metabolic engineering of Saccharomyces cerevisiae for enhanced taxadiene production.*
- **5:35 PM – Markus M. M. Bisschops** (Wageningen University & Research, the Netherlands): *OP9. General stress response & growth-uncoupled production: saving energy and increasing expression.*
- **5:55 PM – Bojan Žunar** (University of Zagreb, Faculty of Food Technology and Biotechnology, Croatia): *OP10. Engineering Debaryomyces hansenii for hypersaline surface display.*

October 23rd, 2025

Session 2 – Fermentation Technology and Bio-Based Compounds Production

(Session chairs: Brigitte Gasser, Marwen Moussa, Anissa Khelfa, José L. Martínez)

8:00 AM – 8:45 AM: Plenary Lecture

- **8:00 AM – Andreas Gombert** (University of Campinas, Brazil): *Microbial ecology and (meta)genomics for a better understanding and improvement of bioethanol production from sugarcane.*

8:45 AM – 10:05 AM: Oral Presentations

- **8:45 AM – Volkmar Passoth** (Swedish University of Agricultural Sciences, Sweden): *OP11. Oleaginous Rhodotorula spp. for biochemicals, food and a safe environment.*
- **9:05 AM – Boris Gilis** (University of Antwerp, Belgium): *OP12. Waste valorisation: A biochemical approach to long-chain dicarboxylic acid production.*
- **9:25 AM – Camille Botcazon** (ATV Technologies, France): *OP13. KOSMETOIL project: developing fermented natural oils for the cosmetic field.*
- **9:45 AM – Nabila Imatoukene** (URD ABI, AgroParisTech, France): *OP14. Development of a tailored culture medium for improved de novo biosynthesis of ferulic acid in fed-batch biphasic fermentation with Saccharomyces cerevisiae.*

10:05 AM – 10:35 AM: Coffee break & Poster session

10:35 AM – 12:35 PM: Oral Presentations

- **10:35 AM – Octavio García Depraect** (University of Valladolid, Spain): *OP15. Acidogenic fermentation as a platform for the sustainable production of organic acids for yeast applications.*
- **10:55 AM – Iris Cornet** (University of Antwerp, Belgium): *OP16. Rhodosporidium kratochvilovae, a promising yeast for the conversion of lignocellulosic inhibitors into microbial oil.*
- **11:15 AM – *Tomás Zubak*** *(IMDEA Energy Institute, Spain): OP17. Yarrowia lipolytica as a robust cell factory for the valorisation of high-salinity effluents via the carboxylate platform: case of microbial oil production.*
- **11:35 AM – José L. Martínez** (Technical University of Denmark, Denmark): *OP18. Evidencing the capability of Debaryomyces hansenii for the use of VFAs from organic digestates as feedstock for bioproduction.*
- **11:55 AM – Emmanuel Omachoko Anthony** (AgroParisTech, France): *OP19. Valorisation of acetic acid-rich forced endive root juice to produce volatile aroma compounds using Kluyveromyces marxianus.*
- **12:15 PM – Christian Kennes** (University of La Coruña, Spain): *OP20. Sustainable co-production of lipids and carotenoids by Rhodosporidium toruloides using carbon dioxide and acetate as alternative carbon sources.*

12:35 PM – 2:00 PM: Lunch break

October 23rd, 2025

Session 3 – Pretreatment, Downstream Processing, Bioreactor Design, and Process Scale-Up *(Session chairs: Valeria Mapelli 🇮🇹, David Moreno 🇪🇸, Ewelina Celińska 🇵🇱, Eugène Vorobiev 🇫🇷)*

🎤 **2:00 PM – 2:45 PM: Plenary Lecture**

- **2:00 PM – Elodie Vlaeminck** (Bio Base Europe Pilot Plant, Belgium): *Scaling yeast fermentation and downstream processing: Sustainable production of triacylglycerols with Y. lipolytica.*

🎤 **2:45 PM – 4:05 PM: Oral Presentations**

- **2:45 PM – Nicola Di Fidio** (University of Bari Aldo Moro, Italy): *OP21. Cardoon and wheat straw biorefinery for the production of lactic acid, oil and β-carotene by integrated thermo-physical and biotechnological approaches.*
- **3:05 PM – Sarah Mahfoud** (Université de Technologie de Compiègne, France): *OP22. Yeast for the future of food and feed: downstream processing for alternative protein recovery.*
- **3:25 PM – Margarita Smirnova** (Norwegian University of Life Sciences, Norway): *OP23. Valorization of lignocellulose side-streams containing HMF and furfural into oils for industrial use via oleaginous yeasts.*
- **3:45 PM – Elise Viau** (Toulouse Biotechnology Institute, France): *OP24. Exploring innovative bioprocess routes for ethyl acetate production by K. marxianus using lignocellulosic hydrolysate.*

☕ **4:05 PM – 4:30 PM: Coffee break & Poster session**

🎤 **4:30 PM – 6:30 PM: Oral Presentations**

- **4:30 PM – Alejandro Berzosa** (University of Zaragoza, Spain): *OP25. Cascade extraction of bioactive compounds from yeast biomass using pulsed electric fields.*
- **4:50 PM – Dana Byrtusova** (Norwegian University of Life Sciences, Norway): *OP26. Fed-batch optimization and scale-up of Rhodotorula and Schizochytrium sp. for bio-based epoxy coatings.*
- **5:10 PM – Oleksii Parniakov** (Elea Technology GmbH, Germany): *OP27. Influence of pulsed electric fields with other procedures on extraction from brewer's spent yeast cells.*
- **5:30 PM – Juan Manuel Martínez** (University of Zaragoza, Spain): *OP28. PEF-assisted extraction of carotenoids: Yield enhancement through autolysis and green solvents.*
- **5:50 PM – Mickaël Villeneuve** (Verrerie Dumas, France): *OP29. Automated bioreactor for process optimization: The BLEW® solution.*
- **6:10 PM – Mohamed Koubaa** (TIMR, ESCOM/UTC, France): *OP30. Sustainable scale-up production of odd-chain fatty acids by Y. lipolytica.*

🍽️ **8:00 PM – 11:30 PM: Gala dinner (optional): L'Hostellerie du Royallieu (9 Rue de Senlis, 60200 Compiègne, France)**

October 24th, 2025

Session 4 – Techno-Economic Analysis, Life Cycle Assessment, and Industrialization

(Session chairs: Elia Tomás Pejó 🇪🇸, Mohamed Koubaa 🇫🇷)

🎤 **8:15 AM – 9:00 AM: Plenary Lecture**

- **8:15 AM – Patrick Carré** (Terres Inovia, France): *Techno-economic analysis of microbial oil production: a case study on odd-chain fatty acids.*

🎤 **9:00 AM – 10:00 AM: Oral Presentations**

- **9:00 AM – Fernanda Thimoteo Azevedo Jorge** (Bio Base Europe Pilot Plant, Belgium): *OP31. Sustainable lactic acid fermentation at low pH using second-generation feedstocks: a techno-economic assessment.*
- **9:20 AM – Marco Vitale** (IMDEA Energy Institute, Spain): *OP32. Toward frameworks for performance evaluation of (yeast-based) sustainable aviation fuel production: gaps and opportunities in science for policy.*
- **9:40 AM – Isabella Pisano** (University of Bari Aldo Moro, Italy): *OP33. Cascading biorefinery of cheese whey permeate: integrated bioethanol and biomethane production through lab-scale validation and techno-economic process modeling.*

☕ **10:00 AM – 10:30 AM: Coffee break & Poster session**

🎤 **10:30 AM – 11:50 AM: Oral Presentations**

- **10:30 AM – Marwen Moussa** (AgroParisTech, France): *OP34. Fermentative production of 2-phenylethanol by yeasts using agro-industrial wastes: a techno-economic and environmental assessment.*
- **10:50 AM – Paola Branduardi** (University of Milano-Bicocca, Italy): *OP35. Production and carbon footprint of microbial oil from waste lemon peel extract.*
- **11:10 AM – Jean-Marc Nicaud** (INRAE, Micalis Institute, France): *OP36. The yeast Yarrowia lipolytica, workhorse chassis for industrial applications.*
- **11:30 AM – Guillaume Le Cloirec** (ATV Technologies, France): *OP37. Scaling-up continuous fermentation: accelerating industrial biomanufacturing with predictive modeling.*

🏆 **11:50 AM – 12:15 PM: Closing ceremony and awards**

🍽️ **12:15 PM – 1:30 PM: Lunch break**

🏭 **1:30 PM – 4:00 PM: Visit to ATV Technologies (optional): Parc technologique des rives de l'Oise, Rue les Rives de l'Oise, 60280 Venette, France.**
https://atv-technologies.com/en/home/

Detailed Abstracts

Oral Presentations

October 22-24, 2025 • Compiègne, France

Session 1 – Strain Engineering for Sustainable Bioeconomy Applications

Plenary Lecture 1.

Improving yeast strains for advanced biosynthetic pathways, optimizing byproduct and waste assimilation, and fostering sustainable production

Rodrigo Ledesma-Amaro[1,2,3,4*]

[1] *Imperial College Centre for Synthetic Biology, Imperial College London, London, United Kingdom of Great Britain and Northern Ireland.*
[2] *Department of Bioengineering and Imperial College Centre for Synthetic Biology, Imperial College London, London, UK.*
[3] *Bezos Centre for Sustainable Protein, Imperial College London, London, United Kingdom of Great Britain and Northern Ireland.*
[4] *Engineering Biology Mission Hub on Microbial Food, Imperial College London, London, United Kingdom of Great Britain and Northern Ireland.*

*** Corresponding author:** r.ledesma-amaro@imperial.ac.uk

Abstract

Traditional bioprocesses are usually carried out by a single microbial species, converting a feedstock into a product of interest. Yields are often limited by incomplete feedstock utilisation, byproduct formation, metabolic burden and inefficiencies in metabolic pathways. However, in nature, ecosystems are colonised by multiple species that cooperate to maximise resource utilisation and performance. Therefore, in the last few years, there has been a high interest in exploring the creation of synthetic microbial communities that can improve bioproduction.

While research in engineering microbial communities is in its infancy, we have now developed tools that allow us to design communities with defined behaviours, such as the capacity to divide labour either in production or feedstock utilisation or to control population ratios to optimise metabolic fluxes across multiple cells.

The latest advances in developing tools to create microbial communities include the development of a cross-feeding platform for improving cooperation [1], a toolbox for population control [2], the creation of communities specialised in using renewable feedstocks [3] or recycling fermentation byproducts [4], and the formation of cross-species communities [5]. These communities performed better than the monocultures in producing various products such as antioxidants, food ingredients, plastics precursors, chemicals, biofuels, etc. Future trends and challenges of the field will be discussed.

References

1. Aulakh, S.K. et al. (2023) Spontaneously established syntrophic yeast communities improve bioproduction. *Nat. Chem. Biol*. 19, 951–961.
2. Peng, H. et al. (2024) A molecular toolkit of cross-feeding strains for engineering synthetic yeast communities. *Nat. Microbiol*. 9, 848–863.
3. Chen, W. et al. (2024) Synthetic, marine, light-driven, autotroph-heterotroph co-culture system for sustainable β-caryophyllene production. *Bioresour. Technol*. 410, 131232.
4. Rafieenia, R. et al. (2025) Designing synthetic microbial communities with the capacity to upcycle fermentation byproducts to increase production yields. *Trends Biotechnol*. 43, 601–619.
5. Park, Y.-K. et al. (2024) Engineered cross-feeding creates inter- and intra-species synthetic yeast communities with enhanced bioproduction. *Nat. Commun*. 15, 8924.

OP1. Easy and efficient tools for multiple gene integrations and deletions in the yeast *Y. lipolytica*

Vinciane Borsenberger[1], Guo Zhongpeng[1], Christian Croux[1], Gilles Truan[1], Sophie Duquesne[1], Alain Marty[1], Cecile Neuveglise[2], Fayza Daboussi[1], Florence Bordes[1]*

[1] *TBI, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France.*
[2] *INRAE, AgroParisTech, Micalis Institute, UMR1319, Université Paris-Saclay, Domaine de Vilvert, 78350, Jouy-en-Josas.*

*** Corresponding author:** florence.bordes@insa-toulouse.fr

Keywords: *Yarrowia*, CRISPR_Cas9, artificial chromosome

Abstract

The efficient use of non-conventional yeasts as cell factories requires powerful genetic tools dedicated both to the deletion and to the expression of multiple genes. However, the multiple integration and gene deletions are often long and time-consuming. We developed some original tools to accelerate the engineering of the yeast *Y. lipolytica,* a recognized chassis for many biotechnological applications. We developed a simple and robust CRISPR/Cas9 multiplexing approach to knock-out multiple genes in one transformation [1], and we designed an artificial chromosome (ylAC) that enables the efficient construction of metabolic pathways in *Y. lipolytica* [2].

- CRISPR/Cas9 multiplexing approach [1]: We exemplified the potency of this approach by targeting two multiple gene families: the well-characterized acyl-CoA oxidase family (POX) and the uncharacterized SPS19 family. For this purpose, we established a simple and reliable method to construct plasmids able to express up to three sgRNA simultaneously in *Y. lipolytica*.
- Artificial chromosome (ylAC) [2]: This YlAC is composed of different modules: two telomeres, an autonomous replication sequence, fragments containing different selection markers and PCR-amplified expression cassettes. The full assembly of ylAC modules is achieved in one step by homologous recombination in *Y. lipolytica*. As a major result, we showed that various combinations of up to eight genes (23 kb) can be rapidly and easily assembled in vivo into a complete and linear supplementary chromosome with a success rate over 90%.

These tools are robust and easy to use and will be convenient to accelerate the development of industrial strains of the yeast *Y. lipolytica*

References

1. Borsenberger, V. et al. (2020) Developing Methods to Circumvent the Conundrum of Chromosomal Rearrangements Occurring in Multiplex Gene Edition. *ACS Synth. Biol.* 9, 2562–2575.
2. Guo, Z. et al. (2020) An artificial chromosome ylAC enables efficient assembly of multiple genes in *Yarrowia lipolytica* for biomanufacturing. *Commun. Biol.* 3, 199.

Acknowledgments

The authors thank Jean-Marc Nicaud for sharing genetic elements, and Toulouse White Biotechnology and ICEO (part of PICT and IBiSA) for screening access.
Funding: TWB, Région Occitanie/Pyrénées-Méditerranée, and FEDER 2014–2020 program.

OP2. Production of vitamin B2 in *Ashbya gossypii* through precision fermentation

Marta López-Rubio*, **Javier Martín-González, Javier F Montero-Bullón, Rubén M Buey, Alberto Jiménez**

Metabolic Engineering Group, Department of Microbiology and Genetics, Universidad de Salamanca, Campus Miguel de Unamuno, Salamanca 37007, Spain.

* **Corresponding author:** m_lopez@usal.es

Keywords: *Ashbya gossypii*, *Bacillus subtilis*, vitamin B2, metabolic engineering, precision fermentation

Abstract

Vitamin B2, also known as riboflavin, is an essential vitamin required to maintain normal metabolism for humans and animals, which must be obtained from the diet. The industrial production of riboflavin relies mainly on the fungus *Ashbya gossypii* and the bacterium *Bacillus subtilis* [1,2]. *A. gossypii* is a filamentous fungus that overproduces vitamin B2 naturally. Vitamin B2 biosynthesis is initiated from two main precursors, GTP and ribulose-5-phosphate, and it is controlled by the RIB genes (Fig. 1). GTP is produced through the purine pathway, and its availability is crucial for vitamin B2 production [2]. On the other hand, vitamin B2 biosynthesis in *B. subtilis* is controlled by the rib operon [1]. Substantial differences in the regulation of the purine and vitamin B2 pathways in *A. gossypii* and *B. subtilis* have been described [3]. Therefore, it is necessary to further investigate the regulatory mechanisms of these biosynthetic pathways to improve the industrial production of vitamin B2.

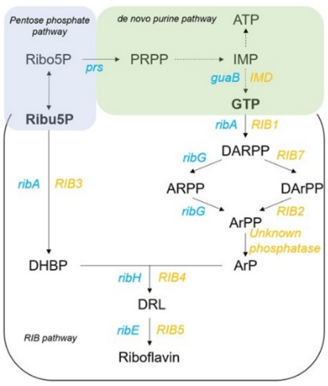

Figure 1. Riboflavin biosynthetic pathway. Dashed lines indicate multistep reactions. *A. gossypii* genes (orange), *B. subtilis* genes (blue). Ribo5P, ribose 5-phosphate; Ribu5P, ribulose 5-phosphate; PRPP, phosphoribosyl pyrophosphate; DARPP, 2,5-diamino-6-ribosyl-amino-4(3H)pyrimidinedione 5′-phosphate; ARPP, 5-amino-6-ribosyl-amino-2,4(1H,3H)pyrimidinedione 5′-phosphate; DArPP, 2,5-diamino-6-ribityl-amino-4(3H)pyrimidinedione 5′-phosphate; ArPP, 5-amino-6-ribityl-amino-2,4(1H,3H) pyrimidinedione 5′-phosphate; ArP, 5-amino-6-ribityl-amino-2,4(1H,3H)pyrimidinedione; DHBP, 3,4-dihydroxy-2-butanone-4-phosphate; DRL, 6,7-dimethyl-8-ribityllumazine.

A. gossypii strains (Table 1) were cultured at 28 °C, 200 rpm in MA2 rich medium. Preinocula were prepared from spores grown in MA2 for 48 h. The integrative cassettes used in this work were assembled using the Golden Gate method. The integrative cassettes comprised recombinogenic flanks targeting either the AFR171W, ABL211W or AGL034C loci, a loxP-KanMX-loxP, and the transcriptional units for guaB, prs and/or ribA from *B. subtilis*, using different promoters. The final integrative cassettes were isolated by SapI digestion and used for *A. gossypii* transformation. The transient expression of a Cre recombinase enabled the loxP-kanMX-loxP marker to be eliminated and reused. Vitamin B2 was extracted with 0.1 N HCl, heat-treated, bead-disrupted, centrifuged, and quantified by reading the absorbance at 445 nm. Biomass was measured as dry weight.

Table 1. *A. gossypii* strains used in this study.

Strain	Genotype	Source
WT	Wild type (ATCC 10895)	Our lab stock
A1501	abl211w:: P_{TSA1}-ribA(*B.subtilis*); P_{SEH1}-prs(*B.subtilis*); P_{SED1}-guaB(*B.subtilis*)	Our lab stock
A1660	afr171w::P_{SED1}-guaB(*B.subtilis*)-T_{PGK1}	This work
A1661	afr171w::P_{SEH1}-prs(*B.subtilis*)-T_{PGK1}	This work
A1662	afr171w::P_{TSA1}-ribA(*B.subtilis*)-T_{PGK1}	This work
A1673	afr171w::P_{SED1}-guaB(*B.subtilis*)-T_{PGK1}; abl211w::P_{TSA1}-ribA(*B.subtilis*)-T_{PGK1}	This work
A1674	afr171w::P_{SEH1}-prs(*B.subtilis*)-T_{PGK1}; abl211w::P_{SED1}-guaB(*B.subtilis*)-T_{PGK1}	This work
A1675	afr171w::P_{SEH1}-prs(*B.subtilis*)-T_{PGK1}; abl211w::P_{TSA1}-ribA(*B.subtilis*)-T_{PGK1}	This work
A1688	afr171w::P_{SEH1}-prs(*B.subtilis*)-T_{PGK1}; abl211w::P_{TSA1}-ribA(*B.subtilis*)-T_{PGK1}; agl034c:: P_{SED1}-guaB(*B.subtilis*)-T_{PGK1}	This work

Ribulose 5-phosphate (Ribu5P) was converted to 3,4-dihydroxy-2-butanone-4-phosphate (DHBP) through ribA expression, ribose 5-phosphate (Ribo5P) was converted to phosphoribosyl pyrophosphate (PRPP) through prs expression, guaB expression diverted IMP towards GTP. These modifications increased the pool of precursors (Fig. 1). The single expression of guaB, prs or ribA in *A. gossypii* showed that guaB and ribA induced the highest increases in riboflavin production. Whereas the expression of prs did not produce any significant change in the riboflavin yield. The double mutant strain which produced the highest levels of riboflavin expressed guaB and ribA. Two different strains expressing the three genes were analysed. A1501 constructed with a single integrative cassette was the one that produced the highest levels of vitamin B2, representing a 2.9-fold increase over the WT production.

We used metabolic engineering to combine the expression of guaB, prs and ribA from *B. subtilis* in *A. gossypii* resulting in the enhancement of the riboflavin precursors. The strain A1501 produced the highest levels of vitamin B2 when expressing guaB, prs and ribA simultaneously.

References

1. Liu, Y. et al. (2023) Metabolic Engineering of *Bacillus subtilis* for Riboflavin Production: A Review. *Microorganisms* 11, 164.
2. Ledesma-Amaro, R. et al. (2015) Metabolic engineering of riboflavin production in *Ashbya gossypii* through pathway optimization. *Microb. Cell Factories* 14, 163.
3. Buey, R.M. et al. (2022) The gateway to guanine nucleotides: Allosteric regulation of IMP dehydrogenases. *Protein Sci. Publ. Protein Soc*. 31, e4399.

Acknowledgments

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OP3. Towards a "sustainable" source of proteins from *Yarrowia lipolytica*

Ewelina Celińska*

Department of Biotechnology and Food Microbiology, Poznan University of Life Sciences, ul. Wojska Polskiego 48, 60-637 Poznań, Poland

*** Corresponding author:** ewelina.celinska@up.poznan.pl

Keywords: Protein, yeast, genetic engineering, bioprocess, stress factors

Abstract

Proteins are fascinating molecules synthesized by living organisms and for living organisms, where they elicit their specific functions (structural, catalytic, signaling, or transporting). Chemical peptide synthesis has made great progress in the field of solid-phase/flow-based procedures, yielding polymers over 100 AAs without any biological machinery. Yet, the bioeconomy is in great need of both bulk and fine polypeptides to be used as foods, drugs, and as industrial catalysts, at a capacity that chemical synthesis cannot meet. Moreover, a sustainable bioeconomy aims at delivering a product in a way that is both economically viable and environmentally responsible (with minimal waste, reduced emissions, supporting long-term ecological balance). At present, chemical synthesis cannot compete with biology in supplying biologically active proteins.

We are interested in producing proteins using *Yarrowia lipolytica* as a host [4, 12], following sustainable bioeconomy assumptions. Both process development and genetic engineering of the host strain are of interest to us. In this talk, I will present different approaches that we used towards making *Y. lipolytica* a competitive bioresource of recombinant proteins (rProts) for industry.

Our early focus was on a raw starch-digesting alpha-amylase (of use in SSF processes) from the rice weevil [1, 2, 3, 5], which later became our reporter protein. We studied the effects of thermal [7] and hyperosmolarity [6, 8] treatment on the rProt production. Based on experimental results, we modelled a pilot-scale bioprocess of the rProt production, and calculated the economic gain from running the process with optimized thermal treatment, in crude glycerol and technical substrates, in a nearly completely waste-free mode [7]. For the host bioengineering, we evaluated genome-dug native signal peptides for their secretory efficiency [3], ran steady-state cultures for comparative transcriptomics experiments [9, 10], which led to the identification of synthesis- and secretion-helpers, that were later used for enhancing rProt synthesis through their co-overexpression [11]. Recently, by high-throughput overexpression screens of transcription factors, we searched for non-specific 'facilitators' of the rProt synthesis [13, 14, 15].

While much has been done, global synthesis of collected data and many more turns of the design-build-learn-test cycle are required to onboard *Y. lipolytica* with biofoundries delivering rProts sustainably.

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OP4. Flo8 – A versatile regulator improving sustainable recombinant protein production in *Komagataella phaffii*

Corinna Rebnegger[1,2], Mirelle Flores-Villegas[2], Sonakshi De[1,2], Diethard Mattanovich[1,2], Brigitte Gasser[1,2]*

[1] *Austrian Centre of Industrial Biotechnology (ACIB), Vienna, Austria.*
[2] *BOKU University, Institute of Microbiology and Microbial Biotechnology, Department of Biotechnology and Food Science, Vienna, Austria.*

*** Corresponding author:** brigitte.gasser@boku.ac.at

Keywords: chassis strain, Flo8, cell engineering, improved productivity, methanol-free process

Abstract

Recombinant protein production is crucial for healthcare and a sustainable bio-based economy. This rapidly growing sector is driven by demand for biotherapeutics, industrial enzymes, and alternative food proteins. Yeast-based expression systems play a key role, including the methylotrophic yeast *Komagataella phaffii* (syn *Pichia pastoris*). Methanol-free production processes of *K. phaffii* are considered more sustainable, as they have significantly lower heat output, a lower energy demand for cooling and oxygen supply, and prevent the use of a toxic inducer. Flo8 is a key transcriptional regulator of flocculation and pseudohyphal growth in yeast. Disruption of *FLO8* in the popular recombinant protein production host *K. phaffii* prevents pseudohyphal growth and reduces cell-to-surface adherence [1], making the *flo8*Δ mutant valuable for research and industry. However, knowledge of the physiological impact of the mutation remained scarce.

Transcriptomic analysis of *FLO8*-deficient *K. phaffii* was carried out by RNAseq, and subsequent bioinformatics data analysis. Cloning was performed using the modular Golden Gate toolkit GoldenPiCS, established for modular cloning in *K. phaffii* [2]. Intracellular EGFP fluorescence was determined by flow cytometry, while secreted recombinant protein titers were determined by microcapillary electrophoresis of culture supernatants. Glucose-limited fed batch cultivations were carried out in multiparallel DasGiP bioreactors, using the model-based optimized feeding profile described in [3].

Transcriptomic analysis of *FLO8*-deficient *K. phaffii* revealed systemic changes. In addition to flocculation genes, Flo8 affects genes involved in cell cycle, mating, respiration, and catabolite repression. One notably upregulated gene in *flo8*Δ was *GTH1*, encoding a high-affinity glucose transporter in *K. phaffii*. Its promoter P_{GTH1} is established as a strong, glucose-regulatable alternative to methanol-induced promoters [3]. Thus, we tested the *flo8*Δ mutant in combination with P_{GTH1} and its improved derivatives (P_{G1X}; [4]) for recombinant protein production in small-scale screenings and bioreactor cultivations. Both P_{GTH1} and P_{G1X} demonstrated significantly elevated expression strength in *flo8*Δ, resulting in substantially enhanced recombinant protein titers. Consistently, secreted protein yields of several different secreted recombinant proteins were strongly enhanced (up to 8-fold higher) as well.

*K. phaffii flo8*Δ has many advantageous characteristics, such as reduced surface growth and increased productivity, making it an excellent chassis strain for recombinant protein production. Higher product titers of *K. phaffii flo8*Δ in combination with reduced energy demand and heat output strongly increase the sustainability of production processes.

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OP5. Biocatalytic depolymerization and upcycling of plastics using engineered *Yarrowia lipolytica*

Eugenia Messina[1], Antonino Biundo[2], Serena Barile[1], Pasquale Scarcia[3], Isabella Pisano[1], Zbigniew Lazar[4], Gennaro Agrimi[1]*

[1] *Laboratory of Microbial Biotechnology (M4B), Department of Biosciences, Biotechnologies and Environment, University of Bari Aldo Moro, Campus Universitario, via Orabona 4, 70125 Bari, Italy.*
[2] *REWOW srl, Via G. Matarrese 10, 70124, Bari, Italy.*
[3] *Department of Biosciences, Biotechnologies and Environment, University of Bari Aldo Moro, Campus Universitario, via Orabona 4, 70125 Bari, Italy.*
[4] *Department of Biotechnology and Food Microbiology, Faculty of Biotechnology and Food Science, Wrocław University of Environmental and Life Sciences, Chelmonskiego 37, 51-630 Wrocław, Poland.*

*** Corresponding author:** gennaro.agrimi@uniba.it

Keywords: Plastic upcycling, *Yarrowia lipolytica*, metabolic engineering, synthetic biology

Abstract

The growing accumulation of plastic waste has become a pressing environmental issue, largely driven by the extensive use and durability of synthetic polymers such as polyurethanes (PUs) and polyethylene terephthalate (PET). These materials are highly resistant to natural degradation, resulting in their persistent presence in ecosystems. Enzymatic depolymerization offers a sustainable and eco-friendly approach to mitigate this problem by converting plastics into reusable monomers that can be further upcycled into valuable products. This study focuses on establishing an effective enzymatic hydrolysis platform for PU breakdown using engineered microbial systems, while also investigating the bioconversion of ethylene glycol (EG)—a key monomer released from PET and PU—into glycolic acid (GA) and yeast biomass, thereby enhancing its valorization potential.

To enhance the enzymatic degradation of polyurethanes (PUs), we applied a synthetic biology approach to construct a modular plasmid library using Golden Gate assembly. This library combined four secretion signals, six polymer-binding modules, and four flexible linkers, all fused to a fungal cutinase. A total of 96 enzyme variants were generated and genomically integrated into *Yarrowia lipolytica*, selected for its robustness and secretion efficiency.

Enzymatic activity was screened on PU-based media, with halo formation indicating polymer breakdown. In parallel, we investigated the bioconversion of ethylene glycol (EG), a monomer released from PET and PU depolymerization, into glycolic acid (GA)—a high-value compound used in cosmetics, pharmaceuticals, and biodegradable materials. ^{13}C-labeling experiments and metabolic flux analysis revealed that acetate enhances EG assimilation via the glyoxylate cycle. Bioreactor conditions were optimized to improve GA production.

Screening of the 96 enzyme variants in *Y. lipolytica* revealed several highly active constructs capable of efficiently degrading PU, as evidenced by clear halo formation on Impranil-containing media. These top-performing variants demonstrated the effectiveness of combining secretion signals and polymer-binding modules to enhance enzymatic activity. Metabolic analysis using ^{13}C-labeled EG showed that co-feeding with acetate significantly improved EG assimilation via the glyoxylate cycle and glycine synthesis, leading to enhanced GA production. Under optimized bioreactor conditions, the engineered strain achieved a GA concentration: 48.41 ± 1.4 g/L; Molar yield: 73%; Productivity: 0.73 g/(L·h). These results highlight the potential of metabolic engineering and process optimization in converting plastic-derived monomers into valuable biochemicals.

This study presents an integrated approach to tackling plastic pollution by combining enzymatic depolymerization with microbial upcycling. It demonstrates that waste polymers can not only be broken down into their monomeric components but also transformed into high-value products through engineered microbial bioprocesses.

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OP6. Turning non-pathogenic yeasts into powerful long-chain dicarboxylic acid production hosts

Jungho Lee[1], Meriam Vandeputte[1], Kristien De Sitter[2], Iris Cornet[3], Inge Van Bogaert[1]*

[1] BioPort Group, Centre for Synthetic Biology, Department of Biotechnology, Ghent University, Ghent, Belgium.
[2] Separation and Conversion Technology, Flemish Institute for Technological Research (VITO), Mol, Belgium.
[3] Biochemical Wastewater Valorization and Engineering (BioWAVE), University of Antwerp, Wilrijk, Belgium.

*** Corresponding author:** inge.vanbogaert@ugent.be

Keywords: *Starmerella bombicola*, long chain dicarboxylic acid, vegetable oil, used cooking oil, fatty acid, lipid metabolism

Abstract

Long-chain dicarboxylic acids (LCDAs) harbouring 16 or 18 carbon atoms are important chemicals used in a wide range of industries, including pharmaceuticals, plastics, and lubricants. However, current production methods are inefficient and non-sustainable, relying on biotransformation strategies that involve expensive downstream processing and pathogenic production strains like *Candida tropicalis*, hampering their implementation in Europe. In order to mitigate these limitations, we propose to employ novel, non-pathogenic yeast strains for LCDA synthesis.

A phylogenetic screen was used to test an underexplored group of non-pathogenic yeasts for their LCDA-producing capacities from vegetable oils. The best strains derived from this screening are currently being turned into more efficient LCDA producers by, amongst others, engineering the lipid metabolism and blocking competing pathways.

Our best-performing yeast, a modified *Starmerella bombicola* strain, produces 5.5 g/l of total LCDA in shake flasks. Three deletions seem to be required for this optimal phenotype.

The fatty acid profile of the vegetable oil, which is enriched in oleic acid and other C18 and C16 fatty acids, could largely be retrieved in the LCDA chain length. Furthermore, pH optimization of the bioprocess in a bioreactor resulted in a significant improvement of the total LCDA titer up to 99.8 g/l in a fed-batch process [1]. We are currently also working on tailoring the fatty acid profile of our LCDAs towards longer chains by overexpression of endogenous or heterologous elongases, while still using C16 and C18-based vegetable oil inputs. Both topics are the subject of a recently filed patent application.

To conclude, by screening for novel strains and optimizing them for LCDA production, we open doors to produce high-grade LCDAs with lower environmental impact, and create a safer production process that allows for implementation in Europe.

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OP7. Can the re-introduction of a functional *GUT1* allele (*GUT1*$_{JL1}$) improve succinic acid production from glycerol in *S. cerevisiae*?

Putu Virgina Partha Devanthi, **Janine Sachs, Andreea Perpelea, Mathias Klein, Elke Nevoigt***

School of Science, Constructor University, Campus Ring 1, 28759 Bremen, Germany

*** Corresponding author:** enevoigt@constructor.university

Keywords: *Saccharomyces cerevisiae*, glycerol, glycerol kinase, succinic acid, reductive TCA cycle

Abstract

Glycerol, a by-product of biodiesel production, is an attractive fermentation feedstock due to its high degree of reduction. We previously improved glycerol utilization in *Saccharomyces cerevisiae* by optimizing the native glycerol-3-phosphate (G3P) pathway or introducing a synthetic dihydroxyacetone (DHA) pathway, which included deletion of *GUT1*, encoding glycerol kinase, to abolish the G3P route. Unlike the G3P pathway, the DHA pathway generates cytosolic NADH, supporting fermentative production of reduced compounds.

Recently, we constructed a strain co-expressing both G3P and DHA pathways by introducing the superior allele *GUT1*$_{JL1}$ into a *gut1*Δ DHA pathway background (CEN DHA$_{PW}$) [1]. This strain showed increased glycerol consumption and ethanol production, indicating an increased glycolytic flux. Here, we investigate the impact of introducing *GUT1*$_{JL1}$ in a strain isogenic to CEN DHA$_{PW}$ but engineered for succinic acid (SA) overproduction.

The SA-producing strain *PYC2*$_{oe}$ was derived from a *gut1*Δ background carrying the DHA pathway and equipped with a cytosolic rTCA module. To enhance SA yield from glycerol, *PYC2*, encoding pyruvate carboxylase, was overexpressed to increase carbon flux into the rTCA pathway [2]. *GUT1*$_{JL1}$ was integrated in strain *PYC2*$_{oe}$ at the *ble* locus (previously used for *GUT1* deletion) via CRISPR/Cas9, resulting in the strain *PYC2*$_{oe}$ + *GUT1*$_{JL1}$. This strain and the reference strain *PYC2*$_{oe}$ were analyzed in shake flask fermentations conducted at 30 °C and 200 rpm for 9 days using a synthetic medium containing 75.6 g/L glycerol as the sole carbon source, 2.27 g/L urea as the nitrogen source, and 30 g/L $CaCO_3$. Samples were collected every 24 h to monitor OD_{600}, pH, and concentrations of glycerol, succinic acid, malic acid, and ethanol via HPLC.

The introduction of *GUT1*$_{JL1}$ improved both glycerol utilization and SA production. The specific glycerol consumption rate increased from 0.21 to 0.30 g/g DCW h, and the maximum SA titer rose from 31.55 g/L to 36.46 g/L, although it was reached later (120 h vs 96 h in the parental strain). This improvement was accompanied by a reduction in growth rate, suggesting a shift in carbon allocation from biomass synthesis toward product formation. This shift is further supported by a 7% increase in SA yield, a 47% decrease in biomass yield, and a 91.26% increase in SA produced per gram of biomass. Overall, these findings indicate that *GUT1*$_{JL1}$ enhances the efficiency of glycerol-to-product conversion by directing more carbon toward SA biosynthesis at the expense of cell growth.

GUT1$_{JL1}$ enhanced succinic acid production, likely by increasing glycerol consumption and redirecting carbon flux toward product formation. However, this came with a trade-off in growth. A two-stage fermentation strategy, separating biomass accumulation from product synthesis, may offer a promising future approach to balance growth and production.

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OP8. Metabolic engineering of *Saccharomyces cerevisiae* for enhanced taxadiene production

Hulya Karaca[1]*, Handan Acelya Kapkaç[2]

[1] *Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Anadolu University, Eskisehir 26471, Turkey.*
[2] *Department of Biology, Faculty of Sciences, Eskisehir Technical University, Eskisehir 26471, Turkey.*

*** Corresponding author:** hulyakaraca@gmail.com

Keywords: Terpenes, taxol, *S. cerevisiae*, mevalonate pathway, metabolic engineering

Abstract

Taxol is a potent anticancer drug originally derived from *Taxus brevifolia*, but its natural extraction faces limitations due to low yield, high cost, and complex purification. Chemical synthesis is impractical due to its lengthy multi-step nature. Microbial production using engineered *Saccharomyces cerevisiae* offers a promising alternative, thanks to its compatibility with cytochrome P450 enzymes involved in taxol biosynthesis. The study focuses on enhancing taxadiene, the first committed precursor of taxol, by metabolically engineering the mevalonate (MVA) pathway. Two background strains were used: SCIGS22a and a newly constructed MVA strain with overexpressed MVA genes. By testing 32 different plasmid strain combinations involving key genes (tHMG1, ERG20, GGPPS, and TS), the aim was to identify optimal upstream–downstream balance for high-level taxadiene production.

Yeast strain SCIGS22a and a newly engineered MVA strain were used as the background chassis. MVA was constructed by chromosomally integrating six MVA pathway genes (ERG10, ERG13, ERG12, ERG8, ERG19, IDI) into SCIGS22a using dual-promoter cassettes. 16 episomal plasmids were designed to express combinations of tHMG1, ERG20, TS (taxadiene synthase), and GGPPS (geranylgeranyl pyrophosphate synthase), with variations including fusion proteins in both orientations. In total, 32 strains were generated by transforming SCIGS22a and MVA with these plasmids. Shake flask cultivations were performed in defined minimal media supplemented with dodecane. Taxadiene was extracted from the dodecane layer and quantified using GC–MS with β-caryophyllene as the internal standard. Colonies were verified by PCR, and constructs were confirmed by sequencing [1].

Taxadiene production varied significantly across constructs. Expression of TS and GGPPS alone led to moderate titers (109.7 mg/L in SCIGS22a; 2.9 mg/L in MVA). Fusion of TS-GGPPS improved titers to 277.8 mg/L in SCIGS22a and 7.9 mg/L in MVA. Adding tHMG1 or ERG20 alone did not consistently enhance titers. However, co-expression of tHMG1, ERG20, and TS-GGPPS fusions in the MVA background (HK12) achieved the highest yield—528.5 mg/L. ERG20-GGPPS and GGPPS-ERG20 fusions yielded up to 2.6× higher titers versus separate expression. Results highlight that proper fusion orientation and background strain pairing critically impact production. MVA background generally performed better with enzyme fusions, suggesting improved metabolic channeling. Minor changes in gene arrangement had substantial effects on final titers [1].

This study demonstrates that optimizing both upstream and downstream modules via combinatorial design significantly enhances taxadiene production in *S. cerevisiae*. The best performing strain reached 528.5 mg/L in shake flask, outperforming previously reported systems. Fusion strategies and balanced pathway flux were key factors [1].

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OP9. General stress response & growth-uncoupled production: Saving energy and increasing expression

Markus M.M. Bisschops*, **Nuran Temelli, Simon van den Akker, Willem H. Baris, Ruud A. Weusthuis**

Bioprocess Engineering, Wageningen University, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands

*** Corresponding author:** mark.bisschops@wur.nl

Keywords: energy requirements, slow growth, zero growth, recombinant protein, stress response, yeast

Abstract

To increase product yield and process economics, the production of specific products, like recombinant proteins and terpenes, should be uncoupled from growth. While this is more straightforward for catabolic products, e.g., ethanol, for metabolic energy-requiring products, this remains challenging. The first step is to achieve stable, virtually non-growing, but metabolically active cultures. For different yeasts, this has been realized using retentostats. In these nutrient-limited cultures, the majority of energy substrate (e.g., glucose) is used for the maintenance energy requirement (MER): energy to maintain cellular homeostasis and activity. Despite the absence of other environmental stresses, yeast cells also activate the general stress response (GSR) under these extremely slow growth conditions. In this work, we set out to find an evolutionary driver behind this GSR activation and explore if it can be used to express and secrete recombinant protein in *Saccharomyces cerevisiae*.

To investigate if the GSR offers an energetic advantage under energy-limited conditions, a strain with reduced GSR was constructed by deletion of the main activating transcription factors Msn2 and Msn4. Cells were exposed to hydrogen peroxide or 53 °C to determine if indeed stress resistance was reduced. Chemostat cultures at different dilution rates and fed-batch cultures were used to determine the effect on maintenance energy requirement. Fed-batch cultures had a constant feed-rate, which over time led to very low growth rates (~0.01 h^{-1}). To test if the GSR can be used to express heterologous genes at a high level in slow-to-non-growing yeast cells, the *HSP12* promoter (P_{HSP12}) was selected, and expression of a fluorescent protein driven by this P_{HSP12} was compared to the expression driven by the widely used, constitutive *TEF1* promoter P_{TEF1} under slow-growing conditions. The effect of both promoters on the secretion of a fluorescent protein was also tested by using the Ksh1 signal peptide.

Deletion of *MSN2* and *MSN4* resulted in reduced resistance against heat shock and H_2O_2. Both chemostat and fed-batch cultures showed that a reduction in GSR significantly increased maintenance energy requirements but did not impact theoretical maximum biomass yields or maximal growth rates. The GSR-promoter P_{HSP12} led to high intracellular fluorescent protein levels in slow-growing fed-batch cultures. The intracellular protein titer increased 15-fold within 2 days of fed-batch cultivation, whereas for the P_{TEF1}-driven protein titers remained virtually constant. The specific production rate (q_P) versus specific growth (μ) correlation furthermore predicts a higher productivity at near-zero growth rates. However, when the target protein was secreted, opposite results were obtained. The reference P_{TEF1} outperformed the P_{HSP12} promoter, leading to higher titers and an inverted q_P, μ correlation. Additional results obtained in even slower-growing, retentostat cultures will also be shared.

We show that an energetic advantage may explain an evolutionary advantage of GSR activation under slow, energy-limiting conditions in yeast. This conserved GSR can also be used to drive heterologous expression specifically in slow to non-growing yeast cells, thereby bringing efficient, growth-uncoupled production processes a step closer.

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OP10. Engineering *Debaryomyces hansenii* for hypersaline surface display

Bojan Žunar[1]*, Antonia Paić[1], Tea Martinić Cezar[1], Renata Teparić[1], Béatrice Vallée[2], Igor Stuparević[1]

[1] *University of Zagreb Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia.*
[2] *Centre de Biophysique Moléculaire, CNRS, UPR 4301, 45071, Orléans Cedex 2, France.*

*** Corresponding author:** bojan.zunar@pbf.unizg.hr

Keywords: *Debaryomyces hansenii*, surface display, NaCl

Abstract

Debaryomyces hansenii is a halo-, xero-, and osmotolerant oleaginous yeast capable of thriving in extreme environments, tolerating up to 4 M NaCl [1]. These unique properties make it an attractive candidate for non-sterile biotechnological applications, including processes that use seawater [2]. Despite its promise, *D. hansenii* lacks essential genetic tools for inducible expression, secretion, and covalent anchoring of proteins to its cell surface, which constrains its use as a microbial chassis in biotechnology.

Leveraging RNA-seq data to identify strong native promoters, we constructed a suite of expression vectors with both constitutive and inducible control. These vectors were employed to engineer *D. hansenii* for surface display of heterologous proteins. We evaluated covalent and non-covalent anchoring strategies by expressing various fusion constructs and assessed display efficiency under different salinity conditions, with a particular focus on the effect of 1 M NaCl supplementation.

The engineered strains demonstrated robust surface display capability, with clear evidence of both covalent and non-covalent anchoring of heterologous proteins to the cell wall. Notably, surface display was enhanced in media containing 1 M NaCl, consistent with the organism's halotolerance. As a proof of concept, we successfully displayed multiple biotechnologically relevant enzymes on the outer surface of *D. hansenii*, effectively converting its cell wall into a catalytically active biointerface.

This work establishes *D. hansenii* as the first osmotolerant yeast chassis engineered for hypersaline-compatible surface display. By expanding its genetic toolkit and demonstrating functional enzyme presentation on its cell surface, we lay the foundation for future applications of *D. hansenii* in non-sterile and high-salinity bioprocesses.

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Session 2 – Fermentation Technology and Bio-Based Compounds Production

Plenary Lecture 2.

Microbial ecology and (meta)genomics for a better understanding and improvement of bioethanol production from sugarcane

Andreas K. Gombert*

Department of Engineering and Food Technology, School of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil.

*** Corresponding author:** gombert@unicamp.br

Abstract

Fuel ethanol has been used in different countries, either as a standalone fuel or blended with gasoline, to propel light vehicles. In comparison to the use of gasoline, the use of ethanol results in up to 90% less greenhouse gas emissions, when produced from sugarcane feedstocks, as currently performed in Brazil. When combined with the electricity obtained from the burning of bagasse, sugarcane alone is responsible for ~16% of Brazil's energy matrix, which is more than the energy obtained using the country's hydroelectric structure. Besides ethanol and electricity, table sugar is a third product from this industry, underpinning its economic, social, and environmental importance.

Unlike most industrial bioprocesses, fuel ethanol production is carried out in non-aseptic setups, due to the low commercial value of the product. Although a single microbial species, namely *Saccharomyces cerevisiae*, is responsible for the conversion of sugars into ethanol, and in spite of several process strategies employed with the aim of maximizing the frequency of this species in the fermentation setup, it is inevitable that contaminating microorganisms also thrive in this environment. Companies make use of different chemicals to counteract (mainly bacterial) contaminants and their consequences, such as sulfuric acid, antibiotics, antimicrobials, dispersants, and antifoaming agents, among others, which adds significant costs to the process and jeopardizes its environmental sustainability.

In such an industrial process, in which the raw material represents ~70% of the final production costs, the yield (mass of ethanol produced divided by the mass of sugars consumed) is the most important parameter, which should be maximized. Increasing the ethanol yield means producing more ethanol from the same area of cultivated sugarcane. The yield has remained quite stable in Brazilian sugarcane biorefineries, reaching values between 85 and 90% of the theoretical maximum, in the last decades, indicating that learning-by-doing has exhausted its possibilities and that we need paradigm disruptions to increase this process parameter. Due to the large volumes of ethanol produced each year, even small increases in the yield can be significant in absolute terms. A better understanding of the microbiota involved in this process may form the basis for the necessary process improvements. For instance, once we know which bacterial species or strains inhabit the process, how their dynamics relate to process conditions, which mechanisms of interaction exist between these microorganisms and the yeast strains that are responsible for converting sugars into ethanol, and finally, how the general microbial dynamics responds to process perturbations, we might start to design strategies with the aim of e.g. eliminating known harmful bacterial contaminants or manipulating their relationship with the yeast cells that coinhabit the fermentation environment. Some recent works indicate that it is even possible to design a microbial community that leads to increased ethanol yields, at the same time protecting the microbiome from invasion by undesired microorganisms. Parallels can be made with e.g. the human gut microbiome.

In this talk, current work in our research group will be discussed, mainly around three different lines: 1) the use of (meta)genomics to unravel the microbial diversity encountered in different sugarcane

biorefineries and how this relates to process conditions; 2) the use of genomics to unravel the pangenome of industrial *S. cerevisiae* strains used for bioethanol production; 3) the perspective of a paradigm shift in industrial practice, with the possibility of starting each production season with the inoculation of a designed microbial community, instead of the current practice of inoculating only yeast strains.

OP11. Oleaginous *Rhodotorula* spp. for biochemicals, food and a safe environment

Volkmar Passoth*, **Sigurdur Davidsson, Johanna Blomqvist, Yashaswini N. Nagaraj, Bettina Müller**

Swedish University of Agricultural Sciences (SLU), Department of Molecular Sciences, Uppsala, Sweden

*** Corresponding author:** volkmar.passoth@slu.se

Keywords: *Rhodotorula*, oleaginous yeasts, microbial oil, carotenoids, fish feed

Abstract

Yeast oil produced from lignocellulose can serve as a sustainable alternative to vegetable oils (VO), whose production is frequently accompanied by monocultures, land use changes, or rainforest clearings. We aim to understand the physiology of lipid production by oleaginous yeasts, optimise the production, and establish novel applications of single-cell oil (SCO) and other compounds [1].

Fermentation methods and methods to analyse intracellular lipid content (based on FT-NIR and FT-IR) were developed [2,4]. Supercritical CO_2-extraction was established to analyse both lipids and carotenoid content [5,6]. As a basis to understand the behaviour of the different strains and species, we sequenced a variety of genomes of *Rhodotorula* species, using Illumina and Nanopore-sequencing [7,8].

Yeast oil was tested in feeding experiments of Arctic char (*Salvelinus alpinus*). Fish were fed a diet where vegetable oil was replaced by yeast oil. Fish growth and health, as well as content of heavy metals and organic pollutants were tested [9,10]. There was a high variability in the formation of lipids and other interesting compounds, e.g., carotenoids, even between very closely related oleaginous yeast strains [1,2]. Torularhodin was the major carotenoid in *R. toruloides* [6]. Most of the yeast chromosomes could be reconstructed. We identified 18-21 chromosomes and several potential circular structures. A high diversity was found; some strains of the same species showed a phylogenetic distance to each other as to those of other species [7,8]. SCO produced by oleaginous yeasts from lignocellulose could replace VO in aquaculture of arctic char. Heavy metals and POP in yeast biomass cultivated on lignocellulose (wheat straw) hydrolysate were below the limits of the regulations defined by the EU. Sensory analysis did not reveal any perceptible difference [9,10]. System analysis showed that VO replacement by SCO is sustainable: production of yeast oil required 10-38% less fossil energy input than the same amount of rapeseed oil [11].

Rhodotorula spp. holds an exceptional potential to produce SCO and other high-value compounds. There are already some examples of possible applications of this group of yeasts; however, taking into account the high diversity, the full biotechnological potential still needs to be discovered.

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OP12. Waste valorisation: A biochemical approach to long-chain dicarboxylic acid production

Boris Gilis[1]*, Muhammad Bilal[1], Kristien De Sitter[2], Inge Van Bogaert[3], Iris Cornet[1]

[1] *Biochemical Wastewater Valorisation and Engineering (BioWAVE), Faculty of applied engineering, University of Antwerp, Groenenborgerlaan 171 Wilrijk, Belgium.*
[2] *Separation and Conversion Technology, Flemish Institute for Technological Research (VITO), Boeretang 200 Mol, Belgium.*
[3] *Centre of Synthetic Biology (CSB), Faculty of Bioscience Engineering, Ghent University, Sint- Pietersnieuwstraat 25, Gent, Belgium.*

*** Corresponding author:** boris.gilis@uantwerpen.be

Keywords: *Candida tropicalis*, long-chain dicarboxylic acids, grease trap waste, bioconversion, biochemicals

Abstract

Grease trap waste (GTW), a lipid-rich byproduct of the food industry, is an underutilised waste stream that contains high levels of free fatty acids (FFAs), which limits its use in conventional applications such as biodiesel production [1]. This study explores the use of GTW as a renewable feedstock for the production of long-chain dicarboxylic acids (LCDAs), as shown in Figure 1, using *Candida tropicalis* ATCC 20962, a robust yeast capable of w-oxidation. This yeast has been modified to block the β-oxidation, thereby preventing it from using lipids as a carbon source for growth and directing lipid metabolism towards LCDAs production. Unlike traditional LCDA production, which often uses non-renewable substrates, the conversion of GTW using *C. tropicalis* offers a sustainable alternative. In addition to evaluating the feasibility of using this feedstock, we optimised the process on shake flask and bioreactor scale, focusing mainly on improving the glucose and GTW feed rates.

Shake flask and bioreactor experiments were conducted to investigate the potential for producing LCDA with GTW as a lipid feedstock, using the yeast *C. tropicalis* ATCC 20962, and to optimise the process. In the shake flask experiments, the glucose feed rate varied from 0.1 to 1.4 g/(L.h), and the initial GTW addition was varied from 20 to 50 g/L. DesignExpert was used to develop an experimental design and evaluate the results. Next, bioreactor experiments were performed, which enabled more precise control of parameters such as pH and dissolved oxygen. First, a method was developed to enable the continuous GTW addition, as this limits foaming and delays the accumulation of toxic components. Then, the optimum conditions were determined by varying parameters such as pH, glucose feed (0.1 - 0.75 g/(L.h) and GTW feed (0.5 - 1 g/(L.h). During the experiments, cell growth (gravimetrically), lipid accumulation (GPC), glucose levels (HPLC), and LCDA concentrations (GC-FID/GC-MS) were monitored.

For the shake flask experiments, 2-factor interaction and reduced-cubic models were obtained. This revealed that the optimal GTW addition was 20 g/L and that the optimal glucose feed rate was 0.38 g/(L.h). Higher glucose levels increased biomass while limiting LCDA production, and excessive GTW inhibits both cell growth and LCDA production. For the bioreactor experiments, a glucose and GTW feed rate of respectively 0.38 g/(L.h) and 0.5 g/(L.h) were further confirmed to be optimal. The applied conditions produced 34.7 g/L of LCDA, corresponding to a productivity of 0.42 g/(L.h) and a yield of 75.9%. Higher glucose feed rates resulted in more biomass and less LCDA production, while lower glucose feed rates resulted in both less biomass and LCDA production. The optimal GTW addition was the lowest value tested, as higher feed rates reduced both biomass and LCDA production. Although the literature suggests an alkaline pH, it had to be limited to 7 to limit foaming and reactor overflow [2].

This study demonstrates that *Candida tropicalis* ATCC 20962 can efficiently convert grease trap waste into long-chain dicarboxylic acids, reaching 34.7 g/L with a productivity of 0.42 g/(L.h). The valorisation of this lipid-rich waste stream offers a sustainable alternative to fossil feedstocks with strong potential for industrial applications.

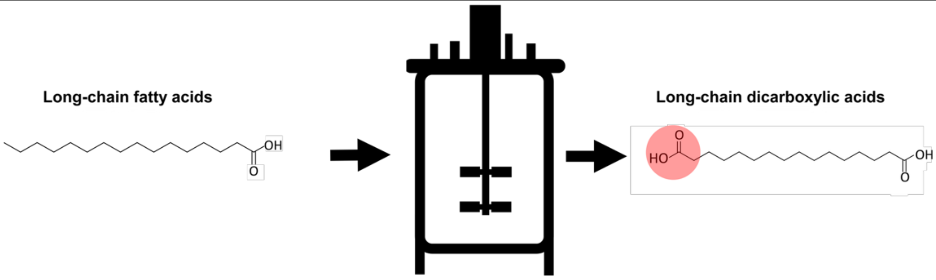

Figure 1. Schematic representation of the bioconversion of long-chain fatty acids (present in grease trap waste) into long-chain dicarboxylic acids. The process occurs in a bioreactor and involves the introduction of a second carboxylic acid group at the terminal end of the fatty acid carbon chain.

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OP13. KOSMETOIL project: developing fermented natural oils for the cosmetic field

Maud Sanchez, Sabine Defosse, Sophie Coorevits, Sabrina Soum, Camille Botcazon*, Jérôme Le Nôtre

ATV technologies, Parc Technologique des Rives de l'Oise, Rue les Rives de l'Oise, 60280, Venette, France.

*** Corresponding author:** camille.botcazon@atv-technologies.com

Keywords: Fermented oil, cosmetics, *Starmerella Bombicola*, *Pseudozyma antarctica*

Abstract

The global cosmetics sector, a major economic driver, demonstrated a 5.5% increase in 2018 with the skincare segment representing 39% of the market share, generating over €200 billion in global revenue. This continuous growth spurred an increasing demand for natural, personalized, sustainable cosmetic products and a need for the development of novel ingredients. Consumers are increasingly rejecting synthetic chemicals, favoring natural formulations in response to societal demands for cleaner, safer products. The rise of clean beauty movements, alongside regulatory efforts such as ISO 16128 for natural ingredient standards, reflects this shift. In this context, the use of fermented oils in cosmetics presents an innovative approach to meet these emerging demands. The KOSMETOIL project aims to address the challenge of controlling the functional properties of oils through fermentation, optimizing the interaction between the fermentation process and the resultant oil functionality.

This approach seeks to enhance the production of functional, natural ingredients for the cosmetics industry, utilizing local plant oils and specific yeasts, such as *Starmerella Bombicola* and *Pseudozyma antarctica*, which are allowed in cosmetic formulations. The project focuses on the fermentation of plant oils such as camellia oil, widely studied and commercially available, to produce a variety of bioactive compounds, including glycolipids, free fatty acids, mono- and diglycerides, and phospholipids, expected to be used as functional ingredients in skincare products. The project addresses challenges in downstream processing, particularly in the extraction and purification of complex mixtures. As such, analytical techniques such as HPLC, and GC are employed for the quantification and characterization of fermented oil components. Furthermore, innovative tools are currently being tested to improve the fermentation process, such as an online UV-fluorescence probe (BioIntelligence Technologies).

The originality of the KOSMETOIL project lies in its comprehensive approach to oil fermentation, which allows the preservation of complex mixtures of bioactive compounds in the final product. The optimization of fermentation conditions will enable the production of diverse mixtures that provide various functional properties depending on the ratio and quantity of each component. The project includes the development of robust methodologies to control the structure-function relationship of fermented oils, incorporating physicochemical characterization and efficiency testing. Furthermore, the project has explored the scaling-up of fermentation processes, from laboratory-scale to pre-industrial-scale production, with a focus on applying the method to local vegetable oils.

The KOSMETOIL project will bridge the gap between laboratory innovation and industrial application, with a sustainable, scalable, and functional approach to cosmetic ingredient production. This research will contribute to the development of innovative and sustainable cosmetic formulations, with potential applications in creams and skincare products.

OP14. Development of a tailored culture medium for improved de novo biosynthesis of ferulic acid in fed-batch biphasic fermentation with *Saccharomyces cerevisiae*

Blandine Godon[1], Julien Couvreur[1], Fanny Brunissen[1], Fanny Brunois[1], Clémentine Fojcik[2], Florent Allais[1], Michel Lopez[1], Nabila Imatoukene[1]*

[1] *URD Agro-Biotechnologies Industrielles (ABI), CEBB, AgroParisTech, 51110 Pomacle, France.*
[2] *Abolis Biotechnologies, Genopole Campus 1, 91030 Evry, France.*

*** Corresponding author:** nabila.imatoukene@agroparistech.fr

Keywords: Ferulic acid, medium optimization, *Saccharomyces cerevisiae*, biphasic fermentation

Abstract

Ferulic acid (FA) is a natural phenolic compound with diverse biological properties, widely used in the food and cosmetic industries. Its production from fermentation is a promising strategy because its extraction from biomass is costly. To enable cost-effective microbial production, medium optimization is mandatory. The objective of this study was to produce FA from D-glucose using an engineered *Saccharomyces cerevisiae* ABG20 strain, with the goal of developing a more economically viable medium and fermentation process. Given the hydrophobic nature of FA, which limits its accumulation in the aqueous phase, an appropriate bioprocessing approach was implemented, involving the cultivation of *S. cerevisiae* using both batch and fed-batch biphasic fermentation strategies. This work proposes a medium formulation for the heterologous production of FA from D-glucose and demonstrates its application in extractive fed-batch fermentation.

FA production was first assessed in a classically defined medium, 2X Yeast Nitrogen Base (YNB) without amino acids, and complex Yeast Peptone Dextrose (YEPD) medium. Finally, as FA has deleterious antimicrobial properties, continuous extraction from the broth using fed-batch biphasic fermentation was implemented.

Our results showed that YEPD medium resulted in the production of 207 $mg{\cdot}L^{-1}$ of FA in a medium composed of 30 $g{\cdot}L^{-1}$ D-glucose, 10 $g.L^{-1}$ yeast extract, 1 $g{\cdot}L^{-1}$ $(NH_4)_2SO_4$, 10 $g.L^{-1}$ peptone, 4 $g{\cdot}L^{-1}$ KH_2PO_4 and 2 $g{\cdot}L^{-1}$ K_2HPO_4. Fed-batch biphasic fermentation system resulted in almost a two-fold increase in FA production compared to batch one (312.6 $mg{\cdot}L^{-1}$ and 176.7 $mg{\cdot}L^{-1}$, respectively). Regarding the distribution of FA and its related intermediates, such as *p*-coumaric acid (*p*-CA) and caffeic acid, during batch and fed-batch fermentations, the acidic pH facilitated the migration of *p*-hydroxycinnamic acids (*p*-HCAs) predominantly into the organic phase, enabling effective detoxification of the broth and efficient recovery of *p*-HCAs. Increased solubility of *p*-HCAs in the fatty alcohol phase can be attributed to their protonation when the pH is lower than their respective pK1 values, which are 4.65, 4.62, and 4.56, for *p*-CA, caffeic acid, and FA, respectively.

Through optimization of the culture medium, continuous extraction of FA, and application of fed-batch processing, we achieved a production yield of 312.6 $mg{\cdot}L^{-1}$ of FA, a production that has not yet been reported in the literature.

OP15. Acidogenic fermentation as a platform for the sustainable production of organic acids for yeast applications

Octavio García-Depraect[1,2]*, Ana P. Chango-Cañola[3,4], Julián Carrillo-Reyes[3], Raúl Muñoz[1,2]

[1] *Institute of Sustainable Processes, Dr. Mergelina s/n, 47011 Valladolid, Spain.*
[2] *Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid, Dr. Mergelina, s/n, 47011 Valladolid, Spain.*
[3] *Instituto de Ingeniería, Unidad Académica Juriquilla, Universidad Nacional Autónoma de México, Blvd. Juriquilla 3001, Santiago de Querétaro, Querétaro 76230, Mexico.*
[4] *Institute of Sustainable Processes, Dr. Mergelina s/n, 47011 Valladolid, Spain.*

*** Corresponding author:** octavio.garcia@uva.es

Keywords: VFAs, lactate, acidogenesis, food waste

Abstract

Acidogenic fermentation is a key biological process for converting organic waste into valuable chemical intermediates within circular bioeconomy frameworks. Beyond its role in the initial stages of anaerobic digestion, this process enables the selective production of short-chain carboxylic acids such as acetate, butyrate, and lactate, which can serve as substrates for yeast-based bioprocesses and other microbial valorization routes. By focusing on the soluble phase of the fermentation, acidogenic fermentation offers a promising pathway to develop resource-efficient and integrated biorefineries. To the best of the authors' knowledge, this study is the first to examine how abiotic differences in real household food waste influence organic acid production during acidogenic fermentation.

Five different batches of real household food waste were used as substrates, each representing a distinct diet composition. A 2.5 L semi-continuous stirred reactor was operated under mesophilic conditions for 56 cycles to evaluate organic acid production via dark fermentation. The process used the indigenous microbiota present in the food waste as inoculum. No pH control was applied during operation. The system was run at a total solids content of 12% and a hydraulic retention time (HRT) of 1.1 days.

Dark fermentation of five distinct batches of real household food waste over 56 semi-continuous cycles yielded a consistent profile of carboxylic acids, despite differences in the substrates' physicochemical properties. Lactic acid was the predominant product across all conditions, with concentrations up to 40 gCOD/L, while acetic, propionic, butyric, and valeric acids were also present in lower amounts. Total acid production varied between batches, reflecting substrate composition, but the overall system remained stable without pH control. The resulting acid mixture, particularly its high lactic acid content, may be suitable for downstream valorization via yeast-based processes employing non-conventional strains.

Acidogenic fermentation of real household food waste with varying composition led to the consistent production of short-chain carboxylic acids, predominantly lactic acid. This approach shows potential for food waste valorization and may serve as a platform for integration into microbial bioprocesses, including yeast-based applications.

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OP16. *Rhodosporidium kratochvilovae*, a promising yeast for the conversion of lignocellulosic inhibitors into microbial oil

Iris Cornet*, **Waut Broos**

Research group BioWAVE, Faculty of Applied Engineering, Department of Biochemical and Chemical Engineering, Groenenborgerlaan 171, 2020 Antwerpen, Belgium.

*** Corresponding author:** iris.cornet@uantwerpen.be

Keywords: wastewater, valorisation, bioconversion, immobilised bioreactor, oleochemicals, substrate cost

Abstract

Oil is used in food, but it is also used to produce oleochemicals such as dicarboxylic acids for polymer production, biodiesel, surfactants, and many more. Microbial oil production has many advantages over plant oil, including the possibility to apply different substrates, a production independent of season, location, weather, and a minimal ecological footprint. The main disadvantage is the substrate cost, as current production uses synthetic sugars. This is why industrial production of microbial oil is currently limited to high-value applications such as functional foods, food additives, and cattle feed. For its application in biofuel and oleochemical production, the cost is too high. Increased use of inexpensive lignocellulosic biomass as a renewable resource leads to increased diluted lignocellulosic waste streams. Our research, therefore, focuses on developing a process for producing microbial oil from dilute lignocellulosic wastewater while cleaning it.

The applied catalyst was *Rhodothorula kratochvilovae* EXF7516, a yeast capable of metabolically degrading the aromatics and organic acids produced during the thermochemical pretreatment of lignocellulosic biomass and converting them into microbial oil [1]. To accumulate sufficient triglycerides inside the cells for economically viable extraction, the cells were repeatedly exposed to fresh, diluted wastewater. This was done by using a new bioreactor concept in which the cells were first immobilised on a porous polyurethane carrier, then contacted with the diluted waste stream, and finally remobilised for lipid extraction. The stability of *R. kratochvilovae* immobilisation was evaluated for different liquid and air flow rates. The recycle flow rate and aeration rate were set, and the pH and temperature were controlled at 30 °C and 5.5, respectively.

During the growth phase, the yeast extract, peptone, and dextrose (YPD) medium was susceptible to infection. However, preparing the YPD medium in lignocellulosic wastewater made unsterile fermentation possible. At a dissolved oxygen (DO) level of 70%, 84% of the monomeric compounds in the wastewater were removed, and it was used as the criterion to start a new cycle of fresh wastewater addition. It was also demonstrated that biofilm stability was greatly influenced by its age and the type of support. Five-day-old biofilms and porous supports reduced the remobilisation of the cells via airflow. After three cycles, an oil content of 24% CDW was obtained. 25% of the carbon consumed during the production phases was used to synthesise microbial oil. This was less efficient than when glucose was used, when up to 55% of the total carbon could be converted into microbial oil [2]. This is likely due to the additional energy required for metabolising aromatic inhibitory compounds.

Although desired yields were not obtained, the research proves that producing microbial oil by *R. kratochvilovae* in a reversibly immobilised cell reactor is technically feasible. Converting components in dilute wastewater into oil improves carbon efficiency and contributes to a circular economy.

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OP17. *Yarrowia lipolytica* as a robust cell factory for the valorisation of high-salinity effluents via the carboxylate platform: case of microbial oil production

Tomás Zubak*, **Elia Tomás-Pejó**

Biotechnological Processes Unit, IMDEA Energy, Avda. Ramón de la Sagra 3, 28935 Móstoles, Madrid, Spain.

*** Corresponding author:** tomas.zubak@imdea.org

Keywords: *Yarrowia lipolytica*, microbial oil, short-chain fatty acids, ham exudate, salt tolerance, bioreactor

Abstract

Yarrowia lipolytica is a promising cell factory with outstanding traits worth to explore. On one hand, *Y. lipolytica* can accumulate microbial oil (MO) from short-chain fatty acids (SCFA) produced via anaerobic fermentation of organic waste under high carbon-to-nitrogen (C/N) ratios. On the other hand, its striking behaviour under high-salinity conditions makes it a brilliant candidate for the valorisation of high-salinity waste streams that remain underutilized despite their potential and relevance for the water economy. This study evaluated MO production using exudate, a high-salinity effluent from ham processing, as culture media supplemented with a carbon source. The direct effects of different NaCl concentrations on yeast growth and bioproduction capacity were identified using both synthetic media and exudate to assess salinity tolerance, scale-up feasibility, and optimized conditions for MO production.

Y. lipolytica ACA DC 50109 was used for MO production. Synthetic media was prepared with yeast nitrogen base, $(NH_4)_2SO_4$ (q.s.f C/N of 4.5 and 100), and 15 g/L of a SCFA (7.22 HAc, 0.72 HPro, 4.60 HBut, 1.22 HVal, and 1.24 HCap) [1]. Exudate (900 mg N/L; 300 g NaCl/L) was diluted until the desired salt (15-120 g/L) and nitrogen (40-300 mg/L) contents, and supplemented with the same SCFA profile and concentration as synthetic media. Shake flask cultures (100 mL) were incubated at 27ºC, pH 6.8, and 150 rpm in an orbital shaker. Promising fermentation conditions were scaled up to a 1L bioreactor (Minifors 2, INFORS) where pH was kept at 6.8 with 2 M H_2SO_4, and dissolved O_2 at 35% with agitation (200-600 rpm) and aeration (0.5-2 L/min). Cultures were inoculated at OD_{600} = 1 and fermentations were finalised when ≥90% of the carbon source was consumed. SCFA were quantified by HPLC; and NaCl by IC. MO content was determined gravimetrically by solvent extraction, and lipid profile by GC-FID according to [2].

Y. lipolytica exhibited remarkable salt tolerance, being able to grow in both synthetic media and exudate with up to 60 g/L NaCl. In synthetic media (C/N 4.5), 15 g/L NaCl resulted in a 43% increase in MO content compared to the same media without NaCl, while higher NaCl concentrations were gradually detrimental. At C/N 100, MO production peaked at 30 g/L NaCl, with a MO content increase of 51% respect to the control. Exudate boosted MO production up to almost 3-fold when compared to standard synthetic media, reaching the highest MO yield with 30 g/L NaCl and a C/N of 100. This optimal condition determined in shake flask was successfully scaled up to bioreactor, where productivity increased 3.12-fold while maintaining similar MO yields. The C/N ratio and salt concentration in the optimized ham exudate condition matched those in the synthetic medium, suggesting a common optimal range for lipid production and pointing to *Y. lipolytica* as an efficient cell factory to valorise challenging residues.

These findings underscore the significant potential of *Y. lipolytica* for valorisation of traditionally underexplored wastes and highlight high-salinity effluents as a cost-effective alternative medium for economic and scalable MO production, offering significant potential for industrial applications and contributing to the fight against water scarcity.

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OP18. Evidencing the capability of *Debaryomyces hansenii* for the use of VFAs from organic digestates as feedstock for bioproduction

Mònica Estrada[1], Elia Tomás-Pejó[2], José Luis Martínez[1]*

[1] *Technical University of Denmark, Department of Biotechnology and Biomedicine, Søltofts Plads Building 223, 2800 Kgs. Lyngby, Denmark.*
[2] *Biotechnological Processes Unit, IMDEA Energy, Avda. Ramón de la Sagra 3, 28935 Móstoles, Madrid, Spain.*

*** Corresponding author:** jlmr@dtu.dk

Keywords: VFAs, fermentation, *Debaryomyces*, microalgae, green transition

Abstract

Volatile Fatty Acids (VFAs) have emerged as promising alternative carbon sources to replace sugars (such as glucose) for microbial production, which can help reduce production costs. VFAs can be sustainably produced through anaerobic fermentation (AF) of organic waste, revalorizing these side streams and thus contributing to the transition towards a circular bio-based economy [1]. Finding microorganisms able to tolerate and consume VFAs is a crucial need to achieve this goal. This study assesses, for the first time, the ability of the halotolerant and oleaginous non-conventional yeast *Debaryomyces hansenii* [2] to metabolize VFAs as the sole carbon and energy source, testing different total VFAs concentrations and acid profiles in synthetic media.

D. hansenii was able to efficiently grow with up to 15 g/L of total VFAs and metabolize all of them when acetic acid was the main acid present in the medium. An improved growth of the yeast was observed when using a real organic VFAs-rich digestate derived from the AF of microalgae biomass, compared to growth in synthetic media. In this case, a biomass yield of 0.43 g/g was achieved.

The capability of *D. hansenii* to produce and accumulate lipids from VFAs was assessed, showing that when the C:N ratio of the medium was increased to 200, accumulating up to 20.87% w/w of lipids, being oleic, palmitic, and linoleic acids the primary acids produced. Interestingly, a significant amount of the odd-chain fatty acid C17 was also detected.

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OP19. Valorisation of acetic acid-rich forced endive root juice to produce volatile aroma compounds using *Kluyveromyces marxianus*

Emmanuel Omachoko Anthony*, Ana-Karen Sánchez-Castañeda, Claire Saulou-Bérion, Marwen Moussa

Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood, 91120 Palaiseau, France

*** Corresponding author:** emmanuel.anthony@agroparistech.fr

Keywords: Agro-industrial residue, fermentation, aroma compounds, yeast, acetic acid

Abstract

The valorisation of agro-industrial residues is increasingly essential due to the huge volumes of agro-waste generated yearly [1,2]. Rising demand for natural products alongside strict regulations against non-biobased molecules in food, fragrance, and cosmetics [3] further supports biomass valorisation. Converting these wastes into valuable products often requires extraction and hydrolysis, processes that release fermentable sugars but also inhibitory compounds like acetic acid, which can be detrimental to *K. marxianus* at elevated concentrations [4]. Some yeast species can internalise extracellular acetic acid to synthesize acetyl-CoA, a key precursor for ester production [5]. In this study, we explore the bioconversion of acetic acid-rich forced endive root (FER) juice extracted via pulsed electric field (PEF)-assisted diffusion into aroma compounds using *K. marxianus*. Particular attention is given to the yeast's tolerance to acetic acid and its capacity to produce isoamyl acetate.

The FER juice used in the study is rich in sugars (12 g/L fructose, 2.9 g/L glucose and 2.7 g/L sucrose) and 11 g/L of acetic acid. Isoamyl alcohol (IAOH) was used as a precursor for isoamyl acetate (IAA) synthesis. All experiments were conducted at pH 5, 30 °C, and 180 rpm in a 250 mL baffled flask with 25 mL of FER juice inoculated with 7.6 $\times 10^5$ cells/mL of *K. marxianus*. Preculture was done in a semi-defined synthetic medium containing glucose, yeast extract, and minerals. Autoclaving was done for 30 min at 120 °C. Initial tests aimed to assess the yeast's ability to grow in the juice without supplementation, compared to a reconstituted medium with yeast extract and minerals in triplicate. Subsequent fermentations were performed in the FER juice with varying concentrations (1-4 g/L) of IAOH in duplicate. Cell growth and viability, sugar and acetic acid consumption were quantified by fluorescence flow cytometry (FFC) and HPLC, respectively, while volatile products were analysed by GC-FID.

Preliminary results showed that *K. marxianus* had a higher maximum growth rate and cell count in the FER juice than in the reconstituted medium (Table 1). Acetic acid did not impair the growth of the yeast. Its concentration decreased steadily, implying possible uptake by the yeast for acetyl-CoA synthesis. The resulting fruity smell confirms production of aroma compounds. This implies that the juice does not require supplementation or detoxification prior to valorisation using *K. marxianus*. Moreover, current results on the addition of isoamyl alcohol to the medium showed that *K. marxianus* can resist IAOH toxicity up to 2 g/L, as cell growth and viability are not impacted (Table 2). IAOH addition significantly improved IAA production compared to the control. It was also observed that the presence of IOAH in the medium enhances ethyl acetate production, probably due to enhanced alcohol acetyltransferase enzyme activity. Future studies will involve in-situ product recovery for enhanced yield.

K. marxianus is able to grow in acetic acid-rich medium with enhanced volatile aroma production in the presence of isoamyl alcohol. It is a promising candidate for valorising acetic acid-rich media and for the production of industrially relevant molecules of interest by fermentation.

Table 1. Growth parameters of *K. marxianus* in FER root juice and reconstituted media.

Media	Maximum cell count log(count/mL)	Maximal growth rate (μ_{max}) (h^{-1})	Residual Acetic acid (g/L)
FER juice	8.74 ± 0.02	0.76 ± 0.02	6.83 ± 0.08
Reconstituted medium	8.52 ± 0.04	0.61 ± 0.00	4.05 ± 0.46

Table 2. Growth parameters and fermentation performance of *K. marxianus* in the presence of acetic acid and isoamyl alcohol.

Condition	Maximum cell count log(count/mL)	Maximal growth rate (μ_{max}) (h^{-1})	Ethyl acetate (mg/L)	Isoamyl acetate (mg/L)	Cell Viability (%)
Control	8.80 ± 0.07	0.76 ± 0.02	392.1 ± 84.1	1.75 ± 0.50	97.0 ± 0.5
1 g/L IOAH	8.87 ± 0.00	0.80 ± 0.03	757.3 ± 89.2	173.8 ± 12.6	94.8 ± 3.9
2 g/L IAOH	8.70 ± 0.05	0.74 ± 0.02	520.1 ± 42.7	218.8 ± 58.9	96.7 ± 0.1

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OP20. Sustainable co-production of lipids and carotenoids by *Rhodosporidium toruloides* using carbon dioxide and acetate as alternative carbon sources

Cecilia Naveira Pazos, Maria C. Veiga, Christian Kennes*

Chemical Engineering Laboratory, Faculty of Sciences and Interdisciplinary Centre of Chemistry and Biology – Centro Interdisciplinar de Química y Biología (CICA), BIOENGIN group, University of La Coruña, E-15008-La Coruña, Spain.

*** Corresponding author:** c.kennes@udc.es

Keywords: oleaginous yeast, lipids, carotenoids, β-carotene, acetic acid

Abstract

The growing global energy demand continues to be met mainly by non-renewable fossil fuels, driving interest in more sustainable biofuels. Although vehicles based on conventional fuels continue to dominate transportation, biofuels are becoming attractive, especially if produced from waste or pollutants [1]. Biodiesel is commonly obtained by transesterification of animal fats or vegetable oils and has similar properties to conventional diesel.

The use of oleaginous yeasts such as *Rhodosporidium toruloides*, makes it possible to produce lipids (microbial oils) without competing with food crops. Using carbon sources such as carboxylic acids derived from pollutants to produce microbial oils can reduce costs. Some oleaginous yeasts may also produce carotenoids, antioxidant compounds that are increasingly in demand. This study evaluates for the first time the ability of *R. toruloides* to produce lipids and carotenoids from acetogenic acetic acid.

The strain *R. toruloides* DSM 4444 was used and grown in potato dextrose broth (PDB) at 33 °C and 150 rpm for 24-36 h. The inoculum was collected by centrifugation and then washed. For production, 2 L bioreactors were used with synthetic medium supplemented with either glucose or acetic acid (10 g/L), at pH 6, 30 °C, with shaking at 150 rpm and aeration of 2 vvm. To test in real conditions, acetic acid obtained by fermentation with *Acetobacterium woodii* and CO_2 as sole carbon source was used; the medium was adjusted to 10 g/L acetic acid and with a C/N ratio of 40. Growth was measured spectrophotometrically (OD_{600nm}) and substrate consumption by HPLC. Lipids were extracted from freeze-dried biomass, subjected to transmethylation, and quantified as FAMEs by gas chromatography. Carotenoids were extracted with organic solvents and determined spectrophotometrically at 448 nm as β-carotene equivalent.

Parallel fermentations were carried out using either glucose or acetic acid as sole carbon source, under equal conditions. In both cases, the yeast grew fast, reaching higher biomass build-up with glucose (OD_{600nm} = 71.9) than with acetic acid (OD_{600nm} = 43.5) (Figure 1), although the maximum substrate consumption was higher with acetic acid (0.53 g/L.h) than with glucose (0.35 g/L.h), indicating high efficiency of both substrates. During growth, *R. toruloides* accumulated up to 27.2% lipids with glucose and 22.3% with acetic acid, composed predominantly of oleic acid in the profile, similar to vegetable oils. As for carotenoids, their content was higher with acetic acid (1.4 mg/g) versus glucose (1 mg/g), indicating that this substrate favors their accumulation. In real acetogenic medium rich in acetic acid derived from CO_2, the yeast also managed to grow well, reaching 0.14 mg β-carotene/g biomass, though only 9.5 % lipids, confirming the potential for valorization of waste streams.

R. toruloides DSM 4444 efficiently utilized glucose and CO_2-derived acetic acid to produce lipids and carotenoids. Lipid content was similar with both substrates, but carotenoids were 40 % higher with acetic acid, supporting its use in sustainable bioprocesses.

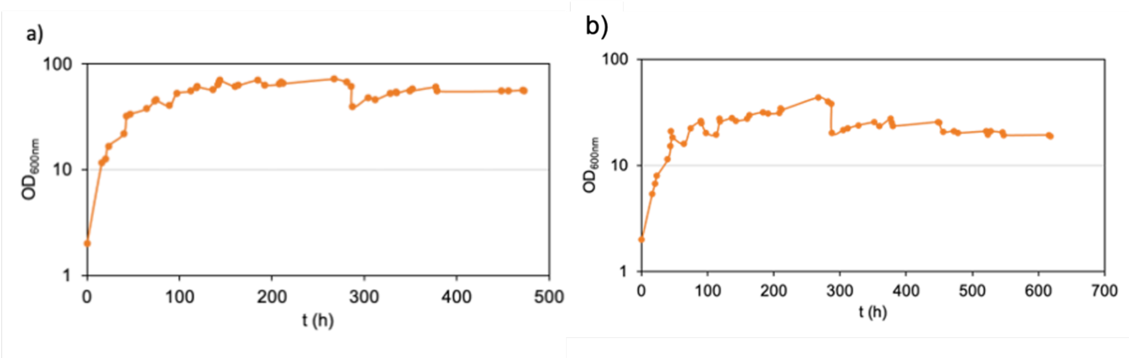

Figure 1. Growth of *R. toruloides* (log scale) in bioreactor with 10 g/L initial glucose (a) or acetic acid (b) (initial OD_{600nm} = 2)

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Session 3 – Pretreatment, Downstream Processing, Bioreactor Design, and Process Scale-Up

Plenary Lecture 3.

Scaling yeast fermentation and downstream processing: Sustainable production of triacylglycerols with *Yarrowia lipolytica*

Elodie Vlaeminck*

Bio Base Europe Pilot Plant (BBEPP), Rodenhuizekaai 1, Ghent, 9042, Belgium

*** Corresponding author:** elodie.vlaeminck@bbeu.org

Abstract

The oleaginous yeast *Yarrowia lipolytica* is widely recognized for its exceptional ability to accumulate triacylglycerols (TAGs) within intracellular lipid bodies. This microbial oil, chemically analogous to vegetable oil, is a valuable renewable product that can be used for diverse applications, including biofuels, chemicals, food, and cosmetics. Despite promising research published over the last decades, scaling microbial TAG production to a commercial, industrial-scale process remains challenging. It requires an integrated approach that ensures technical robustness, economic competitiveness, and environmental sustainability.

This lecture explores the critical factors that determine scalability, focusing on feedstock flexibility, fermentation process design, and downstream processing (DSP). Illustrative examples from laboratory and pilot-scale demonstrations at the Bio Base Europe Pilot Plant (BBEPP, Belgium) will be presented. These trials were conducted within several research projects, including BioSFerA [1] ("Biofuels production from syngas fermentation for aviation and maritime use"), CAPTUS [2] ("Liquid renewable energy carriers from captured carbon emissions"), FUELPHORIA [3] ("Advanced biofuels and renewable fuels from a sustainable value chain"), YAF [4] ("Yeast-based solutions for sustainable aviation fuels"), and HYBRID [5] ("Harnessing pyrolysis and biotechnology to recycle mixed plastic waste to dicarboxylic acids"). These have addressed key challenges such as the pre-treatment of heterogeneous feedstocks (including sugars, syngas, plastic waste streams, …), advanced bioprocessing control to achieve high-cell-density fermentations with high TAGs content, effective cell disruption to release the TAGs from the cells, and energy-efficient purification of the TAGs from the broth using scalable solvent extraction methods. Consequently, the pilot infrastructure at BBEPP enabled the validation of integrated process chains, demonstrating the microbial conversion of renewable feedstocks into high-purity TAGs at a kilogram scale. In addition, these efforts provide essential data for techno-economic analysis (TEA) and life cycle assessment (LCA) to correlate key process metrics (yield, titer, productivity) with cost structures and environmental performance, facilitating informed decisions for industrial deployment.

Ultimately, the successful scale-up of *Y. lipolytica* TAGs production depends on a synergetic combination of innovations across different levels, from strain engineering to fermentation development, process intensification, techno-economic and sustainable scale-up design, and integrated value chain assessments. In this context, pilot-scale operations play a pivotal role in bridging the gap between laboratory research and commercial application, elucidating technological uncertainties, and thereby accelerating the scale-up of yeast fermentation for industrial TAGs production.

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OP21. Cardoon and wheat straw biorefinery for the production of lactic acid, oil and *β*-carotene by integrated thermo-physical and biotechnological approaches

Nicola Di Fidio[1]*, Mattia Colacicco[1], Federico Liuzzi[2], Antonio Caporusso[2], Roberto Albergo[2], Gennaro Agrimi[1], Isabella De Bari[2], Isabella Pisano[1]

[1] *Department of Biosciences, Biotechnology and Environment, University of Bari "Aldo Moro", Via E. Orabona, 4, 70125, Bari, Italy.*
[2] *Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Laboratory for Processes and Technologies for Biorefineries and Green Chemistry, ENEA C.R. Trisaia S.S. 106 Jonica 75026 Rotondella (MT), Italy.*

*** Corresponding author:** nicola.difidio@uniba.it

Keywords: biorefinery, cardoon, wheat straw, lactic acid, oil, β-carotene, oleaginous yeasts

Abstract

Wheat straw and cardoon residues (agro-industrial by-products) offer environmental and economic advantages in comparison to edible crops [1]. Biorefineries fractionate and transform biomass into different bio-products, using low-impact technologies. Through pretreatment, fractionation, and enzymatic hydrolysis, biomass-derived monosaccharides can be converted into various high-value chemicals via fermentation, such as lactic acid (LA), oil, and carotenoids. LA, long used in the food, leather, cosmetic, and pharmaceutical industries, is a building block for chemical synthesis of the biopolymer polylactic acid [2], acetaldehyde, and acrylic acid. Yeast oils are third-generation raw materials for biofuels, bio-lubricants, food additives, pharmaceuticals, cosmetics, and oil-derived chemicals [3]. Yeast-derived carotenoids are lipophilic pigments with antioxidant properties, which find commercial applications in food, pharmaceutical, nutraceutical, cosmetic, and advanced materials sectors [3].

Cardoon was sourced from the Matrica biorefinery located in Porto Torres (Sardinia, Italy), while wheat straw was sourced from the Basilicata region (Italy). Both biomasses underwent steam explosion pretreatment using a 10 L batch reactor (Staketech) available at the ENEA Trisaia. The operational parameters adopted were previously optimised [4]. Enzymatic hydrolysis of the pretreated wheat straw and cardoon was performed in a 10 L stirred-tank bioreactor equipped with a helical impeller. The commercial enzyme blend Cellic CTec2 was applied (pH 4.8, 50 °C, 300 rpm, citrate buffer 0.05 M). Enzyme dosage, biomass loading, and process time were optimised for each biomass. *Lactobacillus brevis* ATCC 14869, *Lactobacillus plantarum* ATCC 14917, *Lactobacillus reuteri* ATCC 23272, *Trichosporon oleaginosus* DSM 11815, and *Rhodosporidium toruloides* DSM 70398 were used. Batch fermentations were performed in 250 mL Erlenmeyer flasks by using specific formulations of culture medium for each microorganism.

For the enzymatic hydrolysis of holocellulose, biomass loading (5-20 wt%), enzyme dosage (5-25 FPU/g glucan), and reaction time (0-96 h) were optimised. The optimal enzyme dosage was 15 FPU/g glucan. The maximum glucose yield (≥90.0 mol% for both biomasses) was obtained at a loading of 5 wt%, while the optimal compromise between glucose yield (>75 mol%) and sugars concentration (>70 g/L) was 15 wt%. For LA production, after a preliminary screening of *Lactobacillus* spp. on different MRS medium/hydrolysate (H) ratios (7/3, 3/7, and 0/1 v/v) and C/N ratio (6-38 g/g), *L. brevis* was the best producer on both the hydrolysates with LA yields ≥85% of the theoretical yield. For oil production, *Trichosporon oleaginosus* achieved an oil content >50 wt%, a cell production >15 g/L, and lipids production, productivity, and yield of >7 g/L, >75 mg L-1 h-1, and >15 wt%, respectively, on cardoon H. For *Rhodosporidium toruloides*, β-carotene and lipid productions were ≥150 mg/L and ≥3 g/L on cardoon H.

An innovative integrated biorefinery model was developed to convert cardoon residues and wheat straw to fermentable second-generation sugars (glucose, xylose), lactic acid, single-cell oil, β-carotene, and nitrogen-rich yeast biomass leftover. It was based on steam explosion, enzymatic saccharification, and fermentation with bacteria and yeasts.

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OP22. Yeast for the future of food and feed: downstream processing for alternative protein recovery

Sarah Mahfoud[1,2]*, Thomas Auxenfans[2], Nabil Grimi[1]

[1] *Centre de Recherche de Royallieu, École Supérieure de Chimie Organique et Minérale, TIMR (Integrated Transformations of Renewable Matter), Université de Technologie de Compiègne, CS 60319, 60203 Compiègne CEDEX, France.*
[2] *Lesaffre Institute of Technology, 59700 Marcq-en-Barœul, France.*

*** Corresponding author:** sarah.mahfoud@utc.fr

Keywords: yeast, extraction, alternative proteins, sustainable nutrition

Abstract

By 2050, the world population is expected to reach 9.7 billion people. In response to this demographic explosion, the demand for nutrient-rich protein sources is booming. Simultaneously, efforts to transition toward a circular economy have amplified the search for sustainable, alternative protein sources. Yeasts have thus emerged as a promising alternative to conventional animal and plant proteins. Growing interest in this microbial biomass is driven by its accessibility and its protein profile reported to be of low allergenicity, high digestibility, and high nutritional value. However, realizing the full potential of yeast proteins for food and feed applications relies on efficient downstream processing to release them while preserving their functional integrity. In this work, we investigate and compare various yeast protein recovery strategies, supported by a comprehensive characterization in terms of both quantity and quality, with relevance to human and animal nutrition.

Recovering intracellular yeast proteins requires their release through different cell disruption treatments. Parametric studies are carried out on mechanical treatments: high-pressure homogenization (500 to 900 bar, 1 to 9 passes), ultrasonication (200 to 400 W, 4 to 16 min treatment time), and bead milling (4 to 10 min treatment time). Non-mechanical treatments are also tested, namely pulsed electric fields and high-voltage electrical discharge. Moreover, different combinations of treatments are established. Process evaluation is based on the analysis of the cell disintegration index (Z), global solubilization, and protein yield. Its specificity and selectivity are assessed with respect to nitrogen solubilization, protein recovery and purity. Furthermore, process-induced proteolysis is evaluated via the determination of the free amino nitrogen/total nitrogen ratio.

Mechanical treatments, high-pressure homogenization, and ultrasonication in particular, allow maximal yeast cell disintegration and protein recovery. At the highest-intensity conditions of these two techniques, the Z index reaches more than 0.8 with a protein yield of around 80% and a nitrogen solubilization of over 70%. Yet, these techniques also result in a proteolysis degree that can exceed 50%. Bead milling and electrical treatments have overall shown to be less effective. Combination treatments are still being tuned as a way of potentially synchronizing the different advantages that the treatments have to offer.

These findings emphasize the critical role of fine-tuning operating parameters to tailor different processing strategies toward the cost-effective recovery of high-quality intracellular yeast proteins for sustainable food and feed applications.

Acknowledgments

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OP23. Valorization of lignocellulose side-streams containing 5-(hydroxymethyl)furfural (HMF) and furfural into oils for industrial use via oleaginous yeasts

Margarita Smirnova[1]*, Boris Zimmermann[1], Mihaela Tanse-Opedal[2], Francesca Di Bartolomeo[3], Susan Maleki[3], Silje Malene Olsen[3], Gunn Broli[3], Dag Helge Hermundsgård[4], Rune Brusletto[4], Volha Shapaval[1]

[1] *Norwegian University of Life Sciences, Realtek, Drøbakveien 31, 1433 Ås, Norway*
[2] *RISE PFI, Høgskoleringen 6b, 7491, Trondheim, Norway*
[3] *SINTEF Industry, Richard Birkelands vei 3, 7034 Trondheim, Norway*
[4] *Arbaflame, Henrik Ibsens gate 90, 0255, Oslo, Norway*

*** Corresponding author:** margarita.smirnova@nmbu.no

Keywords: Oleaginous yeasts, lignocellulosic byproducts, HMF, Furfural

Abstract

Biorefinery processes converting C-rich feedstocks such as lignocellulosic biomass and starch residues often generate side-streams with HMF and furfural [1]. These compounds are formed when high temperatures and strong acids are used. Furthermore, they can be washed from the biomass and found in the washing filtrates. The presence of inhibitors in the washing filtrates makes their reuse more complex and energy-intensive. HMF and furfural are known inhibitors of microbial growth and can significantly reduce the efficiency of metabolic pathways [2], thereby limiting the feasibility of microbial fermentation using such side-streams. Oleaginous yeasts are able to produce single-cell oils from various side streams [3]. In this study, we screened a set of oleaginous yeasts to assess their tolerance, growth, and lipid accumulation using HMF- and furfural-containing side streams. The goal of this study was to identify strains suitable for converting HMF-furfural-rich side streams into microbial oils.

In this study, 8 oleaginous yeast strains from the genera *Yarrowia*, *Cystofilobasidium*, *Rhodotorula*, *Sporidiobolus*, *Phaffia*, and *Debaryomyces* were used. The steam-exploded biomass from Arbaflame was sent to RISE PFI for the washing trials [4]. 4 HMF-furfural containing side streams with different concentrations of HMF and furfural were prepared and used in cultivation trials. Cultivations were performed on three media: control media without inhibitors, Arbaflame washing filtrates media containing carbohydrates and different loadings of HMF and furfural, and synthetic media with added HMF and furfural at different concentrations. Cultivations were done using high-throughput screening systems such as the Duetz-MTPs (Enzyscreen, Netherlands) and BioLector I (Beckman Coulter, USA). The following analysis was performed after the cultivation: estimation of biomass production, total lipid content, fatty acid profile, substrate consumption, and HMF and furfural bioconversion.

Strains of *Y. lipolytica*, *C. infirmominiatum*, *D. hansenii,* and *R. graminis* can tolerate various loading of HMF and furfural without a significant decrease in lipid-accumulation. HPLC results showed that all sugar and acid components of Arbaflame side streams were consumed, and HMF and furfural were transformed into less toxic derivatives. These strains were selected for further screening on 11 synthetic media with different concentrations of HMF and furfural and combinations. It was observed that *C. infirmominiatum* and *D. hansenii* can tolerate HMF (up to 3 g/L) and combinations. *Y. lipolytica* and *R. graminis* can tolerate up to 3 g/L of HMF, while could not tolerate furfural and combinations of both. Total lipid content of *R. graminis* grown in media with 1-2 g/L of HMF was higher compared to the control. Total lipid content of *C. infirmominiatum* grown in the presence of 1-2 g/L HMF and furfural was similar to that of control media.

C. infirmominiatum and *D. hansenii* showed to be tolerant to HMF, furfural, and combinations and were able to transform them into less toxic derivatives and produce lipid-rich biomass. Total lipid content of *R. graminis* was higher in Arbaflame media than in control. This strain was less tolerant to furfural and combinations of HMF and furfural.

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OP24. Exploring innovative bioprocess routes for ethyl acetate production by *K. marxianus* using lignocellulosic hydrolysate

Elise Viau*, Alicia Huesca, Carine Bideaux, Sandrine Alfenore

TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France.

*** Corresponding author:** viau@insa-toulouse.fr

Keywords: Ethyl acetate, lignocellulose microbial valorization, microbial reaction

Abstract

Research on bio-based chemical production is accelerating. Regarding ethyl acetate (EA), while demand will reach 6.86 Mt by 2032 [1], this solvent remains fossil-derived [2]. Microbial production by the yeast *Kluyveromyces marxianus* [3], which can utilize whey (0.368 g_{EA}/g_S [4]) or lignocellulose, offers a promising alternative. While EA production from whey under iron limitation is well-documented [4], lignocellulosic routes remain underexplored, with most studies focused on ethanol [5-7]. Although EA synthesis from cassava bagasse or pomaces has been reported, yields (784 μg_{EA}/kg) or titers (640 μg_{EA}/L) remain low [8-11], highlighting knowledge gaps in glucose and/or xylose metabolism [12-15]. No study has examined xylose/glucose-based EA production by this species under iron-limited conditions. As part of the New Eco-Routes project (ANR-21-CE43-0012-01), this study investigates *K. marxianus* under iron limitation across pure/mixed substrates, aiming to optimize EA production from lignocellulose.

K. marxianus CBS 600 pre-cultures were grown on YPD agar and liquid medium before transfer to mineral medium. Metabolites were quantified in liquid/gas phases during stirred tank experiments to establish kinetics. Temperature was kept at 30 °C, and pH was controlled at 5 via NH_3 (14% v/v). Oxygen limitation was avoided by adjusting both the stirring rate and airflow. Vitamins and trace elements (except iron) were added every 5 g/L of biomass. Biomass was quantified by optical density and cell dry weight, while sugars and metabolites were analyzed via HPLC-UV-RI and GC-FID. Iron limitation was chosen as the EA production trigger. Carbon and oxidoreductive balances were calculated on raw data. Production/consumption rates were obtained and reconciled to recover elemental balances as described previously [16]. Strain behavior and kinetics were compared across glucose/xylose ratios under iron limitation, refining our understanding of *K. marxianus* metabolism in EA synthesis.

Using glucose, xylose, or their combination as substrates in batch mode, under various iron initial values, the maximum EA concentration was 3 g/L in the liquid phase on glucose, versus 1.3 g/L without iron limitation. With mixed substrates, it closed to 2 g/L and results suggest ethanol influenced EA production on glucose/xylose, as noted on whey [13]. Xylose and glucose were not co-consumed due to glucose repression, which seems strain-dependent [3, 17-19]. Literature links pyruvate accumulation to iron-dependent enzyme disruption in the TCA cycle, with EA forming as an overflow mechanism [20-22]. Pyruvate was detected in iron-limited conditions, unlike in non-limited one. Oxygen limitation was manually induced in specific culture conditions, triggering ethanol and EA, though less effectively than iron limitation, as observed in another strain [12]. Based on these findings, batch and fed-batch cultivations helped to investigate the impact of substrate fluxes (glucose/xylose) on EA production.

We explored EA production by *K. marxianus* under lignocellulosic hydrolysate-like conditions, using iron limitation as a trigger. While promising, targeting EA-xylitol production could enhance economic profitability. The ethanol produced post-limitation warrants further study under ethanol-favoring conditions to assess its impact on EA production.

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OP25. Cascade extraction of bioactive compounds from yeast biomass using pulsed electric fields: A tool for the circular bioeconomy

Alejandro Berzosa*, **Javier Marín-Sánchez, Juan Manuel Martínez, Ignacio Álvarez, Cristina Sánchez-Gimeno, Javier Raso**

Food Technology, Facultad de Veterinaria, Instituto Agroalimentario de Aragón-IA2, (Universidad de Zaragoza-CITA), Zaragoza, Spain

*** Corresponding author:** aberzosa@unizar.es

Keywords: Cascade extraction, pulsed electric fields, glutathione, proteins, mannoproteins

Abstract

The recovery of valuable compounds from yeast biomass—particularly when sourced as a byproduct from fermentation industries—offers a strategic opportunity for circular bioeconomy integration. Compounds such as glutathione, amino acids, proteins, mannoproteins, and β-glucans are widely used in food, cosmetics, and biotechnology for their antioxidant and functional properties. However, their extraction remains challenging due to the robustness of yeast cell envelopes. Conventional methods based on complete cell disruption often yield complex extracts and require intensive downstream processing. Pulsed Electric Fields (PEF) have emerged as a promising non-thermal technology capable of selectively permeabilizing cytoplasmic membranes. Additionally, PEF treatments can accelerate yeast autolytic activity, further contributing to compound release. This approach may offer a more controlled and sustainable alternative for facilitating compound recovery with minimal environmental impact.

Yeast biomass, obtained from both laboratory-grown cultures and brewing fermentation byproducts (La Zaragozana, Zaragoza, Spain), was treated with PEF at varying electric field strengths (10-20 kV/cm) and specific energy inputs (10-207 kJ/kg). The objective was to identify treatment conditions capable of inducing electroporation of the cytoplasmic membrane in more than 90% of the yeast population. Following PEF application, incubation parameters—including temperature, pH, and duration—were optimized to enhance the release of intracellular and structural compounds. Resulting extracts were collected and characterized in terms of their chemical composition [1,2].

Electroporation of the cytoplasmic membrane induced by PEF enabled the rapid release of intracellular compounds. The extraction profile was dependent on the molecular weight of the molecules. The first extract, collected immediately after PEF treatment, was enriched in low-molecular-weight compounds such as glutathione and peptides below 3 kDa, and demonstrated antioxidant activity in grape must, allowing a reduction in sulfite content. A second extract, obtained by incubating the same biomass under optimized temperature and pH conditions, exhibited a higher concentration of soluble proteins and free amino acids and proved to be an effective nitrogen source for microbial growth, comparable to commercial supplements. The autolytic processes also promoted partial degradation of the cell wall, leading to the solubilization of structural polysaccharides, particularly mannoproteins. The final insoluble fraction was composed mainly of residual cell wall components, predominantly β-glucans.

PEF-assisted treatment enables a controlled, sequential extraction of intracellular and structural compounds from the same yeast biomass. This cascade approach, combined with the valorization of fermentation byproducts such as spent brewer's yeast, supports the development of sustainable bioprocesses within the bioeconomy framework.

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OP26. Fed-Batch optimization and scale-up of *Rhodotorula toruloides* and *Schizochytrium* sp oils production for bio-based epoxy coatings

Dana Byrtusova[1]*, Margarita Smirnova[1], Ondrej Slany[1], Boris Zimmermann[1], Leszek Michalak[2], Trine Muren[2], Francesca di Bartolomeo[2], Volha Shapaval[1]

[1] *Norwegian University of Life Sciences, Realtek, Drøbakveien 31, 1433 Ås, Norway*
[2] *SINTEF Industry, Richard Birkelands vei 3, 7034 Trondheim, Norway*

*** Corresponding author:** dana.byrtusova@nmbu.no

Keywords: Fermentation, scaleup, *Rhodotorula toruloides*, *Schizochytrium* sp., lipids, extraction

Abstract

Microbial oils are emerging as sustainable alternatives to petrochemical-derived raw materials in applications such as epoxy coatings. In this context, the NordiCoats project investigates the potential of microbial lipids produced by the oleaginous yeast *Rhodotorula toruloides* and the marine thraustochytrid *Schizochytrium* sp. [1] These organisms were selected due to their high capacity for lipid biosynthesis, including polyunsaturated fatty acids (PUFAs), which offer favourable properties for polymer formulations. Importantly, both species have already shown a potential for industrialization, with established cultivation protocols. *R. toruloides* is known for its robust growth on diverse substrates and high lipid accumulation, while *Schizochytrium* is a proven producer of long-chain PUFAs such as DHA [2,3]. This study aims to evaluate the scalability of these strains in fed-batch fermentation production mode to obtain biomass with high lipid content.

Rhodotorula toruloides and *Schizochytrium* sp. were used to test fed-batch strategies for lipid-rich biomass. Five strategies included: (1) high C/N, C+N co-feed; (2) medium C/N, C-only feed; (3) medium C/N, C+N co-feed; (4) medium C/N, C-only pulse feed every 24 h; (5) low C/N, C-only feed. Both strains were scaled up according to the best fed-batch strategy in a 100-L Techfors bioreactor with 6 L inoculum and 54 L low C/N medium. FTIR spectroscopy was used to monitor substrate consumption. Cells were harvested using an industrial centrifuge: *R. toruloides* (3,200×g, 5 min, 15 °C, washed with water), *Schizochytrium* sp. (8,000×g, 15 min, 4 °C, saline wash). Supernatants/biomass were stored at −20 °C, and the biomass was freeze-dried. Lipid content/fatty acids were analysed via transesterification and GC [4]; glucose via D-Glucose Kit (Megazyme). Lipid extraction was developed to be scalable; different solvents and solvent ratios were tested.

In this work, a fed-batch fermentation strategy was optimized in 3 L bioreactors using glucose and organic nitrogen sources as substrates. Low CN feeding strategy resulted in the highest biomass production in *R. toruloides* (over 50 g/L of biomass, 258 h of cultivation), the same as for *Schizochytrium* sp., where 32 g/L of biomass was produced in only 118 h. The most effective approach was then scaled up to a 100 L pilot-scale bioreactor. The process resulted in a biomass concentration of 35 g/L, with lipids constituting 51% of the dry cell weight for *R. toruloides*. Following biomass harvest, lipids were extracted and used for epoxy resin synthesis. From 4.5 kg of freeze-dried yeast biomass containing 1.56 kg of total lipids, 678 g of oil was recovered, corresponding to a 43% extraction yield.

This study reports the best fed-batch strategy for high biomass and lipid production by *R. toruloides* and *Schizochytrium* sp. and highlights the scalability of microbial oil production and extraction processes, reinforcing the potential of *R. toruloides* and *Schizochytrium* sp. as a renewable feedstock for environmentally friendly epoxy coatings.

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OP27. Influence of Pulsed Electric Fields in combination with other procedures on the extraction of valuable compounds from brewer's spent yeast cells

Sofie Schröder[1], Jan-Michel Schulte[1,2], Corinna Stühmeier-Niehe[1], Claudia Siemer[1], Oleksii Parniakov[1]*

[1] *Elea Technology GmbH, Niedersachsenstr. 5, 49610 Quackenbrück, Germany.*
[2] *Hochschule Osnabrück, Albrechtstraße 30, 49074 Osnabrück, Germany.*

* **Corresponding author:** o.parniakov@elea-technology.com

Keywords: Yeast, pulsed electric field, extraction

Abstract

Brewer's spent yeast (BSY) is one of the main and most nutritious by-products in the production of beer. 2 to 4 kg of BSY are produced per 100 L of beer, which is why the processing into nutrient-rich yeast extracts promotes environmental protection and waste management. To access the valuable components, the cells need to be disintegrated. A promising innovative option here is pulsed electric fields (PEF). The exposure of the cells to the electric field increases the transmembrane potential, leading to pore formation in the cell membrane. If the voltage and energy input are high enough, this process leads to electroporation of the cells, which in turn enables the extraction of inner cell components. In this study, cell disruption and extraction were investigated using PEF alone as well as in combination with other procedures.

The PEF treatment was carried out with an Elea PEFPilot™. A field strength of $E = 15$ kV/cm and a specific energy of $W = 90$ kJ/kg were set. This was followed by homogenization using an APV 1000 at 50, 150, 200, or 800 bar to investigate the combination of PEF and homogenization. Finally, the combination with an incubation time after treatment was investigated at 30 °C or 60 °C for 1 or 2 hours. To analyze the extract, all samples were centrifuged at 4700 rpm for 15 min. Various analyses, like serum released, cell disintegration index, soluble extract content, and protein content, were carried out to investigate the disintegration of the yeast cells and the extraction of valuable compounds after the processes.

As expected, the control showed the lowest solids content in the supernatant. Furthermore, PEF alone leads to a clearly visible increase. However, the combination of PEF with other methods, such as homogenization or an incubation time, leads to a further increase in the soluble extract content. Homogenization and incubation time alone do not have the same positive effect as PEF alone. It was also evident here that PEF led to an increase in protein yield. Together with the other methods, this yield could be further increased to up to 40%. In particular, an incubation time at 60 °C had a significant influence on the extraction of the proteins, but such a temperature also leads to denaturation and is therefore not suitable for extracting functional proteins.

In conclusion, PEF leads to an opening of the cells and a higher increase in soluble extract content than homogenizer or incubation time alone. By combining the processes, even more solids can be extracted from the cells. Finally, PEF can increase the protein yield in the extract, but a combination with other methods leads to an even higher yield.

OP28. Pulsed electric field-assisted extraction of carotenoids: Enhancing yield through enzymatic autolysis and green solvents in yeast biomass

Juan Manuel Martinez*, **Alejandro Berzosa, Javier Raso**

Departamento de Producción Animal y Ciencia de Los Alimentos, Tecnología de Los Alimentos, Facultad de Veterinaria, Instituto Agroalimentario de Aragón (IA2), Universidad de Zaragoza, 50009 Zaragoza, Spain.

*** Corresponding author:** j.martinez@unizar.es

Keywords: Pulsed Electric Fields (PEF), pigments, carotenoids, extraction, eutectic solvents, autolysis

Abstract

Carotenoids like astaxanthin and β-carotene are high-value pigments with antioxidant properties and growing demand as natural additives. Yeasts such as *Rhodotorula glutinis* and *Xanthophyllomyces dendrorhous* are promising microbial sources but require efficient extraction methods due to the intracellular and hydrophobic nature of these compounds. Pulsed electric fields (PEF) are a non-thermal technology that applies short, high-voltage pulses (typically microseconds) to microbial suspensions, inducing electroporation of the cell membrane. This increases membrane permeability without causing complete cell lysis, allowing selective release of intracellular compounds. This presentation explores PEF-assisted extraction, emphasizing PEF-induced autolysis and the use of hydrophobic eutectic solvents (hESs) as green alternatives for improved carotenoid recovery from yeast biomass.

Yeasts were cultured in PDB medium and treated with PEF under varied intensities (10-25 kV/cm, 3-135 µs). For *R. glutinis*, fresh biomass was extracted post-incubation in buffer using ethanol. For *X. dendrorhous*, carotenoid recovery from PEF-treated biomass was compared with and without incubation, using both ethanol and terpene-based hESs. Enzymatic activities (esterase and β-glucanase) were quantified post-treatment. Carotenoids were analyzed via UV-vis and HPLC. Extraction parameters (solvent composition, temperature, and time) were optimized using response surface methodology for hES extractions.

PEF treatments induced >90% irreversible membrane permeabilization, creating conditions that promoted enzymatic autolysis [1]. Beyond electroporation, PEF triggered enzymatic activity during incubation, facilitating cell wall degradation and carotenoid release, [2]. Post-treatment incubation proved essential to enhance yields, likely due to the activation of endogenous hydrolases, including esterases, which cleave carotenoid–lipid complexes. Using hydrophobic eutectic solvents (hESs) like thymol/salol further improved extraction efficiency, reaching 79% [3]. Astaxanthin (AST) retained strong antioxidant capacity in hESs (IC_{50} = 0.02 µg/mL), with a synergistic effect observed between thymol and AST. These results support the hypothesis that PEF not only enhances permeability but also initiates enzymatic processes crucial for efficient and sustainable carotenoid recovery from yeast.

PEF enhances carotenoid extraction by inducing membrane permeabilization and enzymatic autolysis. In *R. glutinis*, it facilitates ethanol extraction; in *X. dendrorhous*, combining PEF with incubation and hESs boosts AST yield. This green, scalable method supports circular bioeconomy and industrial use.

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OP29. Automated bioreactor for the optimization of your processes: The BLEW® solution

Mickaël Villeneuve*

Verrerie Dumas, 872 Route de la Gare 37210 NOIZAY, France.

*** Corresponding author:** mvilleneuve@verrerie-dumas.fr

Keywords: bioreactor, fermenter, cell culture, scalable equipment, control software, repeatability, automation

Abstract

Verrerie Dumas has been manufacturing glass components since 1948, from one-off pieces to small and medium series, for various sectors such as agri-food, research, healthcare, petrochemicals, and design. The company stands out for its artisanal excellence, strong CSR commitment, and innovative engineering office, offering tailor-made solutions.

Verrerie Dumas has combined its expertise in engineering—mechanical, electronic, and automation—with glassblowing craftsmanship to deliver equipment tailored to the needs of research laboratories and industries involved in cell culture. Drawing on our experience and know-how in the development of pilot reactors, the new BLEW® range of bioreactor fermenters (Figure 1) offers precise and automated real-time control of key parameters through an intuitive interface.

Adaptable and scalable, BLEW® bioreactors are available in volumes from 1 to 10 liters and offer broad possibilities for upgrades, ensuring reliable and long-lasting equipment. BLEW® bioreactor fermenters provide an optimal environment for cell development through the regulation of pH, temperature, nutrients, and oxygen. They feature a double-walled borosilicate glass reactor, ensuring visibility and homogeneity during heating and cooling processes, along with equipment that enables precise control of all parameters. Fully equipped, the system includes an agitation motor, feed pumps, temperature control, level and pH probes, as well as sparging, sampling, and draining systems. The bioreactors allow the integration of additional sensors for optimal configuration. To facilitate process monitoring and optimization, our bioreactors are also supplied with a SCADA-type control software. This solution includes data centralization, secure backup, parameter monitoring and control via a synoptic interface, as well as error and alarm tracking. It also features a recipe mode, promoting repeatability of operations and process automation.

This equipment supports a wide range of applications: biomass production, strain optimization, culture media screening, fermentation under controlled conditions, and more. Parameters such as temperature, pH, agitation, and dissolved oxygen (DO) levels can be monitored in real time via the interface, with all data centralized and securely stored. This full traceability of medium characteristics, operator actions, executed recipes, and all alarms and errors enables in-depth analysis and continuous process improvement. It proves especially valuable in sustainable bioproduction, where reproducibility and the fine-tuning of culture conditions are key challenges.

The BLEW® range offers a reliable and scalable solution for optimizing fermentation processes. With its precision, traceability, and durability, it is a key tool for yeast valorization in the development of sustainable bioproduction.

Figure 1. Bioreactor fermenter BLEW

OP30. Sustainable scale-up production of odd-chain fatty acids by the oleaginous yeast *Yarrowia lipolytica*

Mohamed KOUBAA[1]*, Jean-Marc NICAUD[2,3]

[1] *Université de Technologie de Compiègne, Ecole Supérieure de Chimie Organique et Minérale, TIMR, Centre de Recherche Royallieu-CS60319, 60203 Compiègne CEDEX, France.*
[2] *Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas, France.*
[3] *Yalicolor, 20 avenue Hoche, 78190 Trappes, France.*

*** Corresponding author:** m.koubaa@escom.fr

Keywords: Odd-chain fatty acids, *Yarrowia lipolytica*, scale-up fermentation, agro-industrial by-products, metabolic engineering

Abstract

Yarrowia lipolytica is a model oleaginous yeast able to produce tailor-made lipids for industrial applications. Among them, odd-chain fatty acids (OCFAs) such as *cis*-9-heptadecenoic acid (C17:1) are gaining attention due to their anti-inflammatory, antimicrobial, and potential biofuel properties [1,2]. Yet, the high cost of substrates hampers microbial OCFA production at scale. This study aims to reduce production costs by engineering *Y. lipolytica* for OCFA synthesis from low-cost carbon sources such as sugar beet molasses and crude glycerol, and to demonstrate the scalability of the process from lab to pilot scale.

The *Y. lipolytica* strain JMY9178 was engineered by overexpressing genes involved in propionyl-CoA biosynthesis and fatty acid desaturation, including OLE1, to enhance OCFA production. Cultivations were performed in both batch and fed-batch modes using a medium based on sugar beet molasses and crude glycerol. Fermentations were carried out at the 5 L scale and successfully scaled up to 3,500 L in stirred-tank bioreactors. Lipids were extracted via a wet route following cell disruption. Fatty acid methyl ester (FAME) profiles were analyzed by gas chromatography, and total lipid content was quantified. Growth kinetics and OCFA production were monitored across all scales.

The engineered strain produced highly similar OCFA profiles at both 5 L and 3,500 L scales, confirming the robustness of the process. OCFA accounted for 68.08% and 68.71% of total fatty acids at 5 L and 3,500 L, respectively. C17:1 was the predominant fatty acid produced by *Y. lipolytica*, representing 48.88% at 5 L and 45.68% at 3,500 L. These values highlight the strain's strong metabolic orientation toward OCFA lipid production. Microscopy at the end of fermentation confirmed intracellular lipid accumulation through visible oil bodies. Similar growth kinetics and fatty acid distribution across scales demonstrated a successful and reproducible scale-up.

Y. lipolytica JMY9178 enables cost-effective and scalable OCFA production, maintaining high yields at pilot scale. The integration of low-cost substrates and metabolic engineering, including OLE1 overexpression, highlights its potential as an industrial lipid platform.

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Session 4 – Techno-Economic Analysis, Life Cycle Assessment, and Industrialization

Plenary Lecture 4.

Techno-economic analysis of microbial oil production: a case study on odd-chain fatty acids

Patrick Carré*

Terres Inovia, France.

*** Corresponding author:** p.carre@terresinovia.fr

Abstract

Microbial oils obtained from oleaginous yeasts hold strong promise for the specialty fatty acid sector. The YaLiOL project evaluated the techno-economic feasibility of producing odd-chain fatty acids (OCFA) via engineered *Yarrowia lipolytica* . The process was designed at pilot-industrial scale with a 150 m^3 fed-batch fermenter running cycles of about 170 hours, followed by recovery operations including centrifugation, high-pressure homogenization, solvent extraction, and stripping. Production was planned for an annual output of approximately 21.8 tons of OCFA-rich oil, but with a modest productivity of 3.3 g/L and a low substrate carbon conversion: 12.5 carbons from substrate were needed for each carbon in oil, considerably less efficient than reported in recent fermentation benchmarks.

A detailed cost analysis shows that substrate cost is the major factor, reaching 26.1 € per kg of oil, with beet molasses alone contributing 5.3 € per kg, sodium acetate at 5.0 € per kg, yeast extract at 4.2 € per kg, sodium propionate at 2.9 € per kg, and glycerol at 2.8 € per kg. Costs for pH correction agents account for 5.3 € per kg, while all other mineral salts and additives combined represent only 0.5 € per kg. The relatively high prices of yeast extract and organic acids, coupled with the carbon conversion inefficiency, drives the overall substrate cost well above that typically found in commodity lipid fermentations. Electricity, at 18.8 € per kg of oil, is the next most significant contributor. Fermenter agitation and compressed air production together are responsible for more than 80% of total electrical consumption. The agitation of the main reactor accounts for around 25% of the power demand, while the compressed air system, which is sized for continuous operation at 0.6 vvm, typically draws about 60% of the electricity used. Steam required for solvent stripping and evaporation accounts for 0.6 € per kg of oil. Amortization, representing the investment costs spread over the projected equipment lifespan, amounts to 14.4 € per kg, while salaries account for 9.4 € per kg, and other operational expenses, including maintenance, insurance, finance, and water, reach 9.5 € per kg. This cost structure reflects the relatively small scale of production and the sophistication of downstream lipid extraction.

Within the context of raw material and energy prices prevailing in the summer of 2023, the full production cost for OCFA reached 78 € per kg of oil. This far exceeds costs typically reported in the literature for standard microbial oils, which often range from $1.4 to $6 per kg, thanks to substantially better substrate conversion—generally between 2.2 and 2.5 carbons from substrate per carbon in oil—and higher fermentation productivities.

Since 2023, market indices have shifted moderately: beet molasses prices have fallen by about 17%, and electricity costs by roughly 5%, while other substrate components remained stable. These changes reduce the current calculated cost of oil only marginally, now standing at close to 76 € per kg. The study's calculations relied on recent economic feasibility studies of microbial lipid fermentations, although none specifically addressed odd-chain fatty acid production. Technical estimates for energy and utility consumption were grounded using classic chemical engineering handbooks and process design guides.

Ultimately, the key bottleneck revealed by the YaLiOL scenario is the poor process performance, notably low yield, magnified by scale limitations. Major advances in strain productivity, process optimization, and scale-up are essential for yeast-derived OCFA oils to reach wider markets beyond specialty applications.

Acknowledgments

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OP31. Sustainable lactic acid fermentation at low pH using second-generation feedstocks: a techno-economic assessment

Fernanda Thimoteo Azevedo Jorge[1,2]*, Sinah Tönjes[2], Evelien Uitterhaegen[2], Karel De Winter[2], Wim Soetaert[1,2]

[1] *Centre for Industrial Biotechnology and Biocatalysis (InBio.be), Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium.*
[2] *Bio Base Europe Pilot Plant (BBEPP), Rodenhuizekaai 1, 9042 Ghent, Belgium.*

*** Corresponding author:** fernanda.thimoteo@bbeu.org

Keywords: lactic acid, fermentation, downstream processing, reductive catalytic fractionation, process simulation, techno-economic analysis

Abstract

Lactic acid (LA) is a key building block for the production of bioplastics and chemicals such as polylactic acid and ethyl lactate (EL), a non-toxic solvent derived from LA esterification with ethanol. However, current commercial production of LA relies largely on first-generation feedstocks and involves substantial base consumption during fermentation, along with costly, energy-intensive, and polluting downstream processes [1,2]. The LAPLACE project addresses these challenges by integrating reductive catalytic fractionation (RCF) of second-generation biomass with low-pH yeast fermentation and engineered strains for EL coproduction.

This study aims to evaluate the techno-economic viability of this process and to benchmark it against current industrial practices, focusing on the potential of low-pH fermentation to reduce base use and streamline downstream purification with fewer steps, lower energy consumption, and less waste. Process simulations were performed in Aspen Plus v14 and SuperPro Designer v13 to model the different biorefinery configurations at an industrial scale (100,000 metric tons of LA per year). Mass and energy balances from the simulations formed the basis for capital and operational costs estimation, and financial indicators—including net present value, payback time, and internal rate of return—were derived from discounted cash-flow analysis.

Techno-economic analysis revealed significant reductions in capital and operational costs for low-pH fermentation, with increased profitability when coproducing EL. Fermentation at pH 3 drastically decreased $Ca(OH)_2$ consumption by 85%, enabling a 10% reduction in fermenter sizes and the elimination of acidulation and gypsum separation equipment. Despite the need for extra equipment, materials and energy for EL coproduction, fermentation at low pH provided significant advantages to the process, leading to a 3.5% reduction in the total capital investment of the integrated EL-LA biorefinery and limiting the increase of annual operational costs to 6.2% compared to a LA benchmark process at neutral pH. EL sales also increased the net profit for the integrated biorefinery, resulting in a 40% higher net present value of US$205.69 million.

Therefore, this work offers key insights into the industrial feasibility of sustainable LA and EL production, demonstrating economic viability and superior performance compared to conventional neutral-pH LA production.

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OP32. Toward frameworks for performance evaluation of (yeast-based) sustainable aviation fuel production: gaps and opportunities in science for policy

Marco Vitale*, **Pedro Gerber Machado, Diego Iribarren**

Systems Analysis Unit, IMDEA Energy, Av. Ramón de la Sagra 3, 28935 Móstoles, Madrid, Spain.

*** Corresponding author:** marco.vitale@imdea.org

Keywords: Sustainable aviation fuel, science for policy, decision-making, modeling, techno-economic analysis, life cycle assessment, process simulation, yeast

Abstract

Sustainable aviation fuels (SAFs) are expected to play a key role in mitigating aviation-related emissions [1]. Novel promising biotechnological production routes are being explored [2] beyond the most technologically mature ones, with which they could be integrated into SAF deployment strategies. To check the sustainability of such fuel production systems, appropriate systemic modeling, assessment and optimization approaches constitute essential work to be carried out. Moreover, policy-related measures will play a fundamental role in guiding technological integration and the transition from conventional aviation fuels to SAFs [3]. In this regard, post-normal scientific approaches taking into account the often-contrasting necessities of the different parties involved in the decision-making processes and affected by their outcomes could offer the methodological path to be taken.

Within this context, this work focuses on evaluating practices at the science-policy interface (SPI) that allow for enhanced sustainability-related policy information and streamlining of research efforts in yeast-based SAF production within the complex advanced biojet fuel production landscape. This study systematically reviews literature on both SAF production, with a special focus on yeast-based solutions, and SPI-related structures, working mechanisms, and practices. It follows a double-diamond approach (Figure 1) and relies on well-established databases (Scopus and WoS) and policy-related tools.

The review reveals a consistent mismatch between the number of outputs in the fundamental empirical enquiry domain and the number of works currently adopted at higher levels through the hypothesized information pathways, especially regarding novel yeast-based routes. In addition, as part of the effort to understand the reasons for this mismatch, literature gaps are identified, including the lack of multi-level methodological frameworks, robust implementation roadmaps, multi-technology assessment models for policy, and complete process models. Finally, following a bottom-up approach, a set of actions is proposed based on lessons from what is currently efficiently exchanged at the SPI.

Our findings are expected to inform the basis for future efforts building collaborative research frameworks that shorten the distance among stakeholders at the SPI, thereby contributing to the advancement of promising yeast-based solutions.

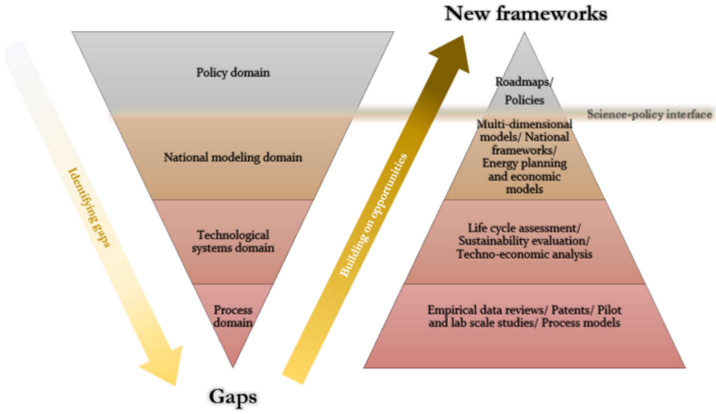

Figure 1. The employed double-diamond approach

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OP33. Cascading biorefinery of cheese whey permeate: integrated bioethanol and biomethane production through lab-scale validation and techno-economic process modeling

Claudia De Micco[1], Mattia Colacicco[1], Matteo Radice[2], Cosetta Ciliberti[1], Matty Janssen[3], John P. Morrissey[4], Gennaro Agrimi[1,5], Maurizio Bettiga[6], Isabella Pisano[1,5]*

[1] *Department of Biosciences, Biotechnology and Environment, University of Bari "Aldo Moro", Via E. Orabona, 4, 70125, Bari, Italy.*
[2] *Universidad Estatal Amazónica, Km 2 ½ Via Puyo-Tena, Puyo, Ecuador.*
[3] *Environmental Systems Analysis, Department of Technology Management and Economics, Chalmers University of Technology, 412 96 Gothenburg, Sweden.*
[4] *School of Microbiology, SUSFERM Fermentation Centre, University College Cork, Cork T12 K8AF, Ireland.*
[5] *Interuniversity Consortium for Biotechnology (CIB), Trieste, 34100, Italy.*
[6] *Italbiotec Srl Società Benefit, 20126, Milan, Italy.*

*** Corresponding author:** isabella.pisano@uniba.it

Keywords: Analysis, waste, *Kluyveromyces marxianus*, cheese whey permeate, ethanol, fermentation, techno-economic

Abstract

The sustainable valorization of industrial waste aligns with Goal 12 of the UN 2030 Agenda [1]. In 2022, EU cheese whey (CW) production exceeded 55 million tons, generating lactose-rich effluents with high organic load and costly disposal [2]. A promising biorefinery approach includes protein extraction, yielding a lactose-rich CW permeate (CWP) [3]. This study characterizes three *Kluyveromyces marxianus* strains for optimal bioethanol production by fermentation. In a cascading biorefinery model, the distilled fermentation residue (FR) is further valorized via anaerobic digestion (AD) for biomethane, integrating waste reduction and renewable energy. This strategy enables multiproduct valorization of CWP in line with circular economy goals. A process simulation for an industrial-scale facility (539 m^3/day) was developed, integrating experimental data from lactose-supplemented fermentations with *K. marxianus* and literature-based parameters to assess techno-economic feasibility [4].

Three *K. marxianus* strains (DSM 5422, 7239, 5572) were cultured in YPD and inoculated at OD_{600} 0.1. Semi-synthetic medium (SSM) and industrial CWP from Distilleria Bartin Srl were used as culture media. Growth kinetics were assessed in microtiter plates. Flask and bioreactor fermentations evaluated ethanol production from CWP/SSM at various temperatures. β-galactosidase activity was measured in permeabilized cells using ONPG hydrolysis. Following bioreactor ethanol fermentation, FR was tested as co-substrate in AD studies. AD tests used CWP, FR, and anaerobic sludge (from Bartin Srl) were conducted at 40 °C in a BRS III BioReactor Simulator (BPC Instruments) under batch and continuous modes. Biomass was measured by OD_{600}/DCW and lactose/ethanol via HPLC. Process modeling in SuperPro Designer divided the overall biorefinery process into three modular sections (filtration, fermentation and distillation, and AD and wastewater treatment) for integrated performance and cost evaluation.

K. marxianus strains showed optimal growth on glucose, followed by lactose and galactose, with improved performance at 37 °C. The best lactose concentration was 13% (w/v). Flask fermentations at 37-42 °C achieved rapid and complete lactose consumption, yielding up to 60 g/L ethanol. Bioreactor fermentation of CWP produced ~58 g/L ethanol, achieving 89% of the theoretical yield and a productivity of 1.38 g/L/h. FR, mainly spent yeast, was subsequently valorized via AD for biomethane production, confirming a stable process. Notably, co-digestion of CWP and FR (70:30 ratio) achieved successful cumulative biomethane production (17.9 ± 1.2 NL) and specific yield (378 ± 24.7 NL/Kg VS). The techno-economic process modelling simulated three main scenarios based on different fermentation yields and microbial robustness. The Minimum Ethanol Selling Price (MESP) was estimated via DCFROR analysis, identifying Scenario 3 as the most economically viable.

K. marxianus enabled high-yield bioethanol production from CWP. A cascading strategy using CWP for ethanol by fermentation and FR for biomethane via anaerobic digestion enhanced both

economic and environmental sustainability, optimizing waste valorization within a circular bioeconomy framework.

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OP34. Fermentative production of 2-phenylethanol by yeasts using agro-industrial wastes: a techno-economic and environmental assessment

Fernanda Thimoteo-Azevedo-Jorge[1,2], Nurdana Orynbek[3], Aiganym Abduraimova[3], Anastasiya Valakhanovich[3], Hector-Adiel Flores-Nestor[3], Marwen Moussa[3], Claire Saulou-Bérion[3], Ana-Karen Sanchez-Castaneda[3]*

[1] *Centre for Industrial Biotechnology and Biocatalysis (InBio.be), Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium.*
[2] *Bio Base Europe Pilot Plant (BBEPP), Rodenhuizekaai 1, 9042 Ghent, Belgium.*
[3] *Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood, 91120 Palaiseau, France.*

*** Corresponding author:** ana.sanchezcastaneda@agroparistech.fr

Keywords: Waste valorisation, Life Cycle Assessment, aroma compound, ISPR

Abstract

2-Phenylethanol (2-PE) is a natural aromatic alcohol with a rose-like scent, widely used in perfumery, cosmetics, and food and pharmaceutical products. Industrial production of 2 PE is currently dominated by chemical synthesis routes, which involve hazardous solvents, harsh reaction conditions, and significant environmental burdens. Alternatively, natural 2-PE extracted from roses is approved for food and fragrance applications but remains economically unviable due to its low yield and high production cost [1]. In this context, microbial fermentation emerges as a promising, eco-friendly approach. *Kluyveromyces marxianus* can convert L-phenylalanine to 2-PE via the Ehrlich pathway [2]. This study [3] investigates the use of agro-industrial byproducts: acid whey, sweet whey, and apple pomace, as low-cost, renewable substrates for 2-PE biosynthesis. The work also integrates in situ product recovery (ISPR) and evaluates process viability through techno-economic and environmental analyses.

K. marxianus CBS600 was cultivated on glucose, apple pomace, and acid and sweet whey to evaluate their suitability for 2-PE biosynthesis from L-phenylalanine. Acid whey was further supplemented with yeast extract and minerals. Cultivations were carried out in shake flasks and 2 L bioreactors under controlled conditions. ISPR was achieved using a membrane contactor with oleyl alcohol as extractant. 2-PE and L-phenylalanine were quantified via LC-MS, while sugars and ethanol were monitored by HPLC. Experimental data supported process simulations in SuperPro Designer for a plant producing 150 tons/year of 2-PE, incorporating fermentation, ISPR, and downstream distillation. Capital and operational costs were estimated from equipment sizing, raw material, and utility demands. A cradle-to-gate life cycle assessment was performed using OpenLCA and the ReCiPe 2016 method, considering European inventory data to quantify environmental impacts per kg of purified 2-PE.

In shake flasks, apple pomace enabled rapid 2-PE biosynthesis, reaching 1.07 g/L in 24 h (44.6 mg/L.h), while acid whey required nutrient supplementation to achieve 2.64 g/L in 72 h, which represents the highest reported titer for whey fermentation. In bioreactors, membrane-based ISPR with oleyl alcohol allowed efficient 2-PE extraction without affecting yeast productivity. Techno-economic simulations for 150 tons/year production showed that supplemented acid whey was the most cost-effective substrate, with a unit production cost of US$186/kg and an internal rate of return of 24.9%. In contrast, apple pomace resulted in higher costs due to drying and handling. Life cycle assessment revealed that whey-based productions had significantly lower environmental impacts than glucose-based routes, and 1500-fold lower greenhouse gas emissions than rose-derived 2-PE. These findings confirm the economic and environmental viability of yeast-based 2-PE production from food byproducts.

K. marxianus enables efficient 2-PE production from acid whey and apple pomace. Techno-economic and environmental analyses confirm the process as a viable alternative to chemical and rose-derived 2-PE. Future work should focus on intensification and pilot-scale validation, with ISPR showing promise for whey-based systems.

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OP35. Production and carbon footprint of microbial oil from waste lemon peel extract

Paola Branduardi[1]*, Vittorio Giorgio Senatore[1], Sofía Martínez-López[2], Essi Paronen[3], Sofia Ceccarossi[1], Mirko Zago[1,3], Immacolata Serra[1], Katri Behm[4]

[1] *Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy.*
[2] *Environmental Department, National Technological Centre for the Food and Canning Industry (CTNC), C. Concordia, S/N, 30500 Murcia, Spain.*
[3] *Soft Chemicals S.r.l., Marnate, Lombardia, 21050, Italy*
[4] *VTT Technical Research Centre of Finland, Tekniikantie 21, 02150 Espoo, Finland.*

*** Corresponding author:** paola.branduardi@unimib.it

Keywords: *Cutaneotrichosporon oleaginosum*, microbial oil production, green solvents, carbon footprint, impact assessment

Abstract

The agricultural sector is one of the leading producers of agro-industrial solid organic waste. This waste, mainly disposed of by incineration or landfilled, could be used for the production of high-value chemicals. In this study, a fermentation process for the production of microbial oil from waste lemon extract (LE), an aqueous side stream deriving from waste lemon peel and pulp processing, was developed and assessed for impacts. Microbial oil can have many and diverse applications, from plasticizers in plastic and rubber compounds to moisturizers in cosmetic formulations.

Characterization of LE revealed that its autoclaving process is effective for increasing the concentration of readily available glucose and fructose, reaching 28.77 ± 0.08 g L^{-1} and 25.68 ± 0.27 g L^{-1}. Nitrogen content was measured too, revealing a C/N ratio of 85, optimal for triggering lipid accumulation in the selected microbial cell factory. The oleaginous yeast *Cutaneotrichosporon oleaginosum* was used as a cell factory, first assessing the optimization of growth and production setting, and then comparing different extraction methods (with and without drying the biomass) when using traditional versus green solvents for product recovery.

C. oleaginosum was cultivated in an unmodified LE-based medium in 2 L bioreactors, resulting in a lipid accumulation of 0.47 ± 0.08 g_{oil} $gCDW^{-1}$. Finally, a new lipid extraction method using green solvents was developed, which allowed the extraction and purification of 11.29 g of oils, corresponding to 35% of the cell dry weight. The carbon footprint of this laboratory-scale production was estimated to be 71 - 434 $kgCO_2eq$ kg^{-1} microbial oil, with electricity consumption of the fermentation step as the main factor. Simulation of the process in a 300 L fermenter suggests that the overall impact can be drastically reduced with scale-up.

The proposed process is promising in terms of production and has the advantage of not competing with edible resources and land use. However, the microbial oil yield and the downstream process must be optimized to make the process sustainable.

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OP36. The yeast *Yarrowia lipolytica*, a workhorse chassis for industrial applications

Jean-Marc Nicaud[1,2]*

[1] *Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas, France.*
[2] *Yalicolor, 20 avenue Hoche, 78190 Trappes, France.*

*** Corresponding author:** jeanmarc.nicaud@gmail.com

Keywords: *Yarrowia lipolytica*, microbial cell factory, metabolic engineering, lipids, lipid derivatives, oils, recombinant protein, chassis strain

Abstract

Key parameters for sustainable bioeconomies are the availability of chassis strains for the production of renewable compounds of interest. Using microorganisms, such as yeast, to produce proteins, enzymes, biofuels, bio-lipids, and high-value lipids and lipid derivatives from renewable resources is a promising approach.

Initially, gene deletions were performed using pop-in/pop-out, followed by pop-in and marker rescue. Then, gene deletions were performed using a dual-guide CRISPR/Cas9 strategy together with a PCR fragment targeting the upstream and the downstream regions flanking the gene to be disrupted [1]. Successful gene disruptions were confirmed by colony PCR. The new chassis strain (JMY9438) was constructed for recombinant protein production. In JMY9451/9452, we further deleted five protease genes. Recombinant protein production was evaluated in strains harboring one, two, or three integrated copies of a target gene, under the control of a hybrid promoter. Protein levels were assessed via quantification of extracellular expression in culture supernatants [1].

Several chassis strains have been constructed for recombinant protein production. The main one was Po1d, JMY1212 (for lipases and enzyme evolution). More recently, the chassis strain JMY7126 was constructed for the use of hybrid-erythritol-inducible promoters [2]. The new industrial chassis strain JMY9438 contained four additional modifications, and the chassis strains JMY9451 and JMY9452 display five additional protease gene deletions for the production of sensitive protein to degradation [3].

The BIMLip team succeeded in engineering the oleaginous yeast *Yarrowia lipolytica*. New tools and metabolic engineering methods will be presented [1, 2]. They have been used for the production of specific lipids [4], for the production of high value proteins such as full-length IgG [5] and the construction of industrial chassis strains for recombinant protein production at industrial scales [3].

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OP37. Scaling-up continuous fermentation: accelerating industrial biomanufacturing with predictive modeling

Guillaume Le Cloirec [1,*], Shannon Hall (Pow.Bio), and the ATV Biotech Team [1]

[1] *ATV technologies, Parc Technologique des Rives de l'Oise, Rue les Rives de l'Oise, 60280, Venette, France.*

*** Corresponding author:** guillaume.lecloirec@atv-technologies.com

Keywords: Continuous fermentation, scale-up, predictive modeling, biomanufacturing, CDMO

Abstract

The bio-manufacturing sector is undergoing a paradigm shift driven by the need for higher productivity, reduced costs, and more sustainable industrial processes. Conventional fed-batch fermentation, while established, faces limitations in operating expenses (OPEX) due to high water and energy consumption, as well as significant capital investment (CAPEX) requirements for large-scale facilities. In response, continuous fermentation is offering a transformative solution, offering superior productivity, improved automation, and reduced downtime, while drastically lowering CAPEX and OPEX.

ATV Technologies leverages its facility and CDMO services to accelerate innovation and industrialization in biotechnology and green chemistry. In partnership with Pow.Bio, ATV has developed a stepwise scale-up strategy for continuous fermentation, progressing from laboratory proof-of-concept to pre-industrial runs. The scale-up trials were conducted using a *Pichia pastoris* strain, with successive validation stages including sterile blank testing, real-condition trials, and integration of predictive modeling tools (CASSIE). Continuous fermentation was successfully demonstrated at 300 L and scaled to 3,000 L for over 300 hours.

Results highlight robust process performance with up to three times the productivity compared to fed-batch fermentation, achieving 25 kg of product over 300 hours. Predictive modeling and dynamic process control enabled by CASSIE were critical to optimize parameters such as feed rates, oxygen transfer, and evaporation management, transforming decision-making from empirical trial-and-error into data-driven strategies. This machine learning-guided approach significantly improved process resilience and adaptability, while enabling facility-specific optimization with minimal retrofitting costs. The successful demonstration of continuous fermentation at pilot scale paves the way toward industrial implementation and redefines industrial biotechnology. So far, ATV has focused its continuous fermentation efforts on *Pichia pastoris*, but through their collaboration, Pow.Bio and ATV now intend to explore additional yeast strains to develop further and optimize the process, and to expand its applicability across different markets. This ambition positions ATV as a versatile partner for sustainable and economically viable solutions across cosmetics and food sectors.

Detailed Abstracts

Poster Presentations

PO1. Development of a platform strain of *Yarrowia lipolytica* optimized for carboxylate utilization and pigment bioproduction

Erik Habek[1], Ivan Dubois[2], Volker Döring[2], Andrew Tolonen[2], Young-Kyoung Park[1*], Tristan Rossignol[1*]

[1] *Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas, France.*
[2] *Génomique Métabolique, Génoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay, 91057 Evry, France.*

* **Corresponding authors:** youngkyoung.park@inrae.fr (Y.-K.P.), tristan.rossignol@inrae.fr (T.R.)

Keywords: *Yarrowia lipolytica*, biosourcing, synthetic biology, acetate, metabolism, bioproduction, evolution

Abstract

Organic wastes are valuable renewable carbon sources that could be recycled into a bioeconomic model. Carboxylates like acetate, which are byproducts of anaerobic fermentation of organic wastes, are abundant and cheaply produced. Yet, they have been limitedly used in biosourcing due to their toxicity to microorganisms. Efficient biosourcing of carboxylates in organic waste necessitates a new approach to organic waste treatment and an optimized microorganism. As part of the ElectroMIC (Electrochemically-assisted MICrobial community metabolic network optimization for biorefinery of organic waste) project, our objective is to produce value-added molecules from acetate-rich treated wastewater by engineering the yeast *Yarrowia lipolytica* [1].

The improvement of *Y. lipolytica* growth on acetate was performed via Adaptive Laboratory Evolution (ALE) using a GM3 continuous fermentation system for 252 days. The acetate concentration in the medium was increased incrementally each time the culture growth stabilized at a specific OD, up to a final acetate concentration of 120 g/L. We have isolated and characterized clones sampled throughout the adaptation process. Growth tests in acetate-containing media were devised at the microplate and flask levels showed strains derived from directed evolution had a shorter lag phase at higher acetate conditions, coupled with a higher acetate consumption compared to the non-adapted starting strain. Their genomes were sequenced, and we expect to identify the key mutations involved in this adaptation for reverse engineering.

To increase the tolerance, uptake, or metabolic utilization of acetate, different heterologous genes from yeasts or bacteria involved in these functions have been identified and integrated for overexpression in a *Y. lipolytica* strain. Golden Gate Assembly [2,3] and CRISPR/Cas9 system [4] tailored for *Y. lipolytica* were used for the engineering . Preliminary data showed that the effect of these integrated genes on growth performance can vary significantly in different acetate concentrations, conferring both beneficial and deleterious effects.

The information gathered on the effects of specific overexpressed genes and the behavior of the strains derived from directed evolution will be merged in order to develop a *Y. lipolytica* strain capable of efficiently utilizing acetate as a primary carbon source, which will become our chassis for pigment bioproduction.

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PO2. Multifunctional fabrics based on *Yarrowia lipolytica* yeast dyes: Introduction to the YaLiGreen project

Camille Eicher[1]*, Mohamed Koubaa[2], Young-Kyoung Park[1], Tristan Rossignol[1]

[1] *Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas, France.*
[2] *Université de Technologie de Compiègne, Ecole Supérieure de Chimie Organique et Minérale, TIMR (Integrated Transformations of Renewable Matter), Centre de recherche Royallieu, CS 60 319, 60 203 Compiègne, Cedex, France.*

*** Corresponding author:** youngkyoung.park@inrae.fr tristan.rossignol@inrae.fr

Keywords: *Yarrowia lipolytica*, prodeoxyviolacein, fabric dyeing, genetic engineering

Abstract

Dyes are positioned as sustainable raw materials for coloring foods, cosmetics, pharmaceuticals, and polymers. Natural pigments extracted from microorganisms represent an attractive alternative to synthetic pigments since they can be produced at high yield in an environmentally friendly way. Among others, violacein is a well-studied dye naturally produced by some Gram-negative bacteria to defend themselves against predators [1]. It displays interesting pharmaceutical properties, including antibacterial, antitumoral, and antioxidant activities [2]. Its production is made possible through the expression of a vio-operon from the precursor, tryptophan. There are some less-studied intermediates of the pathway that are also dyes, such as the green dye prodeoxyviolacein (PV). The project YaLiGreen aims at producing PV by the synthetic biology of yeast and to study its application in fabric coloration and its antimicrobial properties.

To ensure the production of PV, the chassis strain *Yarrowia lipolytica* will be used. This yeast is well known for its capacity to produce a wide range of compounds at high yields and to be genetically engineerable with several available synthetic biology tools [3]. Three genes will need to be integrated into its genome to induce PV production: *vioABE*. Different *vioABE* sequences from different bacteria will be expressed in *Y. lipolytica* for and their production efficiency will be compared. The multigene assembly Golden Gate toolkit will be used to assemble *vioABE* before integration into *Y. lipolytica*'s genome using CRISPR-Cas9. After verifying the correct gene insertion into the genome of the transformants, PV production will be compared among clones, and the best gene combination will be chosen. Then, the metabolic pathways leading to PV synthesis in *Y. lipolytica* will be boosted, including the shikimate and tryptophan synthesis pathways. Industrial byproducts will be used as substrates for *Y. lipolytica*, and the extraction method will be optimized. Finally, antimicrobial properties of PV will be investigated, and it will then be used to generate eco-friendly green dyed fabrics.

The YaLiGreen research project funded by the ANR is dedicated to reducing the carbon footprint of fossil resources through the bioproduction of dyes for fabrics. It combines interdisciplinary and innovative methods mastered by different scientific partners with complementary skills.

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PO3. Towards an innovative enzymatic solution for the valorisation of end-of-life bioplastics

Leonardo Faggian[1]*, Marthinus Wessel Myburgh[1,2,3], Marinda Viljoen-Bloom[2,3], Lorenzo Favaro[1,2,3]

[1] *Waste to Bioproducts Laboratory – DAFNAE, University of Padova, Viale dell'Università 16, 35020, Legnaro (PD), Italy.*
[2] *Urobo Biotech, De Beer Street, Stellenbosch, Western Cape, 7600, South Africa.*
[3] *Stellenbosch University, Microbiology, De Beer Street, Stellenbosch, Western Cape, 7600, South Africa/*

*** Corresponding author:** leonardo.faggian@studenti.unipd.it

Keywords: Industrial strain engineering, waste valorisation, bioplastic hydrolysis, central composite design

Abstract

The bioplastics sector is expected to increase its production capacity from 2.47 million tons in 2024 to about 5.7 million tons in the next five years. The sector is primarily dominated by thermoplastic starch (TPS), poly(lactic acid) (PLA), and poly(butylene-adipate-co terephthalate) (PBAT) to produce a variety of items. As per European regulations, these items are most often disposed of in the organic fraction of municipal solid waste (OFMSW) that is subsequently sent for anaerobic digestion (AD). However, current AD technologies can, in some cases, be ineffective in managing end-of-life bioplastic items [1], forcing many AD plants to remove and send this untreatable fraction (UF) to incineration or landfilling companies. An AD plant in Italy, treating 45,000 tons of OFMSW annually, produces roughly 5,300 tons of UF. This project aims to provide innovative solutions by developing hydrolytic enzyme cocktails that effectively break down bioplastics in the UF, fostering its valorisation.

Three different enzymes, a cutinase-like enzyme [2] (CLEwt) and two engineered variants (CLE_v1 and CLE_v2) were recombinantly expressed through δ integration into the genome of the industrial *S. cerevisiae* strain Ethanol Red® (ERv1). The best 8 transformants for each variant, identified through plate assays, were evaluated using turbidity assays with emulsified PLA and cell-free supernatant (CFS). CFS from the best ERv1 transformant and respective laboratory strain benchmark (*S. cerevisiae* Y294) for each enzyme variant were tested in hydrolysis trials using commercial PBAT/TPS shopper bags. The best performing strain (ERv1T3) was then assessed for the hydrolysis of end-of-life bioplastics from UF. To optimise the hydrolysis of post-consumer bags, concentrated ERv1T3 CFS was mixed with a concentrated CFS containing amylases at different ratios adopting a face-centred central composite designs. Models were obtained (adj R^2>0.95 and terms' p-value < 0.01) for three hydrolysis monomers. Positive transformants were screened for three rounds on polycaprolactone (PCL) agar plates for halo size. Activities in the turbidity assay for the best transformants were up to 8× higher than the *S. cerevisiae* Y294 benchmarks. Hydrolysis trials on commercial bags yielded a significant increase in PBAT monomer release as well as in weight loss for the strain Erv1T3 compared to the same enzyme variant produced in *S. cerevisiae* Y294. UF samples collected at an AD plant contained up to 30% of bioplastics, 80% of which were composed of PBAT/TPS bags. Therefore, amylases were added to obtain an enzyme cocktail; optimal ratios of CLE_v1 and amylases were determined using real post-consumer bags. CLE_v1 was the most impactful component of the enzyme cocktail; moreover, the loading of CLE_v1 significantly impacted the release of glucose from TPS, suggesting a synergistic behaviour with the amylases. The best enzymatic combinations proved to be very effective on the hydrolysis of UF.

Enzyme production in industrial strains of *S. cerevisiae* yielded a marked improvement compared to previous benchmarks. Novel high-producing strains allow for effective technology development on real pre- and post-consumer bioplastics, which are a significant share of the UF, paving the way for end-of-life bioplastic valorisation options.

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PO4. Development of a *Yarrowia lipolytica* co-culture system for ginger essential oil bioproduction

Shirin Bamezai[1,2,3,4] *, Young-Kyoung Park[5], Rodrigo Ledesma-Amaro[1,2,3,4]

[1] *Imperial College Centre for Synthetic Biology, Imperial College London, London, United Kingdom of Great Britain and Northern Ireland.*
[2] *Department of Bioengineering and Imperial College Centre for Synthetic Biology, Imperial College London, London, UK.*
[3] *Bezos Centre for Sustainable Protein, Imperial College London, London, United Kingdom of Great Britain and Northern Ireland.*
[4] *Engineering Biology Mission Hub on Microbial Food, Imperial College London, London, United Kingdom of Great Britain and Northern Ireland.*
[5] *Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, Jouy-en-Josas, 78350, France.*

*** Corresponding author:** sb3019@ic.ac.uk

Keywords: *Yarrowia lipolytica*, co-culture, bioproduction, ginger oil, terpenes, sesquiterpenes, zingiberene, bisabolene, sesquiphellandrene, sustainability, biofungicide, food system

Abstract

Ginger essential oil (GEO) presents a plant-based and biodegradable alternative to synthetic biocontrol agents, with synergistic potential as an anti-phytopathogenic, weed and pest control solution, all whilst having low toxicity to non-target organisms [1]. Although presenting several advantages, its widespread adoption within agriculture has been limited due to key challenges associated with its unsustainable and uneconomical extraction from plant material [2]. Metabolic engineering offers the possibility to develop a stable, inexpensive, and environmentally-friendly bioproduction platform through microbial cell factories. Given the limitations associated with engineering a single strain, this project aimed to develop a proof-of-concept for a modular co-culture system that can effectively produce an alternative to GEO, with major constituent compounds produced at ratios found in nature, utilising specialised strains of the industrial oleaginous yeast *Yarrowia lipolytica*.

Y. lipolytica Po1d and derivative auxotroph strains (S1710, S1280) were utilised in this study. Metabolic engineering strategies were achieved through the use of the YaliCraft toolkit [3], with in silico design of transcriptional units. Constitutive expression of heterologous cassettes was achieved through multicopy genomic integration via zeta sequences. Cultures were grown in shake flasks with dodecane overlay for terpene extraction, and these were then quantified by GC-MS, with biomass measurements determined using OD_{600} and cell dry-weight. Different strategies were employed to determine the ratio of strains in co-culture, including the use of colony-forming units and flow cytometry. Statistical analysis included ANOVA with Tukey's HSD, significance set at $p \leq 0.05$.

This study successfully constructed diverse *Y. lipolytica* strains capable of producing terpene compounds found in GEO, employing two key strategies: gene expression modulation and pathway engineering. In the latter, MVA pathway boosting both in the cytoplasm and peroxisome was explored. To achieve production of three major constituents of GEO at a tunable ratio, a co-culture strategy based on orthogonal auxotrophies was designed. Orthogonal auxotrophies in *Y. lipolytica* were characterised, as well as the impact of amino acid supplementation on growth rate. Successful metabolic engineering strategies for the de novo production of three sesquiterpenes were implemented in separate strains with orthogonal auxotrophies. Co-cultures with desired production profiles were achieved through the fine-tuning of inoculation ratio and media composition.

Successful de novo bioproduction of a range of sesquiterpenes was achieved in *Y. lipolytica*. Initial findings suggest that the co-culture design explored shows potential as a model for a bioproduction platform designed to produce a mixture of value-added compounds.

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PO5. Adaptive laboratory evolution of *Yarrowia lipolytica* for efficient lactate utilization

Monica Squicciarini[1], Gennaro Agrimi[1]*, Jean-Marc Nicaud[2,3]

[1] *Biosciences, Biotechnology and Environment (DBBA) - University of Bari Aldo Moro, Italy.*
[2] *Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas, France.*
[3] *Yalicolor, 20 avenue Hoche, 78190 Trappes, France.*

*** Corresponding author:** gennaro.agrimi@uniba.it

Keywords: *Yarrowia lipolytica*, Adaptive Laboratory Evolution, lactate utilization, genome sequencing

Abstract

Lactic acid is a promising alternative carbon source in microbial biotechnology obtainable from waste material. *Yarrowia lipolytica* has shown potential for lactate utilization, although its efficiency and regulatory mechanisms remain unclear. Initial experiments revealed that the yeast grows poorly on lactate in minimal media but performs well in rich media, suggesting a key role for amino acids in lactate metabolism [1]. To address these growth limitations, Adaptive Laboratory Evolution (ALE) was employed to select strains with enhanced lactate utilization. Genome sequencing of the evolved strain may offer valuable insights into lactate metabolism and its regulatory pathways.

The prototrophic wild-type strain *Y. lipolytica* Y2900 (Po1d LEU2 URA3) was cultivated in rich and minimal media with potassium lactate (pH 7). The minimal medium (YNB+LAT2%) contained yeast nitrogen base, NH_4Cl, potassium lactate, and phosphate buffer. Rich formulations included YP+LAT2%, YP+GLU+LAT2%, and SCM+LAT2%. Cultures were incubated at 28 °C with shaking at 180 rpm in baffled flasks. Pre-cultures were grown in YPD and inoculated at OD_{600} = 0.1. The lag phase was defined as the time for OD_{600} to reach five times the initial value. Lactate consumption was quantified by HPLC using a Rezex ROA Organic Acid H^+ column with 2.5 mM H_2SO_4 as mobile phase at 60 °C. Genomic DNA from three evolved strains and parental Y2900 was extracted using the UltraClean kit (Qiagen). Whole-genome sequencing was outsourced and performed via Illumina MiSeq (2×150 bp). Bioinformatic analysis is underway to identify mutations linked to enhanced lactate metabolism.

Y. lipolytica Y2900 initially exhibited poor growth on lactate in minimal medium (YNB +2% lactate), characterized by a prolonged 120-hour lag phase, low biomass yield (OD_{600} = 5), and limited lactate consumption (15.3%). In contrast, growth in rich media was significantly enhanced, with a reduced lag phase of 24 hours, OD_{600} values reaching up to 52, and over 90% lactate consumption—underscoring the critical role of amino acids. To improve growth in minimal medium, three independent Adaptive Laboratory Evolution (ALE) lines were conducted over nine cycles in YNB + 2% lactate. By the third cycle, the lag phase had decreased to 40 hours, and by the eighth cycle, OD_{600} reached 47.8 with lactate consumption exceeding 96%. The evolved phenotype remained stable after cultivation on glucose and recovery from glycerol stocks. The three evolved strains were physiologically characterized, yielding ~0.5 grams of biomass per gram of lactate consumed. Their lipid profiles were also assessed.

Y. lipolytica underwent Adaptive Laboratory Evolution (ALE) to improve growth on lactate in minimal medium. Evolved strains showed faster growth, shorter lag phases, and near-complete lactate consumption. Whole-genome sequencing is complete; analysis will identify mutations linked to enhanced lactate metabolism.

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PO6. From stress response to riboflavin biosynthesis: Gene expression insights for strain engineering in the halotolerant yeast *Debaryomyces hansenii*

Benjamín Mendoza-Téllez, Diana Villarreal-Huerta, Miguel Angel Rosas-Paz, Viviana Escobar-Sánchez, Claudia Segal-Kischinevzky, James Gonzáles*

Laboratory of Molecular Biology and Genomics, Yeast Microbiology. Faculty of Sciences, National Autonomous University of Mexico. Av. Universidad 3000, Circuito Exterior s/n, Alcaldía Coyoacán, CP 04510, Ciudad Universitaria, Mexico City, Mexico.

*** Corresponding author:** james@ciencias.unam.mx

Keywords: *Debaryomyces hansenii*, riboflavin (vitamin B2), halotolerant yeast, gene expression, stress response, bioproduction.

Abstract

Riboflavin (RF, vitamin B2) is an essential organic compound involved in a wide range of cellular processes across all domains of life. Owing to its biological relevance, RF is widely used as a supplement in the food, pharmaceutical, and cosmetic industries, driving continued interest in the development of efficient microbial production systems. The oleaginous, halotolerant yeast *Debaryomyces hansenii* has emerged as a promising platform for biotechnological applications, including RF biosynthesis. Previous studies have primarily focused on the overexpression of genes directly involved in the RF biosynthetic pathway and the use of alternative carbon sources derived from industrial waste. However, the genetic relationship between stress tolerance and RF production in *D. hansenii* remains poorly understood.

In this study, we analyzed the expression of key RF biosynthetic genes under saline conditions using a stable mutant strain lacking specific stress response genes. Our objective was to elucidate the regulatory mechanisms that link stress adaptation to RF biosynthesis and to optimize a robust strain for sustainable, industrial-scale RF production.

Our results show that the stable mutant exhibited a significant increase in extracellular RF levels upon exposure to NaCl, along with a notable increase in the expression of key genes within the riboflavin synthesis pathway.

These findings reveal a critical genetic component influencing RF production in *D. hansenii*, offering valuable insights for the development of salt-tolerant microbial platforms in industrial bioprocesses, particularly those involving saline wastewater environments.

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PO7. Engineering *Yarrowia lipolytica* for the optimized conversion of microbial lipids into green diesel using a light-driven fatty acid photodecarboxylase

Syeda F. Ahmed[1,2], Cristina Vico-Uribes[2], Elia Tomás-Pejó[2], Elvira Romero[2]*

[1] *Universidad Católica de Valencia San Vicente Mártir, Guillem de Castro 94, 46001, Valencia, Spain.*
[2] *Biotechnological Processes Unit, IMDEA Energy, Avda. Ramón de la Sagra 3, 28935 Móstoles, Madrid, Spain.*

*** Corresponding author:** elvira.romero@imdea.org

Keywords: CRISPR-Cas9 gene-editing, energy transition, fatty acid photodecarboxylase, Golden Gate assembly, green diesel, *Yarrowia lipolytica*

Abstract

The great potential of *Yarrowia lipolytica* in industrial biotechnology has been demonstrated in various studies [1]. This yeast is able to store more than 20% of its dry cell weight as lipids, mostly triacylglycerides, which can be converted into oleochemicals, nutraceuticals and biofuels. Furthermore, *Y. lipolytica* is generally regarded as safe, easily grows on various substrates (e.g., sugars, glycerol, lipids), and its genome data is available. Recently, two studies have shown that this yeast can be used as a host for the heterologous expression of an algal light-driven fatty acid photodecarboxylase (FAP, EC 4.1.1.106) which converts the yeast fatty acids into green diesel (alkanes C15-C17; Figure 1A) [2,3]. The reported yield (12.34 mg/g glucose) was higher than using other enzymes, but only 1.2% of glucose was converted in alkanes. Herein, we present our first efforts to increase the yield of this bioprocess which are focused on ramping up the FAP expression level in the yeast.

The following approach was previously reported [4] but it has not been used for FAP [2,3]. First, the FAP gene will be adapted to codon usage and synthesized, while the commercially available pHR_AXP_GFP vector will be converted into a Golden Gate (GG) version by using mutagenic PCR to add two PaqCI (type IIS) restriction sites. Next, a GG reaction will be performed overnight with the vector, FAP, PaqCI and ligase. As a result, FAP will be ligated in the vector under control of the strong constitutive UAS1B8–TEF(136) promoter. This new construct will be transformed, together with pCRISPRyl vector (Figure 1B), into *Y. lipolytica* PO1f Δku70 cells to achieve the FAP gene integration into the genome by homologous recombination. After auxotroph-based selection and colony PCR, a few positive strains will be cultured in 96 deep-well plates (28 °C, pH 6, 1000 rpm) in yeast nitrogen base and short chain fatty acids medium. Finally, cells will be analyzed by SDS-PAGE to visualize the FAP content.

So far, the work to convert the commercially available pHR_AXP_GFP vector into its GG counterpart has been started. Specifically, to add the two PaqCI restriction sites in the pHR_AXP_GFP_vector, a mutagenic PCR was performed. The primers were designed to add a PaqCI restriction site at both the beginning and the end of the GFP insert (Figure 1B). Thus, the primers (31 bp) are complementary to the PCR template (vector) except for six bases in the middle. As a result, the PCR product is lineal, without most part of the GFP gene, and with a size of 8191 bp (instead of 8873 bp for the circular pHR_AXP_GFP). A band with the expected size was observed in an agarose gel containing the mutagenic PCR reaction, which was cut for performing DNA purification. Next, the Golden Gate reaction will be set up to obtain the FAP construct for yeast transformation and intracellular expression.

This work uses CRISPR-Cas9 gene-editing tool to achieve a high expression level of a novel photodecarboxylase which converts yeast lipids into alkanes serving to replace fossil derived diesel. It has the potential to contribute to stopping climate change, while fostering sustainable economic growth by ensuring the availability of a drop-in biofuel.

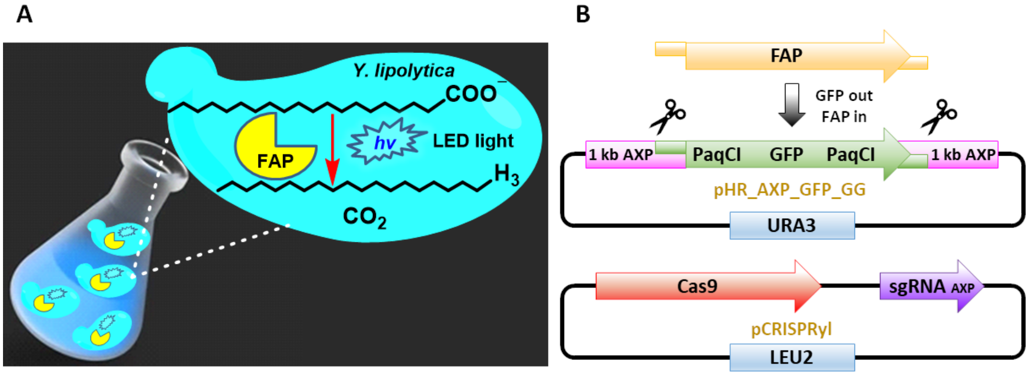

Figure 1. FAP reaction (A) and Golden Gate assembly for CRISPR-Cas9 gene-editing (B).

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PO8. Enhancing brewing yeasts through combined sexual breeding and adaptive evolution: A case study on β-lyase activity

Chiara Nasuti[1], Viola Ceramelli Papiani[1], Kristoffer Krogerus[2], Davide Tagliazucchi[3], Lisa Solieri[1,4]*

[1] *Lactic acid bacteria and Yeast Biotechnology (LYB) Lab, Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, 42122, Reggio Emilia, Italy.*
[2] *VTT Technical Research Centre of Finland Ltd, Tekniikantie 21, PO Box 1000, FI-02044 VTT, Espoo, Finland.*
[3] *Nutritional Biochemistry Lab, Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, 42122 Reggio Emilia, Italy.*
[4] *NBFC, National Biodiversity Future Center, 90133 Palermo, Italy.*

*** Corresponding author:** lisa.solieri@unimore.it

Keywords: Sexual breeding, adaptive evolution, Non-GMO, β-lyase activity, yeast strain improvement, fermentation, brewing

Abstract

Sexual breeding and adaptive evolution (AE) are non-GMO strategies that leverage natural mechanisms underlying biodiversity to improve industrial yeast strains. Traditionally, these approaches have been applied independently. In this study, we combined them and demonstrated that this integrated strategy is effective in enhancing brewing-relevant traits, particularly those governed by monogenic or polygenic inheritance. As a proof of concept, we applied the approach to improve the capacity of brewing strains to convert cysteine-conjugated hop precursors into volatile thiols, which contribute fruity and tropical aromas highly valued in modern beer styles. In *Saccharomyces cerevisiae*, β-lyase activity is encoded by the polymorphic *IRC7* gene. The short allele (*IRC7^S*) encodes a truncated, non-functional protein, whereas the long allele (*IRC7^L*) encodes a functional 400-amino acid enzyme. This trait represents a suitable target for the combined breeding and AE approach proposed in this work.

β-lyase activity was assessed using L-cysteine, S-ethyl-L-cysteine, and S-methyl-L-cysteine as substrates in a spectrophotometric assay based on NADH oxidation at 340 nm. Micromanipulator and spore-to-spore method were used to cross a monosporic clone (MSC) of a commercial *S. cerevisiae* strain (ale4) with NBRC1948, a natural *S. eubayanus* × *S. bayanus* hybrid. Hybridization was confirmed by PCR targeting housekeeping genes and enzymatic restriction of the ITS region. Adaptive evolution was carried out in YNB medium under nitrogen-limiting conditions using cysteine as the sole nitrogen source. The experiment lasted 14 weeks: 3 cycles at 5 mM, 5 at 10 mM, and 6 at 15 mM cysteine, each lasting one week. Mutants were selected based on reduced growth inhibition. Growth curves were used to assess performance in cysteine-only (15 mM) medium. Small-scale fermentations were carried out in 10°P hopped wort (5 g/L hops) at 20 °C.

ale4 showed high β-lyase activity on cysteine and its conjugates and was selected as the parental candidate alongside the cryotolerant brewing strain NBRC1948. A MSC of strain ale4 with the functional *IRC7^L* allele was crossed with NBRC1948, producing an interspecific hybrid, named CN1. Hybrid formation was confirmed by species-specific PCR and ITS restriction analysis. CN1 overcame parents in β-lyase activity. Initial CN1 strain showed partial growth inhibition in media containing cysteine as a unique nitrogen source, like the parents. AE over 14 weekly cycles with increasing cysteine led to isolate 20 mutants of CN1 which outperform ed parents and the initial hybrid CN1 in cysteine-based nitrogen utilization. Small scale fermentation validated the growth of evolved strains.

Our combined sexual breeding and adaptive evolution strategy led to an interspecific hybrid with enhanced β-lyase activity. Evolution under cysteine stress yielded mutants with improved cysteine assimilation and growth. These non-GMO evolved strains are promising starters for producing aroma-rich beers.

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PO9. Towards the use of CO_2-derived dihydroxyacetone (DHA) as a carbon and energy source for *S. cerevisiae*-based bioprocesses

Nabamita Nandi, Mathias Klein, Elke Nevoigt*

School of Science, Constructor University, 28759 Bremen, Germany.

*** Corresponding author:** enevoigt@constructor.university

Keywords: baker's yeast, metabolic engineering, dihydroxyacetone catabolism, *DAK1*, adaptive laboratory evolution, ethanol production, *GUT1*, *UBR2*

Abstract

Reliance of bio-industries on first-generation plant-based carbon sources for fermentations using *Saccharomyces cerevisiae* raises concerns regarding food security and biodiversity. Dihydroxyacetone (DHA), a C3 carbohydrate that could be derived from CO_2 in the future [1], is a promising carbon and energy source. DHA has shown to be toxic to yeast. However, it was demonstrated that the overexpression of *DAK1* in *S. cerevisiae* improves DHA tolerance and growth by converting DHA to DHAP, an intermediate of central carbon metabolism (CCM) [2]. In the past, our group constructed derivatives of the strain CEN.PK113-1A with *DAK1* overexpression and other genetic modifications (*C. jadinii FPS1*, $GUT1_{JL1}$ and $UBR2_{CBS}$) for efficient glycerol consumption. As these modifications might facilitate the utilization of DHA, it was interesting to explore the performance of our strains regarding tolerance to and growth on DHA.

The strain considered most suitable for the aim of this work was created based on a previously constructed CEN.PK113-1A derivative, which carries a modified version of the endogenous G3P pathway for glycerol metabolism and contains the superior alleles $GUT1_{JL1}$ and $UBR2_{CBS}$ [3]. In this strain, we overexpressed *DAK1*. In addition, a heterologous glycerol facilitator (*C. jadinii FPS1*) was also expressed. Both expression cassettes were integrated into the *YGLCτ3* locus using CRISPR/Cas9 method. The resulting strain was named CEN $G3P_{PW}$+$DAK1_{OE}$. Cultures were grown in synthetic Verduyn medium supplemented with DHA (0-400 mM), with or without 6% glycerol. Growth profiling was carried out using the Growth Profiler 960, and the values were used to calculate growth rates. Shake flask fermentations were performed in DHA-only media to assess metabolic outputs under oxygen-limited conditions. HPLC analysis quantified DHA consumption and ethanol production.

The strain CEN $G3P_{PW}$+$DAK1_{OE}$ grew surprisingly well (μ_{max} = 0.24 h^{-1}) in synthetic medium with 100 mM DHA as sole carbon source. In synthetic medium containing glycerol and DHA, growth declined with increasing DHA concentration, but a few technical replicates resumed growth in glycerol + either 300 or 400 mM DHA, indicating adaptive evolution. The best isolate 400R1T2 was again pre-grown in synthetic glucose medium, and the cells were afterwards used to inoculate synthetic medium with 400 mM DHA as the sole carbon source. It exhibited a growth rate of 0.2 h^{-1}, whereas the non-evolved reference strain did not show any growth. Shake flask fermentation with the isolate 400R1T2 resulted in complete depletion of 36 g L^{-1} DHA and produced 6 g L^{-1} ethanol (ethanol yield = 0.2 g/gDHA). Analysis of the mutations in the evolved isolate responsible for the enhanced phenotype is underway.

Engineered and evolved *S. cerevisiae* strains with significant improvements in DHA tolerance and utilization were established. A significant yield of ethanol from DHA forms a starting point towards yeast-based bioprocesses using DHA as a feedstock in the future.

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PO10. Valorization of waste substrates for vitamin biosynthesis in *Ashbya gossypii*

Javier Martín-González*, Marta López-Rubio, Javier F Montero-Bullón, Rubén M Buey, Alberto Jiménez

Metabolic Engineering Group, Department of Microbiology and Genetics, Universidad de Salamanca, Campus Miguel de Unamuno, Salamanca 37007, Spain

*** Corresponding author:** javiermartin@usal.es

Keywords: *Ashbya gossypii*, riboflavin, folates, waste oil, lignocellulosic hydrolysates, metabolic engineering.

Abstract

Ashbya gossypii is a filamentous fungus and a natural riboflavin overproducer, making it an attractive organism for industrial vitamin B2 production. This characteristic has supported the development of advanced genetic engineering tools, establishing *A. gossypii* as a robust microbial chassis for the heterologous production of high-value metabolites in industrial biotechnology [1-3]. Riboflavin and folate biosynthesis share the precursor GTP, synthesized via the purine pathway from ribose-5-phosphate and ATP. However, their secondary precursors are ribulose-5-phosphate for riboflavin and chorismate for folates (Figure 1.A). *A. gossypii* shows metabolic flexibility and can grow on various carbon sources, such as low-cost oils, without requiring genetic modifications. It also grows on xylose-rich lignocellulosic wastes, although this requires overexpression of its criptic xylose assimilation pathway (XR–XDH–XK) (Figure 1.B), which was used to produce terpenes and biolipids, but not yet vitamins [1-2].

A. gossypii strains (Table 1) were cultured at 28 °C, 200 rpm in MA2 medium with glucose, waste cooking oil (WCO), or xylose-rich lignocellulosic hydrolysates. Preinocula were prepared from spores grown 48 h in glucose-MA2 and washed with Triton X-100. Media for folate production were supplemented with 0.002% pABA. WCO was filtered and emulsified (20%) with 0.5% Tween 40. Gene overexpression or subexpression of native genes was achieved by genomic integration of transformation cassettes containing loxP-KanMX-loxP and a promoter sequence, amplified by PCR with gene-specific primers and integrated upstream of the ATG codon by homologous recombination. Riboflavin was extracted with 0.1 N HCl, heat-treated, bead-disrupted, centrifuged, and quantified by UHPLC-DAD (WCO) or spectrophotometry (glucose/lignocellulose). Folates were extracted from cells and culture medium, spiked with labeled folic acid, deglutamylated, purified with SAX, and quantified by UHPLC-Orbitrap MS. Biomass was measured as dry weight.

A. gossypii can grow on low-cost oils as sole carbon sources. Given this, *A. gossypii* was cultured using WCO, where the WT strain showed strong growth and produced high riboflavin levels [4], close to those of engineered strains grown on glucose [5]. For folate biosynthesis, metabolic flux was redirected from riboflavin, the naturally overproduced metabolite in *A. gossypii*, toward folate synthesis. A previously engineered strain [3] produced the highest titer of folates in WCO reported in a modified strain [4]. For riboflavin synthesis from lignocellulosic residues, the XR–XDH–XK pathway was overexpressed. Xylulose-5-phosphate was converted to ribulose-5-phosphate through RPE1 overexpression. ADE12 downregulation diverted IMP toward GTP. These modifications increased the pool of both precursors (Figure 1.C). Overexpressing RIB1 and RIB3 further enhanced riboflavin synthesis, reaching high titers when using xylose as a carbon source.

A. gossypii can efficiently grow on WCO and xylose-rich lignocellulosic residues as a carbon source. Producing high levels of valuable metabolites such as riboflavin and folates, specially using WCO. These results highlight the potential of *A. gossypii* as a sustainable microbial platform for the recycling and valorization of some industrial wastes.

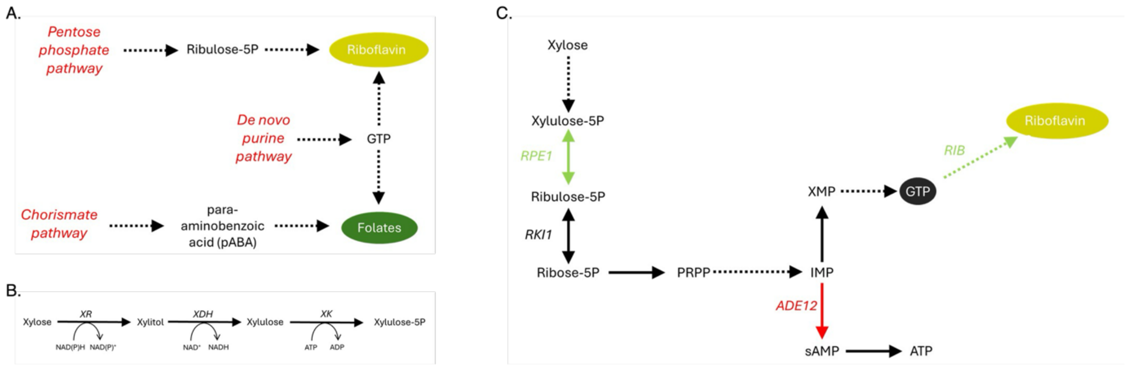

Figure 1. A. Biosynthetic pathways leading to riboflavin and folates in *A. gossypii*. **B.** Xylose assimilation pathway in *A. gossypii*. **C.** Engineering strategy for riboflavin synthesis from xylose in *A. gossypii*.

Table 1. List of the *A. gossypii* strains used in this work.

Strain	Genotype
WT	Wildtype (ATCC 10895)
A493	P_{GPD1}-*FOL2*; P_{GPD1}-*FOL3*; *ade12*Δ; *met7*Δ; P_{RIB7}-*RIB1*
A665	P_{GPD1}-*GRE3*; P_{GPD1}-*XKS1*; P_{GPD1}-*XYL2*
A1284	P_{GPD1}-*GRE3*; P_{GPD1}-*XKS1*; P_{GPD1}-*XYL2*; P_{GPD1}-*RPE1*
A1587	P_{GPD1}-*GRE3*; P_{GPD1}-*XKS1*; P_{GPD1}-*XYL2*; P_{GPD1}-*RPE1*; P_{RIB7}-*ADE12*
A1699	P_{GPD1}-*GRE3*; P_{GPD1}-*XKS1*; P_{GPD1}-*XYL2*; P_{GPD1}-*RPE1*; P_{RIB7}-*ADE12*; P_{GPD1}-*RIB1*; P_{GPD1}-*RIB3*;

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Acknowledgments

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PO11. Coordination of cell wall production and proteasome assembly by Mub1 in *Saccharomyces cerevisiae*

Nada Šupljika[1], Antonia Paić[1], Tea Martinić Cezar[1], Ana Novačić[1], Renata Teparić[1], Beatrice Vallée[2], Bojan Žunar[1], Igor Stuparević[1]*

[1] *University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia*
[2] *Centre for Molecular Biophysics, CNRS, ORLEANS, France*

*** Corresponding author:** istuparevic@pbf.hr

Keywords: cell wall, Mub1, *Saccharomyces cerevisiae*; ubiquitin proteasome system, calcofluor white

Abstract

Yeasts have developed complex regulatory systems to maintain their cell wall, which functions as the main interface with the environment. Despite its importance, the link between this regulatory network and broader cellular processes remains poorly understood. This study investigates Mub1 in *Saccharomyces cerevisiae*, an adaptor protein of the E3 ubiquitin ligase Ubr2, which has previously been linked to the regulation of proteasome genes by the transcription factor Rpn4. mub1Δ cells show increased resistance to standard cell wall stressors compared to wild-type cells. This increased tolerance is attributed to the activation of multiple transcription factors that suppress cell wall remodeling. Importantly, Mub1 influences not only Rpn4 but also a broad range of other transcription factors, making it a novel regulatory hub linking cell wall integrity to the ubiquitin-proteasome system.

The study utilized *Saccharomyces cerevisiae* strains (BY4741, BMA 41) and *E. coli* NEB Stable for genetic manipulations. Plasmids were constructed using HiFi Assembly and restriction enzymes. Yeast cells were characterized via flow cytometry, confocal microscopy, and RT-qPCR using specific primers. Total RNA was isolated using the hot phenol method, and strand-specific RNA sequencing was performed on prepared libraries using Illumina NovaSeq, followed by data analysis using various bioinformatics tools for differential gene expression and functional annotation.

In *S. cerevisiae*, the deletion of the MYND-type zinc finger protein Mub1 — an adaptor protein for the E3 ubiquitin ligase Ubr2— leads to a pronounced tolerance to common cell wall stressors such as heat, Calcofluor white, Congo red, and SDS, which exceeds the sensitivity of the wild type. The increased resistance in mub1Δ strains is attributed to the activity of several transcription factors that inhibit cell wall remodeling, a process that leads to maladaptation under prolonged stress. Surprisingly, this effect is independent of the canonical Mub1 client Rpn4 and other players such as Ssd1 and Rrp6. RNA- sequencing revealed that Mub1 functions as a broad regulatory hub that influences a network of transcription factors beyond proteasome regulation and directly links cell wall robustness to the ubiquitin- proteasome system in yeast [1].

This study shows that Mub1 is an important regulator linking yeast cell wall remodeling and the ubiquitin-proteasome system. Mub1 influences several transcription factors that affect wall resistance. Overexpression of Mcm1 or Swi4 leads to an improvement in stress sensitivity and opens up new ways to improve the stress tolerance of yeast.

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PO12. Genetically modified yeasts as systems for selenium nanoparticle production

Livia-Teodora Ciobanu[1,2], Andrei Bărboi[3], Diana Constantinescu-Aruxandei[2], Bogdan Trica[2], Florin Oancea[2], Ileana Cornelia Farcasanu[1,3*]

[1] *Interdisciplinary School of Doctoral Studies, University of Bucharest, 36-46 Mihail Kogalniceanu Bd, 050107 Bucharest, Romania.*
[2] *National Institute for Research & Development in Chemistry and Petrochemistry—ICECHIM Bucharest, 202 Spl. Independentei, 060021 Bucharest, Romania.*
[3] *Faculty of Chemistry, University of Bucharest, 90-92 Sos. Panduri, 050663 Bucharest, Romania.*

*** Corresponding author:** ileana.farcasanu@chimie.unibuc.ro

Keywords: selenium nanoparticles, biogenic synthesis, yeast surface display, Cys-rich peptides

Abstract

The dual nature of selenium as both beneficial and detrimental is intrinsically associated with a narrow therapeutic window. Nano-selenium has gained recognition as a novel alternative to selenium with reduced toxicity and increased reactivity. Microbial synthesis of selenium nanoparticles (SeNPs) broadens their biomedical and agricultural applications, allowing targeted drug delivery and low-risk biofortification. Microorganisms provide stabilizing biomolecules that additionally enhance the bioactivity and biocompatibility of these nanostructures [1]. Due to their genetic versatility, yeasts can be utilized as biotechnological platforms that can be manipulated in favor of both SeNPs production and cellular mechanism investigation [2]. This study investigates the possibility of using the yeast surface display technique for the expression of cysteine-rich selenite-processing peptides as an improved method for the production of customized biogenic SeNPs.

DNA encoding for cysteine-rich proteins of fungal origin, i.e., *Saccharomyces cerevisiae* metallothionein Cup1 (entire coding sequence and Cys-rich fragments) and *Trichoderma reesei* hydrophobins, were amplified through Polymerase Chain Reaction (PCR) and cloned into the pYD1 vector, which is specifically designed for yeast surface display of recombinant proteins. Some of the recombinant constructs were transformed into *S. cerevisiae* yeast cells. To assess the influence of sodium selenite (Na_2SeO_3) on the transformants' growth, the strains were grown on Na_2SeO_3-enriched media. The heterologous expression of the metallothionein and its subsequences was observed through fluorescent microscopy, due to a green fluorescent protein (GFP) integrated in their structure. The cell morphology and selenium accumulation by the transformants were assessed by electronic microscopy with Energy Dispersive X-Ray (EDX).

The sizes of the PCR products confirmed the specific amplification of the DNAs of interest, ranging from ~ 0.7-0.9 kb in the case of CUP1 and its subsequences and ~ 0.5 kb in the case of hydrophobin sequences. Fluorescent microscopy confirmed the expression of the chimeric constructs. The macroscopic characteristics of Na_2SeO_3-exposed yeast transformants, i.e., red pigmentation, indicated selenite reduction. Electronic microscopy images of yeast transformants in relation to the EDX assay confirmed the synthesis of biogenic SeNPs, while the normal cell morphology was maintained.

Genes encoding selenite-processing peptides were successfully cloned into the pYD1 yeast surface display plasmid. The expression of the recombinant proteins in *S. cerevisiae* was confirmed. Biogenic SeNPs were obtained through genetically modified yeast-mediated synthesis. Future perspectives will address SeNPs extraction and characterization.

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PO13. Application of a fluorescent H_2O_2 biosensor to identify and mitigate intracellular redox stress for more sustainable bioprocesses

Valentin Schachinger[1,2]* Jennifer Staudacher[1,2], Victor Mendes Honorato[1], Brigitte Gasser[1,2]

[1] *Department of Biotechnology, Institute of Microbiology and Microbial Biotechnology, BOKU University, Vienna, Austria.*
[2] *Austrian Centre of Industrial Biotechnology (ACIB), Vienna, Austria.*

*** Corresponding author:** valentin.schachinger@boku.ac.at

Keywords: *Pichia pastoris*, redox engineering

Abstract

Yeasts are widely used organisms for commercial, heterologous protein production, especially *Pichia pastoris* (syn. *Komagataella phaffii*). This yeast is specifically known for its naturally high secretion efficiency, which is commonly engineered with countless approaches to enhance its protein production capabilities further. High production, however, comes with drawbacks for the cells, such as the generation of H_2O_2 during oxidative protein folding. These might lead to reduced viability and cellular stress responses, which in turn diminish protein quality, yield, and the efficiency of the bioprocess. Therefore, using in vivo biosensors to measure stresses becomes invaluable for choosing bioprocess and cell engineering targets, as dye-based detections are often nonspecific and do not allow for real-time measurements.

Microscale cultivations were performed in a BioLector I (M2p-Labs / Beckman Colter) employing three different filter modules. A 620 nm excitation filter was used to access scattered light and biomass levels. Predominantly oxidized HyPer7 signal was measured using a 488 nm excitation / 520 nm emission, while a 400 nm excitation / 510 nm emission filter was used for measuring the reduced form. All experiments were carried out in flower plates at 25 °C, 1200 rpm, and 1 mL volume. Fed-batch cultivations were conducted in 1.8 L DASGIP benchtop bioreactors (Eppendorf). Four bioreactors were operated in parallel for each process: one contained the parental strain and the other three were used for the evaluation of the best redox engineered overexpression clones selected from microscale screenings.

In this work, we tested the applicability of a genetically encoded, fluorescent, H_2O_2-responsive biosensor, named HyPer7 [1], which we have recently adapted for use in *P. pastoris*. The cultivations in microbioreactors with online monitoring of the biosensor oxidation/reduction status showed a clear difference in redox stress between wild-type and recombinant protein production strains. Indeed, high-producing strains also showed higher oxidation signals. Further engineering with redox stress abolishing strategies reduced the obtained oxidation signals again, pointing towards reduced cellular stress. The redox-engineered strains also exhibited increased protein titers and biomass in fed-batch bioreactor cultivations.

Our data shows the applicability of a redox biosensor to identify and mitigate intracellular redox stresses occurring during recombinant protein production. H_2O_2 biosensors are also envisioned to be applicable to increase productivity and sustainability of other types of bioproduction processes, where reactive oxygen species pose a problem.

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PO14. Leveraging carbon-conservative pathways for improved yields

Viktor C. Persson*

Division of Biotechnology and Applied Microbiology, Lund University, Sweden.

*** Corresponding author:** biotech.viktor@outlook.com

Keywords: xylose, weimberg, lignocellulose, *Saccharomyces cerevisiae*, yeast

Abstract

Lignocellulose, a major byproduct of agriculture and forestry, is rich in sugar polymers [1]. Upon hydrolysis, it yields mainly glucose—easily utilized by microbes—and xylose, which is less efficiently used [2]. Despite advances using XR/XDH and XI pathways, these routes lose ~33% of carbon due to decarboxylation reactions. In contrast, oxidative xylose pathways like the Weimberg and Dahms pathways offer higher carbon efficiency at the cost of energy efficiency [3]. The Weimberg pathway, found primarily in bacteria from xylose-rich but carbon-scarce aerobic environments, converts xylose to α-ketoglutarate without carbon loss. However, expressing it in yeast remains challenging. This poster presents an overview of the topic, as well as recent progress on engineering the Weimberg pathway in *S. cerevisiae*.

The Weimberg pathway was added using an optimized protocol for multiplex CRISPR using in vivo homologous recombination and PCR adapters [4]. Bottlenecks were evaluated using microtiter plates to screen multicopy plasmids carrying genes encoding proteins for the individual pathway reactions. The final strain was exposed to laborative evolution, analyzed with metabolomics and genomics to identify significant changes, and benchmarked compared to a reference XR/XDH strain in bioreactors with continuous monitoring of growth using a home-made spectrophotometer attachment for the liquid handler.

Several advances have been made recently in the expression of the Weimberg pathway in yeast, including identifying new homologues with improved activities [5]. These findings, as well as our group's recent experimental findings, are also summarized in the contribution. In short, the Weimberg pathway has the potential to improve carbon yields of lignocellulosic bioprocesses by avoiding the carbon loss in decarboxylation reactions for the formation of TCA intermediates.

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PO15. Yeast surface display as a sustainable strategy for heavy metal removal from water

Lavinia Liliana Ruta*, **Andrei Barboi, Ileana Cornelia Farcasanu**

Faculty of Chemistry, University of Bucharest

*** Corresponding author:** lavinia.ruta@chimie.unibuc.ro

Keywords: *Saccharomyces cerevisiae*, heavy metals, waste bioremediators

Abstract

Heavy metals in water are a major environmental concern due to their toxicity and persistence. This study, conducted at the University of Bucharest, investigates the use of *Saccharomyces cerevisiae* for bioremediation. Known for its metal-binding properties and resilience, this yeast shows promise in removing metals like lead, cadmium, and mercury from polluted water. We examine its biosorption capacity, metal specificity, and tolerance under lab conditions, with a focus on optimizing uptake and exploring metal recovery. Our findings support the potential of yeast-based systems as a sustainable, low-cost solution for water purification.

We employed the yeast surface display technology to engineer *S. cerevisiae* for enhanced heavy metal uptake. Synthetic peptides and metal-binding proteins were genetically fused to the Aga2p protein, enabling their expression on the yeast cell surface via the Aga1-Aga2 anchoring system. The constructs were introduced into yeast cells using standard transformation protocols. Expression and surface localization were confirmed by fluorescence microscopy. Metal uptake assays were performed in controlled conditions using synthetic water wastes containing defined concentrations of heavy metals (e.g., Cd^{2+}, Cu^{2+}, Ni^{2+}), followed by quantification through atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS).

Engineered *S. cerevisiae* strains expressing synthetic metal-binding peptides on their surface showed significantly enhanced uptake of heavy metals compared to wild-type controls. Metal uptake was confirmed by ICP-MS analysis. Surface expression of peptides was validated by fluorescence microscopy, showing >90% of cells displaying the fusion synthetic peptides. The biosorption efficiency was highest at pH 5.5–6.0 and decreased at higher ionic strengths. These results demonstrate the potential of yeast surface display as a platform for targeted and efficient bioremediation of heavy metal-contaminated water. Advantages over traditional methods include lower cost, reusability, and reduced secondary waste. These results highlight the potential of yeast surface display as a sustainable and efficient alternative for heavy metal bioremediation.

Engineered *S. cerevisiae* strains displaying synthetic peptides via the Aga2 system efficiently remove heavy metals from water. This method outperforms wild-type yeast and rivals conventional treatments, offering a sustainable, low-cost, and scalable bioremediation solution with potential for metal recovery.

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PO16. Exploring xylose sensing and utilization in *Saccharomyces cerevisiae* through modulation of the Snf3p glucose receptor

Bruna Bolzico[1,2]*, Viktor Persson[3], Raul Comelli[1,2], Marie Gorwa-Grauslund[3]

[1] *Grupo de Procesos Biológicos en Ingenieria Ambiental, Facultad de Ingenieria y Ciencias Hídricas (FICH), Universidad Nacional del Litoral (UNL), Santa Fe, Argentina.*
[2] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.*
[3] *Biotechnology and Applied Microbiology, Department of Process and Life Science Engineering, Lund University, Lund, Sweden.*

*** Corresponding author:** bbolzico@fich.unl.edu.ar

Keywords: D-Xylose, sugar signaling, *Saccharomyces cerevisiae*, Snf3p receptor

Abstract

Through extensive metabolic engineering, *Saccharomyces cerevisiae* has been enabled to ferment D-xylose, an abundant sugar found in lignocellulosic biomass, allowing its conversion into biofuels. In *S. cerevisiae*, sugar sensing and signaling are closely linked to metabolic control, facilitating fast and efficient glucose uptake and catabolism. However, despite being actively metabolized, the regulatory response to xylose does not fully align with a fermentative pattern, which may hinder its optimal utilization. This highlights the need for a better understanding of how *S. cerevisiae* senses xylose. In this work, we aimed to evaluate D-xylose sensing at the cell surface level by exploring signals from the Snf3p low-glucose receptor. We modulated the signaling activity by deleting and overexpressing SNF3 and assessed its impact on xylose utilization in an engineered *S. cerevisiae* strain.

We used the previously established HXT2p-GFP biosensor [1,2] for the in vivo monitoring of the Snf3p signaling pathway using flow cytometry. The physiological performance of SNF3-wild-type, SNF3-deletant, and SNF3-overexpressing strains was compared in shake flask experiments using xylose as the carbon source. Growth, sugar consumption, and by-product accumulation were monitored over time.

Collectively, our data revealed that Snf3p receptor can recognize xylose extracellularly and activate downstream signalling in both non-engineered and xylose-metabolizing *S. cerevisiae* strains. In a strain lacking SNF3 and expressing the XR–XDH pathway for xylose catabolism, the biosensor response suggested that xylose-derived intracellular metabolites contribute to induction of the Snf3p-responsive HXT2 reporter and can partially bypass the requirement for the Snf3p receptor. Overexpression of SNF3 caused constitutive HXT2 signaling in the absence of sugar; however, in the presence of xylose, induction was further boosted, supporting the notion that xylose is sensed by the membrane receptor. Activation of this signaling pathway was functionally linked to xylose metabolism, as evidenced by increased biomass formation and reduced acetate production upon SNF3 deletion, whereas SNF3 overexpression resulted in the opposite phenotype during xylose utilization.

These findings reinforce that xylose is weakly recognized by *S. cerevisiae* via the low glucose receptor Snf3p. Investigating xylose signaling may uncover regulatory constraints limiting its use. This could enable the engineering of novel regulatory targets for improved utilization of non-conventional sugars in fermentative bioprocesses.

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PO17. New chassis strains of *Yarrowia lipolytica* for recombinant protein production

Djamila Onésime[1]*, Esteban Lebrun[1], Goran Stanajic Petrovic[1], Ewelina Celińska[2], Jean-Marc Nicaud[3,4]

[1] *Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas, France.*
[2] *Department of Biotechnology and Food Microbiology, Poznan University of Life Sciences, ul. Wojska Polskiego 48, 60-637 Poznań, Poland.*
[3] *Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas, France.*
[4] *Yalicolor, 20 avenue Hoche, 78190 Trappes, France.*

*** Corresponding author:** djamila.onesime@inrae.fr

Keywords: *Yarrowia lipolytica*, chassis strain, recombinant protein, protein expression, protease deletion, microbial cell factory

Abstract

Yarrowia lipolytica has emerged as a robust non-conventional yeast platform for the production of recombinant proteins and other high-value biomolecules. Its strong metabolic capacity and resistance to environmental stress make it an attractive host for industrial biotechnology. However, to meet the increasing performance demands of protein expression systems, further strain optimization is needed—particularly in terms of genetic stability, proteolytic background, and engineering flexibility.

We constructed new *Y. lipolytica* chassis strains (JMY9438 and JMY9451/9452) with three auxotrophies to facilitate advanced genetic manipulations. To reduce proteolytic degradation of secreted proteins, we deleted using a dual-guide CRISPR/Cas9 strategy] [1], five extracellular protease genes and a peroxidase gene in the JMY9451/9452 background. Recombinant protein production was evaluated in strains harboring one, two, or three integrated copies of a target gene, under the control of a hybrid promoter [2]. Protein levels were assessed by quantifying extracellular expression in culture supernatants.

The triple-auxotrophic strains demonstrated efficient transformation and stable integration of target constructs. Expression levels increased with the number of gene copies up to two; no additional gain was observed with a third copy. The protease-deletion strain with a single gene copy yielded the highest recombinant protein production per cell. In contrast, the strain with two copies and intact proteases showed the highest total protein output. These results suggest a trade-off between protease activity and gene dosage in optimizing expression [3].

We developed optimized *Y. lipolytica* chassis strains specifically designed for the production of recombinant proteins. Protease deletions provide a favorable background for secretion-based expression, and triple auxotrophies support flexible engineering [3]. These strains offer a versatile and robust platform for industrial biotechnology applications.

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PO18. Marine yeasts as biotechnological platforms: Focus on *Yarrowia lipolytica* YlTun15

Boutheina Bessadok*, **Saloua Sadok**

Institut National des Sciences et Technologies de la Mer- Laboratoire de Biotechnologie Bleue et Bioproduits Aquatiques B3Aqua-La Goulette, University of Carthage, Tunis-Tunisia.

*** Corresponding author:** boutheina.bessadok@instm.rnrt.tn

Keywords: Marine yeast, *Yarrowia lipolytica*, enzyme, biosurfactant, biochemical composition, application.

Abstract

Marine yeasts, isolated from diverse marine environments, exhibit unique adaptations such as high osmotolerance, thermostability, and resistance to industrial inhibitors, making them valuable for biotechnological applications. Their capacity to grow in seawater media offers sustainable benefits by reducing freshwater use and enabling the utilization of marine biomass, notably in bioethanol production. Additionally, these yeasts show enhanced biosynthesis of bioactive compounds for different uses (e.g., pharmaceuticals, nutraceuticals, etc.) [1-2]. *Yarrowia lipolytica* has shown versatility in biotechnological processes. However, in Tunisia, the exploitation of marine yeast remains limited. This study highlights the potential of *Y. lipolytica* YlTun15, isolated from the gills of farmed *Dicentrarchus labrax*, emphasizing its marine origin and attributes that support sustainable bioprocesses and innovative biotechnologies in the region.

To explore its potential, *Yarrowia lipolytica* YlTun15 (Acc. N° MF327143.1) was cultured in various media to extract bioactive compounds and assess potential applications. A comprehensive biochemical analysis was first conducted in accordance with ISO 17025:2017 standards, including assessments of minerals via optical emission ICP, lipids, proteins, amino acids, and carbohydrate content [3], as well as monitoring growth kinetics and biomass productivity [4]. Subsequent research focused on enzyme [5], pigment [6], and biosurfactant synthesis [7]. Given its unique metabolic profile, fatty acid composition was analyzed in detail to evaluate its suitability for biodiesel production. Finally, random mutagenesis was employed to explore the potential for enhancing biodiesel-related traits among resulting variants [8]. The data were subjected to 5% variance analyses using SPSS 24.0 software.

The YlTun15 strain exhibits a distinctive biochemical profile with 25% carbohydrates and a high lipid content of 37% dry matter. Its mineral composition is dominated by potassium (80%), with calcium (13%) and magnesium (7%). Protein content reached 26%, with 64.4% of essential amino acids. Cytotoxicity assays on HEK 293 cells indicated enhanced viability, supporting its safety [3]. The carotenoid fraction revealed the presence of astaxanthin and fucoxanthin as major compounds. YlTun15 also produces biosurfactants (7.7 mg) capable of degrading diesel. In addition, it secretes an extracellular alkaline protease with an optimal pH of 9 and a temperature of 45 °C, which has been identified as a serine protease [5]. Fatty acid analysis revealed an SFA>PUFA>MUFA profile, consistent with biodiesel requirements under international standards. Finally, random mutagenesis yielded two promising mutant variants, MY-2 and MY-3, with enhanced potential for biodiesel production [8].

The marine YlTun15, in wild and mutant forms, demonstrates considerable potential as a multifunctional microbial resource owing to its GRAS status, diverse biochemical profile, and ability to produce high-value compounds. Current research focuses on optimizing targeted metabolites for incorporation into fish feed to enhance aquaculture performance.

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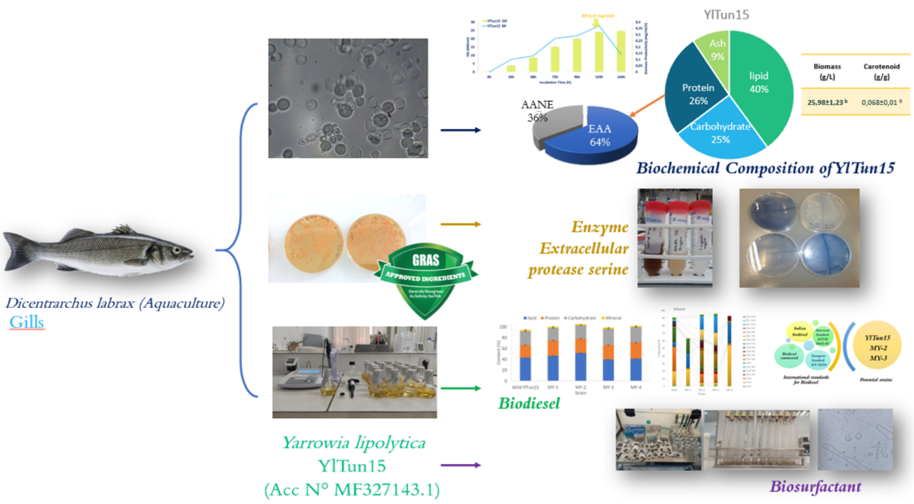

Graphical abstract. Promising application of Yltun15

PO19. Brewer's spent grain and crude glycerol: Sustainable substrates for 2-phenylethanol production by *Yarrowia lipolytica*

Sara Mitri[1,2], Nicolas Louka[2], Tristan Rossignol[3], Richard Maroun[2], Mohamed Koubaa[1*]

[1] *Université de Technologie de Compiègne, Ecole Supérieure de Chimie Organique et Minérale, TIMR (Integrated Transformations of Renewable Matter), Centre de Recherche Royallieu, CS 60319, CEDEX, 60203 Compiègne, France.*
[2] *Centre d'Analyses et de Recherche, Unité de Recherche Technologies et Valorisation Agro-alimentaire, Faculté des Sciences, Université Saint-Joseph de Beyrouth, Mar Roukos - Dekwaneh, P. O. Box 1514 - Riad El Solh, Beirut 1107 2050, Lebanon.*
[3] *Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas, France.*

*** Corresponding author:** m.koubaa@escom.fr

Keywords: 2-Phenylethanol, brewer's spent grain, *Yarrowia lipolytica*, biorefinery, microbial fermentation, crude glycerol

Abstract

2-Phenylethanol (2-PE) is a natural aromatic alcohol used in cosmetics, food, and the pharmaceutical industries. To reduce production costs and support sustainability, this study explores the use of brewer's spent grain (BSG) and crude glycerol (CG), two agro-industrial by-products, as substrates for 2-PE production by genetically modified *Yarrowia lipolytica*, which is capable of starch degradation and fermentation via the shikimate pathway.

A genetically engineered *Y. lipolytica* strain (JMY9385) expressing amyloglucosidase (AMG) was developed to hydrolyze starch in BSG. Fermentations were conducted in BSG based media with various YE and CG concentrations. Optimization used a central composite design. 2-PE and sugar levels were quantified by HPLC; elemental analysis determined C/N ratios, and data were analyzed using ANOVA.

The engineered strain JMY9385 produced up to 1.52 g/L of 2-PE in BSG-based medium supplemented with 2.87 g/L YE and 45.9 g/L CG after 168 h. Compared to the chassis strain, JMY9385 showed enhanced starch hydrolysis and 2-PE synthesis without the addition of glucose or enzymes. CG had a significant positive effect, while YE had a limited impact.

This study demonstrates efficient 2-PE production from low-cost BSG and CG using engineered *Y. lipolytica*, without the need for expensive precursors [1]. The optimized process is cost-effective, sustainable, and demonstrates the potential of agro-industrial waste in microbial aroma biosynthesis.

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PO20. Bioproduction of 2-phenylethanol by *Yarrowia lipolytica* on sugar beet molasses as a low-cost substrate

Sara Mitri[1,2], Nicolas Louka[2], Tristan Rossignol[3], Richard Maroun[2], Mohamed Koubaa[1*]

[1] *Université de Technologie de Compiègne, Ecole Supérieure de Chimie Organique et Minérale, TIMR (Integrated Transformations of Renewable Matter), Centre de Recherche Royallieu, CS 60319, CEDEX, 60203 Compiègne, France.*
[2] *Centre d'Analyses et de Recherche, Unité de Recherche Technologies et Valorisation Agro-alimentaire, Faculté des Sciences, Université Saint-Joseph de Beyrouth, Mar Roukos - Dekwaneh, P. O. Box 1514 - Riad El Solh, Beirut 1107 2050, Lebanon.*
[3] *Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas, France.*

*** Corresponding author:** m.koubaa@escom.fr

Keywords: 2-phenylethanol, *Yarrowia lipolytica*, agro-industrial by-product, fermentation, natural aromas, sugar beet molasses

Abstract

2-phenylethanol (2-PE) is a valuable aromatic alcohol with diverse applications in cosmetics, food, beverages, and pharmaceutical industries. Currently, 2-PE is produced either through chemical synthesis or by extraction from plant materials. However, both conventional production methods have their own limitations. Therefore, there is a need for more eco-friendly and cost-effective approaches to produce natural 2-PE. Biotechnological routes, particularly microbial fermentations, hold promise for the natural production of 2-PE, especially when utilizing low-cost substrates.

In this study, 2-PE was produced by de novo synthesis via the shikimate pathway, using the yeast *Yarrowia lipolytica* in a medium composed of sugar beet molasses (SBM) and yeast extract (YE) as carbon and nitrogen sources, respectively. A genetically engineered strain was generated, in which the SUC2 gene was transformed, expressing the invertase enzyme, enabling *Y. lipolytica* to efficiently utilize SBM as a cost-effective substrate. Fermentations were optimized via response surface methodology. Specifically, a central composite design enabled the optimization of the concentrations of the carbon and nitrogen sources. 2-PE production and sugar consumption were analyzed using HPLC, and biomass was monitored via OD_{600}.

The engineered *Y. lipolytica* strain JMY9398 successfully utilized sucrose in sugar beet molasses, producing up to 0.71 g/L of 2-PE under optimized conditions (44.14 g/L SBM, 3.2 g/L YE). Compared to 0.13 g/L produced by the chassis strain JMY8032. Response surface analysis showed that SBM had a strong positive effect, whereas YE had a nonlinear influence. Sugar consumption was complete within 48 h, and the optimal C/N ratio was ~16.5, supporting efficient 2-PE biosynthesis.

This work shows the successful use of engineered *Y. lipolytica* strain to convert low-cost sugar beet molasses into 2-PE via de novo biosynthesis [1]. The optimized process offers a sustainable and cost-effective method for natural fragrance production, demonstrating potential for industrial application through the valorization of agro-industrial by-products.

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PO21. Exploring yeast biodiversity and process conditions for optimizing ethylene glycol conversion into glycolic acid

Vittorio Senatore,[1] Riccardo Milanesi,[1] Fiorella Masotti,[1] Stefania Pagliari, [1] Luca Campone, [1,2] Immacolata Serra, [1,2] Paola Branduardi[1,2]*

[1] *Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy.*
[2] *NBFC, National Biodiversity Future Center, Palermo, Italy.*

*** Corresponding author:** paola.branduardi@unimib.it

Keywords: *Saccharomyces cerevisiae*, *Scheffersomyces stipitis*, glycolic acid, ethylene glycol, biodiversity, polyethylene terephthalate

Abstract

Plastics have become an indispensable material in many fields of human activity, with production increasing every year; however, most plastic waste is still incinerated or landfilled, and only 10% of the new plastic is recycled even once. Among all plastics, polyethylene terephthalate (PET) is the most produced polyester worldwide; ethylene glycol (EG) is one of the two monomers released by the biorecycling of PET. While most research focuses on bacterial EG metabolism, this work reports the ability of *Saccharomyces cerevisiae* and nine other common laboratory yeast species not only to consume EG, but also to produce glycolic acid (GA) as the main by-product [1].

S. cerevisiae's ability to metabolize EG was first investigated during growth in the presence of glucose; secondly, a two-step bioconversion of EG to GA was optimized by a design of experiment (DoE) approach, to understand the determining process parameters. Then, to improve the process performance, nine non-*Saccharomyces* yeasts were screened for high EG consumption with high GA production. This ultimately allowed the design of a single-step bioprocess in 2-L bioreactors with the best producer.

The two-step bioconversion of EG to GA by *S. cerevisiae* was optimized by a DoE approach, obtaining 4.51 ± 0.12 g/L of GA with a conversion of 94.25 ± 1.74% from 6.21 ± 0.04 g/L EG. To improve the titer, screening of yeast biodiversity identified *Scheffersomyces stipitis* as the best GA producer, obtaining 23.79 ± 1.19 g/L of GA (yield 76.68%) in bioreactor fermentation, with a single-step bioprocess.

This work laid the foundations for studying the physiology of EG metabolism in different yeasts, revealing yeasts as an interesting biorefinery platform for the upcycling of EG to GA. Further studies are required to understand better the genes and metabolic pathways involved in EG oxidation.

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PO22. Characterization of Rhodotorula toruloides metabolism for up-cycling of ethylene glycol to glycolic acid

Valeria Mapelli[1,2]*, Vitorio Giorgio Senatore[1], Alīna Reķēna[3], Petri-Jaan Lahtvee[3], Paola Branduardi[1,2]

[1] *Department of Biotechnology and Biosciences, University of Milan-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy.*
[2] *NBFC, National Biodiversity Future Center, Palermo, Italy.*
[3] *Department of Chemistry and Biotechnology, Tallinn University of Technology, Akadeemia Tee 15, Tallinn, Estonia.*

*** Corresponding author:** valeria.mapelli@unimib.it

Keywords: *Rhodotorula toruloides*, polyethylene terephthalate, ethylene glycol, glycolic acid, agro-food, waste valorization

Abstract

The agro-food chain generates a substantial amount of waste, which includes not only lignocellulosic biomass but also plastic, used for both protective films and packaging. Thanks to advances in enzymatic hydrolysis, it is now possible to envision an upcycling process valorising each waste stream through microbial fermentation.

We first explored the ability of the oleaginous red yeast *Rhodotorula toruloides* to catabolize ethylene glycol (EG) obtained by the hydrolysis of polyethylene terephthalate (PET), in the presence of glucose in controlled batch bioreactors. Secondly, we focused on the physiology of EG catabolism in the presence of xylose as the sole carbon source, as well as in a medium containing both glucose and xylose, which mimics the potential sugar composition of hemicellulose waste.

Our results [1] show that EG is metabolized to glycolic acid (GA) in all tested conditions. Remarkably, we report that the consumption of EG improves xylose-based bioprocess, possibly alleviating a cofactor imbalance by regenerating NAD(P)H. Consumption of EG in the presence of glucose starts after the onset of the nitrogen limitation phase, and results in a yield of 100 % (mol GA/mol EG). In addition, *in silico* analyses integrated with the available omics data allowed us to identify a set of genes putatively involved in EG metabolism in *R. toruloides*.

Based on our results, *R. toruloides* can be considered a promising candidate for the production of GA from EG. Nonetheless, its robustness and the improved fitness on xylose could be coupled to develop efficient hemicellulose-based biorefineries, where *R. toruloides* could be exploited both for the production of microbial oils from residual biomasses and to produce GA from EG.

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PO23. Fed-batch fermentation and biomass recycling strategy to achieve high-level production of *Yarrowia lipolytica* lipase Lip2 in the methylotrophic yeast *Pichia pastoris*

Shilpa Mohanty[1,2], Babbal[1,3], Shivani Chauhan[1], Mohini Talwar[1], Yogender Pal Khasa[1*]

[1] *Department of Microbiology, University of Delhi South Campus, New Delhi-110021, India.*
[2] *Laboratory of Optobiotechnology, School of Biotechnology, Jawaharlal Nehru University, New Delhi-110067, India.*
[3] *International Centre for Genetic Engineering and Biotechnology, ICGEB Campus, Aruna Asaf Ali Marg, New Delhi-110067, India.*

*** Corresponding author:** ypkhasa@south.du.ac.in

Keywords: High-cell density cultivation, *Pichia pastoris*, cell retention, *Yarrowia lipolytica* lipase Lip2, biomass recycling, fed-batch fermentation

Abstract

Pichia pastoris is a methylotrophic yeast with efficient protein secretion systems and strong promoters. However, oxygen limitation, reduced biomass, and higher heat generation contribute to extended fermentation runs. This triggers a cascade of unfavourable events such as cell lysis and protein degradation, hampering volumetric product yields, necessitating alternate protein synthesis strategies. In this study, a novel *Pichia*-based cell recycling strategy was introduced as a substitute for the batch and fed-batch fermentation approaches. Using *S. cerevisiae* α-secretion signal, an enhanced expression of the *Yarrowia lipolytica* lipase Lip2 was targeted in *P. pastoris*. Lipase-like Lip2 is crucial for transesterification reactions in the food, textiles, and cosmetics industries. Given the commercial significance of Lip2 and its relevance in green chemistry, the *Pichia*-cell recycling strategy enhanced its volumetric yields compared to conventional fed-batch fermentation conducted in this study.

The *Y. lipolytica* Lip2 gene was cloned in the *E. coli* and *P. pastoris* expression vectors. The pET28a-Lip2 and pETSUMO-Lip2 were expressed in *E. coli* BL21 (DE3) cells, and the solubility profile was analyzed by ultrasonication. The pPICZαA-Lip2 was transformed in *P. pastoris* X-33 cells via electroporation. The expression was conducted in methanol-based medium (BMMY) till 120 h at 30 °C. The activity of Lip2 was calculated via *p*NPP assay. The fed-batch fermentation of the hyper-producer was conducted in a 2.5L BMMY in a BioFlo 115 bioreactor (NBS). The cell retention studies included growing cells till OD_{600} 10 in glycerol-based medium and resuspending in BMMY till 48 h. The cells were harvested; supernatant (Lip2) was stored, and the cells were resuspended in BMMY for 48 h. This step was repeated for 5 cycles. Control was set up where Lip2 expression was undertaken in shake-flask till 240 h. Cell retention was performed for 60, 72, 84, and 96 h to optimize yields till 300, 360, 420, and 480 h of induction.

The pET28a-Lip2 expression yielded inclusion bodies. Lip2 fused with the SUMO tag did not improve solubility. Thus, Lip2 expression was targeted in *P. pastoris*. The pPICZαA-Lip2 expression in BMMY showed successful Lip2 secretion at ~35 kDa. The tributyrin agar plate assay showed a hydrolysis zone, indicating Lip2 activity. The expression of the hyper producer at induction OD_{600} 10 and 20 yielded 438.83 and 420.09 mg/L of Lip2 with an activity of 220.89 and 203.78 U/mL, respectively. Lip2 was glycosylated, as proven by *in vitro* deglycosylation analysis. The fed-batch fermentation resulted in a maximum WCW of 332.67 g/L (78 h) and Lip2 of 5.25 g/L at 60 h post-induction. Cell retention studies at the 72 h interval yielded 1794 mg/L of Lip2 after termination of 5 cycles. In control, 465 mg/L Lip2 was obtained at 144 h with a decline to 31 mg/L at 360 h. Cell recycling, with over 100% cycle efficiency, ensured a maximum volumetric productivity of 4.98 mg/L/h and a specific product yield of 47 mg/g DCW [1].

The *Pichia* cell recycling strategy is superior to the conventional methods. The continuous removal of spent medium maintains product quality, especially for proteins that are heat/pH-sensitive or have a tendency to form aggregates. It has the potential to generate high product yields without high-scale bioreactors and long fermentation runs [2].

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PO24. High-cell-density fermentation of *Yarrowia lipolytica* on hexadecane for the production of triacylglycerol

Antonia Keil[1,2]*, Elodie Vlaeminck[2], Evelien Uitterhaegen[2], Karel De Winter[2], Wim Soetaert[1,2]

[1] *Department of Biotechnology (BW25), Centre for Industrial Biotechnology and Biocatalysis (InBio.be), Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium.*
[2] *Bio Base Europe Pilot Plant (BBEPP), Rodenhuizekaai 1, 9042 Ghent, Belgium.*

*** Corresponding author:** antonia.keil@bbeu.org

Keywords: *Yarrowia lipolytica*, hexadecane, triacylglycerol, plastic valorization

Abstract

Global plastic production has increased exponentially since the 1950s, reaching an annual production of 366.9 Mt in 2020. By 2050, 4,725 Mt of plastics will have accumulated [1], much of which is not separated by polymer type, making recycling difficult and resulting in landfilling, mismanagement, or incineration [2]. A promising valorization strategy for this mixed plastic waste is thermochemical pyrolysis, which yields a mixture of hydrocarbons [3], followed by yeast bioconversion into higher-value products [4] (Figure 1). This study utilized hexadecane, a representative mid-chain hydrocarbon found in pyrolysis oil, for triacylglycerol (TAG) production by the oleaginous yeast *Yarrowia lipolytica* in a pulse-feed bioreactor fermentation strategy towards high cell densities.

A wild-type *Y. lipolytica* strain with a deletion of MHY1, implemented to prevent hyphal growth, was used in this study. The cells were cultivated on 4.2 g/L YNB medium with 3 g/L yeast extract, and 5 g/L $(NH_4)_2SO_4$ in shake flasks and in the bioreactor with an additional 5 g/L KH_2PO_4. Initial batch concentration of hexadecane in the shake flask experiments was 2, 10, and 20 vol%. Bioreactor cultivations were conducted in 3.6 L Eppendorf DASGIP® glass reactors, starting with the previously selected batch concentration of hexadecane. Two duplicate fermentations at two different pH set-points were conducted to investigate by-product accumulation. The process parameters, including dissolved oxygen, agitation speed, and air flow, were controlled, while the off-gas composition was monitored using GC-MS.

After one day of *Y. lipolytica* cultivation on various initial hexadecane batch concentrations in shake flasks, the same cell dry weight was reached. Thus, confirming that concentrations of up to 20 vol% cause no inhibition effect. The native adaptation mechanisms of *Y. lipolytica* for improved hydrophobic substrate uptake in a biphasic fermentation led to a high degree of emulsification in the bioreactor. Due to this, high cell densities were reached after 90 h of cultivation while TAGs were simultaneously accumulated. The reduction of the pH set-point prevented the accumulation of citrate as a by-product, thereby significantly improving biomass and TAG yields.

Y. lipolytica exhibits exceptional adaptation mechanisms to hydrophobic, water-immiscible fermentation feedstocks such as hexadecane, enabling high cell densities not previously demonstrated with any other microbe.

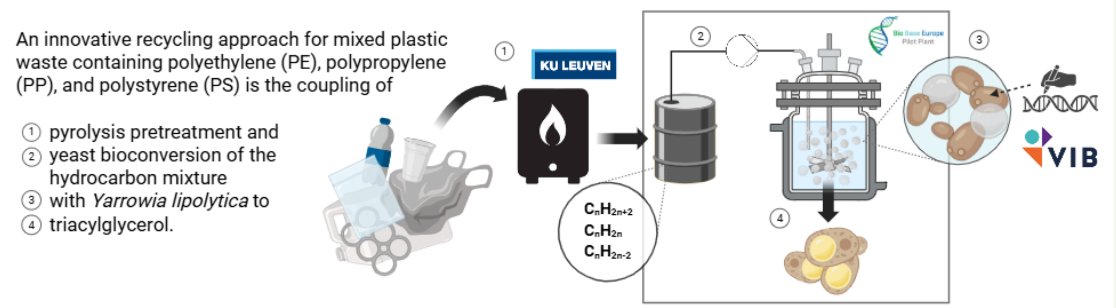

Figure 1. Triacylglycerol production from mixed plastic waste via coupled pyrolysis and fermentation.

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PO25. Model-based optimization of crude glycerol bioconversion by oleaginous yeasts

Roberto Albergo[1]*, Antonio Caporusso[1], Linda Bianco[1], Egidio Viola[1], Nicola Di Fidio[2], Isabella De Bari[1]

[1] *Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Laboratory for Processes and Technologies for Biorefineries and Green Chemistry, ENEA C.R. Trisaia S.S. 106 Jonica 75026 Rotondella (MT), Italy.*
[2] *Department of Biosciences, Biotechnology and Environment, University of Bari "Aldo Moro", Via E. Orabona, 4, 70125, Bari, Italy.*

*** Corresponding author:** roberto.albergo@enea.it

Keywords: Crude glycerol, *Yarrowia lipolytica*, *Rhodosporidium toruloides*, response surface methodology, circular bioeconomy

Abstract

In a sustainable bioeconomy, the identification of low-cost and renewable feedstocks is essential to improve the sustainability performance of bioprocesses. Crude glycerol (GLY), accounting for approximately 10% w/w of biodiesel production, represents a promising carbon source due to its low market value, high availability, and suitability for microbial fermentation without the need for costly pretreatments. Various yeasts can utilize GLY to produce value-added compounds such as organic acids, microbial lipids, polyols, and carotenoids. In this study, the biotechnological potential of crude glycerol was explored using two wild-type yeasts: *Y. lipolytica* DSM8218 [1] for citric acid (CA) production, and *R. toruloides* DSM70398 [2] for the simultaneous production of lipids and carotenoids. The aim was to optimize the fermentation parameters for each process and evaluate the scalability of the optimized conditions, providing new insights into microbial valorisation of crude glycerol.

The study was split into two parts. *Y. lipolytica* DSM 8218 was cultivated in flasks batch fermentations using crude glycerol as the carbon source and either yeast extract or ammonium sulfate as nitrogen sources to generate citric acid. An analytical approach based on Response Surface Methodology (RSM) was applied to assess the influence of varying glycerol levels (ranging between 20 and 40 g/L) and C/N ratio (from 60 to 180) on citric acid production. *R. toruloides* DSM 70398 was grown in flasks with ammonium sulfate as the nitrogen source for lipid and carotenoids synthesis. A design incorporating three factors and three levels, known as Box-Behnken design, was utilized to explore the impacts of glycerol concentration (30–60 g/L), C/N molar ratio (10–30), and temperature (25–33 °C). The optimal conditions for both microorganisms were validated through experiments carried out in bench-scale bioreactors.

The highest yield of citric acid for *Y. lipolytica* was obtained by utilizing 33 g/L of glycerol and maintaining a C/N ratio of 141 with the addition of yeast extract. During fed-batch fermentation in a 2 L stirred tank reactor, a transition from producing mannitol to citric acid occurred due to increased agitation at 800 rpm, accompanied by a change in cell morphology from pseudo-mycelial to round, yeast-like cells. In the case of *R. toruloides*, optimal conditions involving 49 g/L of glycerol, a C/N ratio of 22, and a temperature of 29 °C resulted in a predicted carotenoid concentration of 24.5 mg/L and a lipid concentration of 2.28 g/L. Experimental verification confirmed the production of carotenoids and lipids at 26.2 ± 1.5 mg/L and 2.05 ± 0.21 g/L, respectively, demonstrating consistency with the expected values without any statistically significant differences ($p < 0.005$, $n = 3$).

Crude glycerol is a viable and sustainable feedstock for microbial production of high-value biochemicals. Using *Y. lipolytica* and *R. toruloides* under optimized conditions demonstrated efficient conversion into citric acid, lipids, and carotenoids. The results highlight the feasibility of integrating such processes into circular bioeconomy models.

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PO26. *Yarrowia lipolytica* as a dual platform for sustainable protein and tailored lipid production from carboxylates

Paula de la Huerta Bengoechea*, **Elia Tomás Pejó**

Biotechnological Processes Unit, IMDEA Energy, Avda. Ramón de la Sagra 3, 28935, Madrid, Spain.

*** Corresponding author:** paula.delahuerta@imdea.org

Keywords: *Yarrowia lipolytica*, single-cell proteins, short-chain fatty acids, lipids

Abstract

The rising global food demand intensifies production systems, causing emissions, deforestation, biodiversity loss, and land degradation. Protein intake is projected to reach 87 g/person/day by 2031 [1], intensifying pressure on conventional sources and underscoring the need for sustainable alternatives such as yeast single-cell proteins (SCP). Similarly, global consumption of oils such as soybean, sunflower, and rapeseed oils is expected to rise. *Yarrowia lipolytica* is a promising candidate for both SCP and oil production from organic wastes due to its oleaginous trait and high protein content, superior to many plant and animal sources. It also offers a favourable essential amino acid (aas) profile and produces valuable nutrients such as omega-3s, making it suitable for food and feed. Furthermore, *Y. lipolytica* can utilize short-chain fatty acids (SCFAs) present in anaerobic fermentation digestates as alternative carbon sources to the costly glucose-based media.

Y. lipolytica ACA DC 50109 was used for SCP and oil production. Synthetic media was prepared with yeast nitrogen base (7.5 g/L $(NH_4)_2SO_4$: q.s.f C/N of 7.5) and 29.2 g/L SCFAs based on previous reports [2]. Real media (RM) derived from SCFA-rich autofermented digestates with 32.8 g/L of SCFAs was also used undiluted (URM) and diluted (50% v/v, 50RM). C/N ratios of 7.5 and 33 were studied. Shake flask cultures (100 mL) were incubated at 28 °C, pH 6.8 and 150 rpm to compare the effect of the SCFA concentration in the production of SCP and oil profile. Promising conditions were scaled up to 1-L bioreactor where pH was kept at 6.8 with 2 M H_2SO_4, and dissolved O_2 at 35% with agitation (200-600 rpm) and aeration (0.5-2 L/min). Cultures were inoculated at OD_{600} = 1 and fermentations ended when ≥90% of carbon source was consumed. SCFA were quantified by HPLC. SCP and oil content were determined by BCA protein analysis and GC-FID, respectively.

In synthetic media, *Y. lipolytica* produced 15 % w/w SCP and fatty acid (FA) profile was dominated by C18:1t (28 % w/w) followed by C18:2t and C16:0 (both 15% w/w) with 5 % w/w presence of odd-chain fatty acids (OCFA). In shake flaks fermentation with 50RM, SCP increased to 22 % w/w and predominant FA were C18:2c and C18:1t, accounting for 29 % w/w and 25 % w/w, respectively. URM fermentation resulted in only a slight increase in both lipids and SCP production, but significant differences in the FA profile were observed. In this case, C18:1t accounted for 32 % w/w and C18:2c was 16 % w/w of the total. In both RM fermentations, OCFA increased to 7 % w/w. Reactor results validated lipid and SCP levels, indicating that upscaling had no significant effect. The different produced FA demonstrated the influence of the initial SCFAs and media composition on lipid synthesis. However, SCP production remained consistent in all conditions, highlighting *Y. lipolytica* potential as SCP source.

Y. lipolytica efficiently valorizes SCFA-rich media. The distinct presence of FA in the yeast with diverse applications (e.g., C18:1t offers health benefits and C18:1t is valuable for industrial uses in cosmetics and bioplastics) demonstrates the possibility to produce tailored industrially-relevant FA profiles combined with SCP.

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PO27. Expression and bioprocess optimization of engineered human IL-2 for cost-effective targeted cancer immunotherapy

Shubham Sharma, **Yogender Pal Khasa***

Department of Microbiology, University of Delhi South Campus, New Delhi- 110021, India

*** Corresponding author:** yogi110@gmail.com

Keywords: Interleukin-2, *Pichia pastoris*, *E. coli*, Tregs, cancer immunotherapy, PEGylation, recombinant protein, SUMO fusion, bioeconomy

Abstract

According to the American Cancer Society's 2024 report, cancer caused 9.7 million deaths worldwide in 2022, with over 20 million new cases reported, projected to rise to 35 million annually by 2050. High treatment costs—up to $72,000 for immunotherapy and $1,000–$12,000/month for chemotherapy—limit accessibility. Interleukin-2 (IL-2), produced by activated $CD4^+$ T cells, stimulates cytotoxic $CD8^+$ T and NK cells, making it a promising cancer immunotherapy [1]. However, its clinical use is restricted by severe side effects such as renal toxicity, vascular leak syndrome, and expansion of regulatory T cells (Tregs) via high-affinity IL-2Rα (CD25) binding [2]. To address this, a mutant hIL-2 with reduced CD25 affinity and retained IL-2Rβγ binding was developed. Expression was carried out in *E. coli* and *Pichia pastoris*—with *E. coli* offering high yield and *P. pastoris* enabling secretion and native-like post-translational modifications, aligning with bioeconomic production goals.

The native hIL-2 gene was cloned into pET vectors for *E. coli* and pPICZαA (with/without C-terminal 6×His tag) for *P. pastoris*. A Small Ubiquitin-like Modifier (SUMO) fusion tag was used in *E. coli* to enhance solubility. A site-directed mutagenesis library targeting CD25-binding residues was also created. Expression in *E. coli* BL21 (DE3) under IPTG induction led to inclusion body formation for native hIL-2, while SUMO-tagged hIL-2 showed improved solubility. Inclusion body proteins were solubilized using 8 M urea, refolded, and purified via Ni-NTA affinity chromatography. For yeast expression, *P. pastoris* X-33 was transformed with pPICZαA-hIL-2 constructs by electroporation. Zeocin-resistant colonies were induced with methanol in BMMY and BMGY media at varying OD_{600} values. Secreted proteins were collected from the supernatant, analyzed by SDS-PAGE, and purified using Ni-NTA chromatography via the C-terminal His tag.

Expression in *E. coli*: Native hIL-2 expression in *E. coli* resulted in high yields but was localized largely in inclusion bodies. Fusion with SUMO tag improved solubility significantly, allowing for recovery of functional protein after nickel affinity purification. The use of denaturation and refolding protocols enabled the retrieval of structurally intact and active protein from inclusion bodies. Expression studies of various IL-2 mutants were carried out in *E. coli*, followed by refolding and biophysical characterization (fluorescence spectroscopy).

Expression in *P. pastoris*: *P. pastoris* transformants successfully secreted hIL-2 into the culture medium, with expression levels influenced by induction OD and methanol concentration. The 6×His-tag facilitated easy purification with high purity and acceptable yield. The absorbance maxima of native IL-2 and mutant IL-2 produced in *E. coli* and *P. pastoris* were overlapping.

Human IL-2 is expressed as inclusion bodies in *E. coli*; however, the SUMO tag enhanced its solubility. In the case of *P. pastoris*, hIL-2 was successfully expressed and secreted in extracellular medium with a maximum yield between 90 to 100 μg/ml.

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PO28. Bioconversion of agro-byproducts into industrial products via solid-state fermentation

Widya Nur Habiba*, **Hajar Naciri, Moises Couder-García, Yusmel Gonzalez Hernandez, Patrick Perré, Rafik Balti**

Université Paris-Saclay, CentraleSupélec, Laboratoire de Génie des Procédés et Matériaux, Centre Européen de Biotechnologie et de Bioéconomie (CEBB), 3 rue des Rouges Terres, 51110, Pomacle, France

*** Corresponding author:** widya-nur.habiba@centralesupelec.fr

Keywords: Solid-state fermentation, agro-industrial residues, bioproducts, biomaterials, circular economy

Abstract

Solid-state fermentation (SSF) is an ancient yet highly relevant biotechnological process, offering advantages such as low water consumption, reduced energy and capital investment, and sustainable biomass valorization. At the Pomacle-Bazancourt biorefinery site near Reims, France— bringing together around 15 industrial companies over more than 250 hectares and strongly connected to local agricultural cooperatives—a large volume of agro-industrial residues remains underutilized. These agro-industrial residues comprise rapeseed cake, hemp oil cake, wheat and alfalfa byproducts, along with beetroot pulp.

This study investigates the potential of SSF to convert these agricultural byproducts into high-value products, including bioactive compounds (vitamins, enzymes, antibiotics), flavoring agents, natural pigments, and biomaterials. Different microbial strains were assessed, combined with physical and chemical pretreatments of substrates. Experiments were first performed in Erlenmeyer flasks and subsequently scaled up in packed-bed bioreactors. Growth kinetics, as well as heat and mass transfer, were systematically evaluated. In addition, Digital Twin simulations were applied to support scalability assessment and process optimization.

Preliminary experiments revealed promising applications in food, feed, cosmetics, and textile industries. Optimization efforts focused on maximizing yields while balancing biological performance with industrial feasibility. A parallel market analysis was conducted to assess economic potential.

The results highlight SSF as a circular bioeconomy strategy that promotes industrial symbiosis, reduces waste, and generates added value from agro-industrial residues. This approach offers a sustainable pathway for transforming byproducts into viable economically and environmentally valuable products.

Acknowledgments

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PO29. New strategies to produce proteins for the food industry using yeast

Romain El Andaloussi*, **Emile Gresset, Emma Navarro, Louis Mouterde, Florent Allais, Nabila Imatoukene**

URD Agro-Biotechnologies Industrielles (ABI), CEBB, AgroParisTech, 51110 Pomacle, France.

*** Corresponding author:** romain.elandaloussi@agroparistech.fr

Keywords: Animal protein, sustainable, novel food, yeast, fermentation, medium optimization, scale-up

Abstract

Due to population growth, the demand for dietary protein is increasing. However, traditional production methods present limits in terms of sustainability. Precision fermentation offers an innovative solution by employing engineered micro-organisms to produce proteins of interest for food applications. In order to obtain food-grade ingredients with this technique, all the production process must follow specific regulations, like the novel food in Europe. We studied the production of an animal protein (confidential) largely used in food, in a yeast (confidential) expression system, different than *S. cerevisiae* and *P. pastoris*. Two ways of expressing the protein were studied: one by secretion in the culture medium, the other by accumulation in the cells, in order to examine the best method for industrialization. The production was performed in chemically defined mineral media to control nutrient composition and food-grade compatibility, improve yield, and reduce batch-to-batch variability.

We constructed two expression vectors, for secretion and accumulation, and transformed a yeast strain. The good integration of the gene of interest and the plasmid construction were assessed by Sanger sequencing. Different mineral media were screened, and the protein production was assessed by SDS-PAGE and the Bradford method. An experimental plan was conducted to identify the essential elements and optimize the media composition, using a Plackett-Burman experimental design.

SDS-PAGE method helped us identify the overall protein expression profile, and the Bradford method allowed us to quantify the soluble proteins. We were able to validate the interest of minimum mineral media against a complex organic one. The experimental plan showed a possibility to reduce the number of elements in the medium composition, allowing us to have a cost-efficient way of protein production for future scale-up study.

We will need to study the protein production in bioreactors to assess the possibility of industrial production. Additionally, only the total protein production was assessed, and purification methods will be developed to determine the content of the protein of interest. We will also develop a method to characterize the protein of interest.

PO30. Circular bioproduction of itaconic acid from volatile fatty acids using a genetically engineered *Yarrowia lipolytica* strain

Cosetta Ciliberti[1]*, Anna Martina Fiorentino[1], Zbigniew Lazar[2], Gennaro Agrimi[1] , Isabella Pisano[1]

[1] *Department of Biosciences, Biotechnologies and Environment, University of Bari Aldo Moro, Campus Universitario, via Orabona 4, 70125 Bari, Italy Interuniversity Consortium for Biotechnology (CIB), 34100 Trieste, Italy*

[2] *Department of Biotechnology and Food Microbiology, Faculty of Biotechnology and Food Science, Wrocław University of Environmental and Life Sciences, Chelmonskiego 37, 51-630 Wrocław, Poland*

*** Corresponding author:** cosetta.ciliberti@uniba.it

Keywords: *Yarrowia lipolytica*, itaconic acid, synthetic biology, bioeconomy, volatile fatty acids, waste valorization

Abstract

Itaconic acid (IA) is a high-value dicarboxylic acid used in the production of biopolymers and other industrial materials. Microbial fermentation represents a sustainable route for IA synthesis, but common hosts such as *Aspergillus terreus* and *Ustilago maydis* are limited by several challenges. *Yarrowia lipolytica* is a non-conventional yeast with recognised industrial potential and is widely used as a heterologous platform. This study focuses on the metabolic engineering of *Y. lipolytica* to redirect intracellular fluxes and optimize transport mechanisms for the selective production of IA. In addition to glucose and glycerol, the engineered strain was tested for IA production using volatile fatty acids (VFAs) derived from the anaerobic digestion of the organic fraction of municipal solid waste (OFMSW), aiming to valorise waste streams in a circular bioeconomy framework.

Metabolic engineering was performed using the YaliCraft toolkit, a CRISPR-Cas9-based platform for marker-free, multi-gene editing in *Y. lipolytica*. To reduce citric (CA) and isocitric acid (ICA) secretion, YlYHM2 and YlCEX1 genes were inactivated. Heterologous transporters from *A. terreus* (mttA, mfsA) and *U. maydis* (mtt1, itp1) were introduced to improve mitochondrial export of *cis*-aconitate and extracellular secretion of IA. Complete IA biosynthetic pathways from both fungi were expressed and compared in a shared genetic background. The optimised strain was cultivated in bioreactors using glucose and glycerol, employing a fed-batch strategy [1]. To evaluate IA production from waste-derived carbon sources, synthetic VFAs solutions were tested using acetate, propionate, and butyrate individually (5–20 g/L) and as a simulated mixture, supplemented or not with amino acids. Biomass, substrate consumption, IA titers, and by-products were measured.

Inactivation of native transporters significantly reduced CA and ICA secretion, enhancing flux toward IA. Both fungal pathways were functionally expressed in *Y. lipolytica*, with a synergistic increase in IA observed upon co-expression. The engineered strain, YM4B493, achieved 0.343 mol IA/mol glucose and 0.256 g/L/h IA productivity in bioreactor cultivation. When tested on individual VFAs in flasks, no growth or production inhibition was observed with acetate and butyrate, with butyrate yielding the highest IA titers and conversion efficiency. In contrast, propionate caused inhibition at higher concentrations, resulting in poor growth and low IA productivity. A simulated VFAs mixture were also tested under two culture conditions, with and without amino acids, showing no significant differences in IA production, which were 4.22 ± 0.12 g/L and 3.84 ± 0.54 g/L, respectively. These findings confirm the strain's robustness and potential for waste-derived IA production using YM4B493 strain.

The engineered *Y. lipolytica* strain efficiently produced IA from conventional and waste-derived substrates. Butyrate supported the highest yield, while propionate impaired growth and production. Consistent IA production from VFAs mixtures, regardless of amino acid addition, highlights the strain's versatility in circular bioeconomy applications.

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PO31. An efficient process strategy to co-produce microbial oil and carotenoids utilizing biowaste-derived sugar-rich media

Ishtiak Malique Chowdhury*, Cristina Álvarez, José Luis Fernández-Rojo, María José Negro, Antonio D. Moreno

Advanced Biofuels and Bioproducts Unit, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas, Avda. Complutense 40, 28040 Madrid, Spain.

*** Corresponding author:** ishtiakm.chowdhury@ciemat.es

Keywords: biowaste, oleaginous yeasts, fed-batch bioprocessing, microbial oil, carotenoids

Abstract

Sustainable aviation fuels (SAF) represent the only viable short-term alternative to fossil fuels in the aviation industry. With the growing number of air travellers, the demand for SAF is expected to rise significantly. To meet this increasing demand, yeast cells such as *Rhodosporidium toruloides* will play a crucial role in converting sugar derived from biowaste into SAF precursors, including microbial oils. In addition, *R. toruloides* can simultaneously co-produce carotenoids, allowing the development of a more cost-competitive multi-product bioprocessing approach. To lower overall production costs, optimizing culture conditions is an effective strategy to improve conversion yields. This research aims to address two challenges simultaneously: 1) the effective conversion of biowastes to obtain sugar-rich media for 2) the co-production of microbial oil and carotenoids using the yeast *R. toruloides*.

Agro-food waste was subjected to homogenization, crushing, and centrifugation to obtain the soluble fraction, which was then used as media for yeast growth. On the other hand, lignocellulose-based pruning residues were pretreated by steam explosion (190 °C, 10 min, and 40 mg H_2SO_4/g residue) and then subjected to enzymatic hydrolysis to obtain the resulting hydrolysate [1]. These two sugar-rich media were used to cultivate *R. toruloides* under a fed-batch culture strategy in 0.5 L bioreactors at 30 °C, pH 6, and maintaining the dissolved oxygen level at 20% from saturation [2]. Cell viability, sugar concentration, organic acid concentration, intracellular lipid content, cell biomass concentration, and total carotenoid content were monitored during the cultivation process.

R. toruloides was grown first in agro-food waste to promote cell growth. After sugar depletion, the lignocellulosic hydrolysate was fed continuously to trigger lipid accumulation. This strategy takes advantage of the different C/N ratios of these substrates to boost cell growth and lipid production, respectively. By using the continuous feeding strategy, *R. toruloides* accumulated up to 52-55% (w/w) of total lipids and up to 575 μg/g dry cell weight of carotenoids. Final lipid conversion yields from consumed sugars accounted for up to 0.28-0.30 g/g, which represents 85-90% of the theoretical production.

The results presented herein show the high potential of combining different waste sources as well as optimizing the operational strategies to develop robust and efficient bioprocessing approaches.

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PO32. Bioconversion of fruit waste into bioethanol and single-cell protein using *Saccharomyces cerevisiae* strains

Anahit Shirvanyan[1,2,3]*, Ayaulym Daniyarova[4], Anait Vassilian[1,5], Anna Poladyan[1,2,4], Kumar Gopalakrishnan[6], Duman Orynbekov[4], Kairat Bekbayev[4], Karen Trchounian[1,2,3,4]

[1] *Research Institute of Biology, Yerevan State University, 1 A. Manoogian str., 0025 Yerevan, Armenia.*
[2] *Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University, 1 A. Manoogian str., 0025 Yerevan, Armenia.*
[3] *Microbial Biotechnologies and Biofuel Innovation Center, Yerevan State University, 1 A. Manoogian str., 0025 Yerevan, Armenia.*
[4] *Research School of Food Engineering, Shakarim University, st. Glinka, 20A, Semey, Kazakhstan, 071410.*
[5] *Department of Ecology and Nature Protection, Faculty of Biology, Yerevan State University, 1 A. Manoogian str., 0025 Yerevan, Armenia.*
[6] *Institute of Chemistry, Bioscience and Environmental Engineering, Faculty of Science and Technology, University of Stavanger, Box 8600 Forus, 4036 Stavanger, Norway.*

*** Corresponding author:** anahitshirvanyan@ysu.am

Keywords: *Saccharomyces cerevisiae*, fruit waste, biomass and single-cell protein production, bioethanol, fermentation efficiency, bioethanol production economics.

Abstract

Rising energy consumption, environmental pollution, global warming and food insecurity issues highlight the urgent need for industrial strategies focused on waste management, bioenergy, and alternative food sources. *Saccharomyces cerevisiae* serves as an efficient biotechnological platform for producing food products and biofuels, including bioethanol and biodiesel [1]. First-generation bioethanol, made from sucrose- or starch-rich materials, is easy to produce but limited by feedstock availability. In contrast, second-generation bioethanol from lignocellulosic biomass offers a promising alternative due to its high polysaccharide content. Fruit waste (FW), a lignocellulosic substrate rich in sugars and nutrients, offers a favorable medium for yeast-based bioconversion [2]. This study evaluates FW as a substrate for single-cell protein (SCP) and bioethanol production using *S. cerevisiae* ATCC 9804 and ATCC 13007 strains under varying substrate concentrations, pH, and oxygen levels.

S. cerevisiae ATCC 9804 (isolated from wine) and ATCC 13007 (isolated from beer, STA+) were purchased from Microbial Depository Center of Armbiotechnology Scientific and Production Center of NAS RA. FW (orange, kiwi, apple, and banana peels) were hydrolyzed at 120 °C for 45 min using autoclave and the filtrate (hydrolysate) was used in the study. Yeast growth in FW hydrolysates was assessed via specific growth rate (SGR) using spectrophotometer at 600 nm. Total sugar concentration was determined using a colorimetric method based on the interaction with phenol and sulfuric acid. The total nitrogen and protein content were determined using the Kjeldahl method. Ethanol concentration was determined using the potassium dichromate oxidation method. Fermentation efficiency (FE, %) is defined as the ratio between the observed and stoichiometric ethanol yield coefficients. The Minimal Ethanol Selling Price (MESP, € kg^{-1}) was calculated using a Discounted Cash Flow Rate of Return analysis.

The highest specific growth rate (0.5 ± 0.01 h^{-1}) was observed at pH 6.5 in non-diluted hydrolysates, which corresponds to ~250 g L^{-1} carbohydrates and 0.2 g L^{-1} total nitrogen concentration. The maximal protein production (21 g L^{-1} or ~50% protein by dry weight) was recorded during 24 h cultivation of *S. cerevisiae* ATCC 13007 under oxygen-limited conditions at pH 6.5. In contrast, the maximum ethanol production (109 ± 4 g L^{-1}) was observed at 6 h for both strains under aerobic conditions. The maximal FE was observed at 24 h cultivation of ATCC 13007 in 2-fold diluted hydrolysate under oxygen-limited conditions at pH 6.5. ATCC 9804 strain exhibits the highest FE during 48-hour cultivation in 5-fold diluted hydrolysate under aerobic conditions at pH 6.5. The lowest MESP (€0.87/kg) at pH 6.5 was achieved during 6-hour aerobic cultivation of ATCC 13007, while at pH 3.0, the lowest MESP (€1.05/kg) was attained through 24-hour aerobic cultivation of ATCC 9804 strain in a non-diluted sample.

Thus, FW can serve as a substrate for SCP and bioethanol production using *S. cerevisiae* ATCC 13007 and ATCC 9804 strains. Nitrogen supplementation and combination with fruit processing industries could further enhance SCP production and process efficiency. The proposed technology will align with the circular economy, addressing several global challenges.

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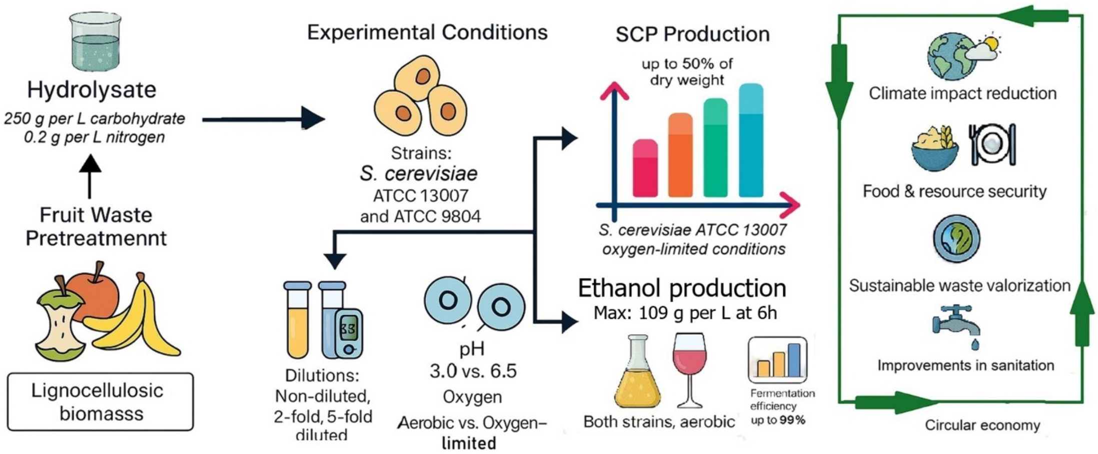

Graphical abstract

PO33. Combining nutrient-limiting conditions and osmotic stress for carotenoids production using the oleaginous yeast *Rhodosporidium toruloides*

Ana Susmozas, Daniela Durán, Ishtiak Malique Chowdhury, José Luis Fernández-Rojo, María José Negro, Antonio David Moreno*

Advanced Biofuels and Bioproducts Unit, Department of Energy, CIEMAT. Avda. Complutense 40, 28040 Madrid, Spain.

*** Corresponding author:** david.moreno@ciemat.es

Keywords: Cheese whey; antioxidants; colorants; red-yeasts

Abstract

Carotenoids are natural pigments of commercial interest due to their attractive coloration and antioxidant properties. As an alternative to current chemical carotenoid production or extraction from plant sources, these pigments can be produced sustainably by culturing oleaginous yeasts such as *Rhodosporidium toruloides* in agro-industrial residues.

In this study, *R. toruloides* was cultured in synthetic media mimicking the chemical composition of cheese-whey. With the aim of maximizing carotenoid production, different conditions were assessed including: 1) different initial inoculum concentrations simulating a single-batch (cell growth and carotenoids accumulation in one stage) and a two-batch process (cell growth and carotenoid accumulation separately); 2) different media with two different salt and total sugars concentrations, simulating a non-concentrated and a concentrated cheese whey streams; and 3) different nutrient supplementation. Sugar consumption, cell growth, cell viability, and total carotenoid production were evaluated under all different culture conditions. The results were then analyzed by a Design of Experiment (DoE) with the aim of determining the best conditions for maximizing carotenoid production, as well as sugar consumption and the final cell biomass concentration.

The results show different sugar consumption, cell growth, and total carotenoids accumulation profiles depending on the culturing conditions. In general, lower sugar consumption rates were observed, independently of the nutrient content, when using media with increased sugars and salt concentrations, which may result from the higher osmotic pressure of the media. The DoE assessment predicted a model showing a good fit for sugar consumption and cellular biomass production, with R^2 values of 89.3% and 85.1%, respectively. However, R^2 for carotenoid production was 59.5%. The model highlights the initial inoculum concentration having a positive effect ($p < 0.05$) on sugar consumption and final cell biomass concentration. However, no statistically significant effect was observed for carotenoid production under the tested conditions, reaching the highest carotenoid accumulation of about 1.2 mg/g of dry cell weight when using the higher inoculum concentration and no nutrient supplementation.

Cheese whey represents a suitable medium for carotenoid production using *R. toruloides*. The initial inoculum concentration was critical for promoting carotenoid production under nutrient-limiting conditions and osmotic stress, being a two-stage batch process, the strategy showed higher yields.

Acknowledgments

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PO34. A reliable protocol for microscale cultivation of strictly aerobic yeasts

Maria Jagodzińska, Maria Gorczyca, Ewelina Celińska*

Department of Biotechnology and Food Microbiology, Poznan University of Life Sciences, Wojska Polskiego 48, 60-637 Poznań, Poland.

*** Corresponding author:** ewelina.celinska@up.poznan.pl

Keywords: *Yarrowia lipolytica*, microscale cultivation, down-scaling, oxygen transfer, high-throughput cultures

Abstract

Microscale cultivation systems are essential tools for accelerating bioprocess development and strain screening. However, aerobic yeasts such as *Yarrowia lipolytica* present unique challenges when scaled down due to their high oxygen demand, tendency toward flocculation and adhesion, and acidogenic metabolism.

A comprehensive literature review was conducted using peer-reviewed studies focused on microscale cultivation formats, including conventional 96-well MTPs, flasks and tubes, square-MTP (Duetz system [1,2]), BioLector FlowerPlates, and microfluidic systems [3,4]. Key experimental parameters were extracted from recent studies by Celińska and Gorczyca (2024) [1] and Gorczyca et al. (2024) [2], providing a benchmark protocol for reliable, repeatable, and pH-stable micro-volume cultivation. Numerical data justifying the selected conditions were provided.

Square-MTP with sandwich lids and maleic acid buffering emerged as a reproducible and adequate configuration addressing *Y. lipolytica*'s physiological demands. Common limitations such as poor oxygen transfer, pH fluctuations, and inter-well variability were addressed through system design and media optimization. Stable, batch-to-batch repeatability was reached.

Microscale cultivation of *Y. lipolytica* is feasible with careful protocol adaptation. The use of validated systems, such as the presented protocol, ensures physiological relevance and full phenotype development. While not free from limitations, the proposed protocol offers a reasonable approach to reliably compare multiple phenotypes in parallel cultures of this demanding species.

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PO35. Unlocking the complexity of yeast fermentation broth through selective pervaporation and fractional condensation of volatile compounds

Pedro Arana-Agudelo, Violaine Athès, Cristian Trelea, Marwen Moussa*

Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood, 91120 Palaiseau, France.

*** Corresponding author:** marwen.moussa@agroparistech.fr

Keywords: Pervaporation, fractional condensation, volatile compounds, fermentation, yeast

Abstract

Fermentation processes are a cornerstone of the bioeconomy. Interest in using them towards the sustainable production of bio-based volatile organic compounds (VOCs) from renewable biomass resources is growing. Ethyl acetate (EA) is an industrially relevant VOC whose bio-based production has grown in interest as an alternative to the traditional oil-based production. The microbial production of EA by yeasts such as *K. marxianus* is limited by the cytotoxic effects that EA accumulation causes [1]. Extractive fermentation has emerged as a promising strategy to recover ethyl acetate while it is produced. Organoselective pervaporation (PV) offers attractive merits in this context [2]. Despite the organophilic character of PV membranes, water is the main constituent of the permeate as EA and other metabolites such as ethanol and acetate are diluted (< 10 g/L). The permeate can be condensed in multiple stages to obtain enriched fractions in targeted compounds.

A polydimethylsiloxane (PDMS) membrane with a total surface area of 50 cm^2 was used. Vacuum pervaporation experiments were conducted in recirculation mode using feed solutions at different temperatures and a permeate pressure of 3 mbar. The membrane permeance was determined and used for process modeling. Two in series condensers were set (C1 and C2). The temperature C1 was varied in the range [-80 °C - -10 °C], whereas the temperature of C2 was set at -80 °C. A simplified scheme of the experimental apparatus is illustrated in Figure 1. The mathematical model was an ordinary differential equation system. The phase equilibria in the condensers were defined as flash problems solved by minimizing the free Gibbs energy of the multiphase system. The model was simulated in MATLAB 2023b.

Figure 2 shows that the amount of EA condensed in C1 increases as the condensation temperature decreases. At -80 °C, EA is completely condensed in C1. The molar fraction of EA in C2 is maximal (0.96) at around -50 °C, which shows that the targeted EA is preferentially condensed in C2. The developed model made it possible to predict EA concentration during fractional condensation coupled to pervaporation and is valuable for optimizing EA recovery from fermentation media containing complex mixtures of volatile metabolites.

In this work, we studied the recovery of EA from aqueous solutions. A mathematical model for multi-stage pervaporation was developed. It brings important outputs towards the development of an integrated model dealing with the ultimate extractive fermentation process.

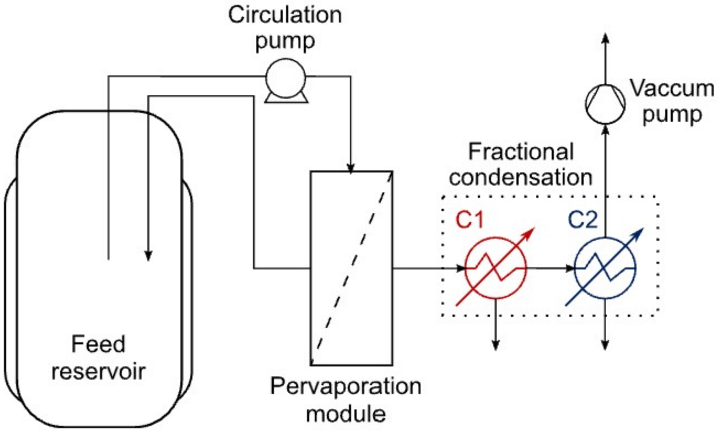

Figure 1. Scheme of the exp. apparatus

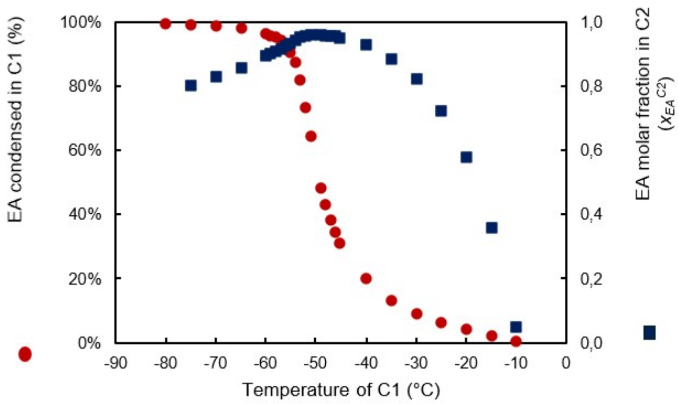

Figure 2. Condensed ethyl acetate in C1 as a function of the temperature of C1

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PO36. Valorisation of lignocellulosic residues into biofuels via integrated chemical and biological approaches

Antonio Caporusso[1]*, Federico Liuzzi[1], Roberto Albergo[1], Nicola Di Fidio[2], Egidio Viola[1], Isabella De Bari[1]

[1] *Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Laboratory for Processes and Technologies for Biorefineries and Green Chemistry, ENEA C.R. Trisaia S.S. 106 Jonica 75026 Rotondella (MT), Italy.*
[2] *Department of Biosciences, Biotechnology and Environment, University of Bari "Aldo Moro", Via E. Orabona, 4, 70125, Bari, Italy*

*** Corresponding author:** antonio.caporusso@enea.it

Keywords: Biorefinery, oleaginous yeasts, biofuels, lignocellulosic biomass, steam-explosion

Abstract

The growing need to reduce reliance on fossil resources calls for a shift toward sustainable production models based on renewable feedstocks [1]. In this context, biorefineries represent an innovative production model based on integrated processes capable of converting sustainable biomass into a wide range of high-value bioproducts. In particular, for the bioconversion processes, the wide availability of yeasts enables many processes of industrial interest. In fact, yeasts are versatile biocatalysts able to convert carbon sources into products of industrial interest [2]. The most relevant processes include the production of biofuels such as biodiesel and green diesel, as well as valuable compounds like carotenoids and organic acids. The integrated valorisation of bioresources, in particular of residues and side stream products, not only reduces environmental impact but also promotes a circular, bio-based economy, combining sustainability, innovation, and industrial competitiveness.

Wheat straw was used as feedstock. After chemical characterization, it underwent steam explosion at 203 °C for 5 min, following parameters optimized in literature. The pretreated biomass was enzymatically hydrolysed using Cellic™ CTec2 (Novozymes, Denmark) in a 2 L bioreactor at pH 4.8, 50 °C, and 180 rpm. The resulting hydrolysate served as the growth medium for the oleaginous yeast *Lipomyces tetrasporus* DSM 70314. Culture conditions, nitrogen source, and C/N ratio were optimized in 250 mL Erlenmeyer flasks. Optimal parameters were scaled up in 50 L bioreactors. Lipids were extracted from the yeast biomass and converted into oleochemicals such as biodiesel and green diesel.

This study optimized the conversion of wheat straw into microbial lipids by *L. tetrasporus* DSM 70314. Enzymatic hydrolysis reached 89% yield, at 17% solids/liquid ratio and 15 FPU. At the bench scale, three nitrogen sources (soy flour, urea, yeast extract) were tested at C/N ratios of 40–200. Under optimal conditions, soy flour and C/N 160, 37% biomass and 20% lipid yield (54% intracellular) were obtained. Nitrogen sources mainly affected biomass and lipid profile, while C/N ratio influenced lipid yield. Soy flour promoted 55% higher unsaturated fatty acids, while urea or yeast extract favoured saturated ones. Scaling to 50 L bioreactor yielded 23% lipid (72% of the theoretical maximum) with 68% lipid content. The extracted oil was converted into biodiesel and green diesel with properties comparable to palm or soy oils, suggesting its potential as a substitute for vegetable oils in advanced biofuel production.

These results demonstrate the potential of *L. tetrasporus* to convert wheat straw into lipids suitable for advanced biofuels. Optimal nitrogen source and C/N ratio significantly improved lipid yield and quality, making this process a promising route for sustainable biodiesel and green diesel production.

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PO37. Screening of cell disruption and solvent extraction strategies for high-yield microbial oil recovery from *Yarrowia lipolytica*

Marta Yabar[1,2]*, Elodie Vlaeminck[1], Karel De Winter[1], Evelien Uitterhaegen[1], Koen Quataert[1], Wim Soetaert[1,2]

[1] *Bio Base Europe Pilot Plant (BBEPP), Rodenhuizekaai 1, Ghent, 9042, Belgium*
[2] *Centre for Industrial Biotechnology and Biocatalysis (InBio.be), Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Ghent, 9000, Belgium*

* **Corresponding author:** marta.yabar@bbeu.org

Keywords: *Yarrowia lipolytica*, microbial oils, dry route extraction, wet route extraction, cell disruption, green solvents

Abstract

The aviation sector is facing increasing challenges due to the urgent need to reduce CO_2 emissions, driving the development of sustainable aviation fuels (SAF) as low-carbon alternatives to conventional jet fuel. Using oleaginous microorganisms, renewable carbon sources can be converted into microbial oils, which serve as SAF precursors. In this context, the oleaginous yeast *Yarrowia lipolytica* has gained great interest as a potential strong microbial platform for bio-based fuel production. However, the recovery of these intracellular microbial oils remains a major bottleneck in the economic and energy feasibility of microbial oil production processes. This screening aims to explore and compare different cell disruption pretreatments and solvent extraction strategies for microbial oil recovery from *Y. lipolytica*, to identify greener and more scalable downstream processing approaches [1-2].

A fermentation using *Y. lipolytica* was carried out to produce biomass rich in intracellular microbial oils. To recover this oil, a two-step approach was applied. First, the biomass was subjected to different cell disruption pretreatments - including mechanical and chemical pretreatments. Secondly, each pretreatment route was followed by solvent extraction, using both conventional and greener solvents.

Microscopy analysis after the pretreatment routes tested revealed substantial differences in disruption efficiency between methods. Mechanical pretreatment led to a higher degree of cell lysis. Interestingly, none of individual pretreatment methods tested resulted in complete disruption. Regarding the solvents tested, the reference system-chloroform: methanol 2:1-showed the highest extraction efficiency, as expected. However, several greener solvents, including acetone, cyclopentyl methyl ether, and diisopropyl ether also resulted in promising recovery yields while offering a more favorable environmental profile.

This study shows that mechanical disruption methods enhance the extraction of intracellular microbial oils from *Y. lipolytica*. Several greener solvents showed promising performance and may help reduce environmental and energy demands compared to conventional solvent systems.

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PO38. High-throughput screening and scale-up of yeast–feedstock combinations for bioprocess applications

Roberto Romano[1,2]*, Gaurav Rajauria[1,2], Francisco Victor Santos da Silva[1,2], John Morrissey[1,2]

[1] *School of Microbiology, University College Cork, Cork T12 K8AF, Ireland.*
[2] *SUSFERM Fermentation Science Centre, University College Cork, Cork T12 K8AF, Ireland.*

*** Corresponding author:** 122145086@umail.ucc.ie

Keywords: Circular economy, yeast, *Kluyveromyces marxianus*, waste product, high-throughput

Abstract

In biorefinery design, selecting the microorganism to be cultivated and the feedstock used to nourish it are key aspects of the process [1]. In line with circular economy principles, waste products—such as whey permeate or lignocellulosic materials—offer both economic and environmental advantages as fermentation feedstocks. However, these substrates are typically undefined media with variable and often unknown compositions, which can differ from batch to batch. This variability makes it challenging to select a microorganism that can fully exploit the feedstock. Moreover, different microorganisms are suited to different applications, making preliminary screening essential for each process. This project presents a fast and reliable high-throughput method to assess multiple yeast–feedstock combinations, aiming to identify the most promising strain for each substrate and optimize growth parameters such as temperature and nitrogen source.

The initial experimental setup was tested on 32 strains of the yeast *Kluyveromyces marxianus*, known for its industrial potential [2]. These strains were screened in parallel on three different media—whey permeate, potato liquor, and apple pomace—using 96-well plates. Ten of the best-performing strains were then further analyzed in the BioLector microbioreactor to optimize growth conditions in whey permeate by adjusting temperature and nitrogen source. Two strains were subsequently scaled up to a 500 mL bioreactor. The same experimental design was later applied to a broader range of yeast species with potential for industrial use. Differences in growth across the three feedstocks were assessed, and the top-performing strains for each substrate were scaled up to the microbioreactor for growth parameter optimization, followed by scale-up to a 500 mL bioreactor.

From the initial screening of *K. marxianus* strains, growth on apple pomace and potato liquor showed little variation among strains. In contrast, growth on whey permeate differed significantly. The ten strains with the highest biomass after 24 hours were scaled up to the BioLector for further analysis. In this second screening, two strains demonstrated superior performance in both final biomass and growth rate. Temperature and nitrogen source were then optimized to maximize growth rate. Scale-up in a 500 mL bioreactor confirmed the process's scalability. From the broader yeast species set, three top-performing strains were selected for each feedstock based on biomass after 24 hours. Their scale-up in the BioLector provided insights into growth optimization. Finally, the best strain for each feedstock—based on growth rate and biomass—was scaled up to the bioreactor stage.

This project has demonstrated how significantly yeast growth can vary between species—and even between strains of the same species—highlighting the importance of a structured workflow in biorefinery process design. The method proved to be both fast and reliable, with results successfully scalable in the lab up to a 500 mL bioreactor.

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PO39. Assessment of lignin content in Lithuanian agro-food industry by-products: Potential for enzymatic and fungal (mould-based) extraction and application opportunities in the circular economy

Aušra Šimonėlienė[1]*, Vilma Šipailaitė-Ramoškienė[2], Tomas Makaveckas[3]

[1] *Department of Food and Agrotechnology, Faculty of Informatics, Engineering and Technologies, Kauno kolegija, Pramonės pr. 22, Kaunas, LT-51189, Lithuania.*
[2] *Centre for Engineering Studies, Faculty of Informatics, Engineering and Technologies, Kauno kolegija, Pramonės pr. 22, Kaunas, LT-51189, Lithuania.*
[3] *Alytus Faculty, Kauno kolegija, Studentų g. 17, Alytus, LT-62252, Lithuania.*

*** Corresponding author:** ausra.simoneliene@kaunokolegija.lt

Keywords: Lignin analysis, fermentation, enzymes, chemical composition, sustainable food resource utilization

Abstract

Scientific studies have shown that agricultural residues are rich in lignin, cellulose, and hemicellulose, making them a promising raw material for paper and bio-composite production [1-3]. Lignin can be extracted enzymatically [4], a mild and environmentally friendly method that enables the efficient recovery of this valuable compound from biological waste for industrial use [5,6]. This study aimed to assess the lignin content in various by-products generated by the Lithuanian agro-food industry, to evaluate the potential for its enzymatic extraction, and to determine the suitability of these materials as raw resources within the circular economy framework. The analyzed biomass residues included straw, brewers spent grain (a by-product of beer production), coffee husks, buckwheat husks, and walnut shells. These materials were selected based on their availability in Lithuania and their potential biochemical composition.

Waste from the Lithuanian agro-food industry, including buckwheat hulls, walnut shells, coffee bean hulls, brewers' spent grain, and wheat straw, was studied. The lignin content in the samples was determined using the Klason method, based on the hydrolysis of biomass components with concentrated sulfuric acid. The principle of this method involves removing carbohydrate fractions, while the remaining acid-insoluble residue is considered to be lignin. The samples are first treated with 72% sulfuric acid at room temperature, then diluted and heated to ensure complete hydrolysis. The samples were dried and ground into a fine powder. Then, the samples were hydrolyzed with 72% sulfuric acid at room temperature for 1 hour. Later, the acid was diluted to approximately 3% concentration, and the samples were heated in an autoclave at 121 °C for 1 hour. Following hydrolysis, the residue was filtered, dried, and weighed to calculate the lignin content.

The lignin content varied significantly among the biomass samples. The highest lignin content was found in buckwheat husks at 35.16%, followed by walnut shells at 29.10%, and coffee husks at 20.06%. In contrast, brewers' spent grain and straw samples contained much lower lignin amounts, slightly above 8%. Nevertheless, even 8% lignin content in straw and brewers' spent grain is a significant source considering their abundance in agriculture and brewing industries. To achieve more sustainable lignin extraction and improve its efficient use for further industrial applications, it is recommended to apply enzymatic methods or white-rot fungi. These biotechnological approaches allow gentler and more environmentally friendly lignin extraction, reducing environmental impact and enhancing raw material utilization efficiency. These results highlight the potential of underutilized local biomass residues based on circular bioeconomy principles, aiming for sustainable resource management and waste reduction.

The lignin content varied significantly among the biomass samples: the highest lignin content was found in buckwheat husks (35.16%), followed by walnut shells (29.10%) and coffee husks (20.06%), while brewers' spent grain and straw samples had lower lignin content, slightly above 8%.

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PO40. The structure of β(1,3)/(1,6)-glucan and the susceptibility of *Cyberlindnera jadinii* cells to immunolabeling with β(1,3)-glucan monoclonal antibodies in relation to growth medium composition - an attempt to modulate the potential therapeutic properties of yeast biomass

Anna Bzducha-Wróbel[1]*, Pavol Farkaš[2], Sandra Bieliková[2], Alžbeta Čížová[2], Marzena Sujkowska-Rybkowska[3]

[1] *Institute of Food Sciences, Department of Food Biotechnology and Microbiology, Warsaw University of Life Sciences, Poland.*

[2] *Institute of Chemistry, Slovak Academy of Sciences,* [3]*Institute of Biology, Department of Botany, Warsaw University of Life Sciences, Poland.*

*** Corresponding author:** anna_bzducha_wrobel@sggw.edu.pl

Keywords: Glucan, synthesis, carbon sources, nitrogen sources, immunolabelling, structure analysis

Abstract

The increasing antibiotic resistance among pathogens is prompting research into new therapeutic agents for treating and preventing animal diseases. This aligns with the European Green Deal and the Farm-to-Fork strategy. Yeast biomass, rich in immunostimulatory polysaccharides like β(1,3/1,6)-glucan, is recognized as a functional feed additive that may enhance an animal's immune response. It is indicated that the chemical structure of yeast glucan is important for its biologically active properties. However, the relationship between the structure of the mentioned polysaccharide and its biological activity is not fully understood. Similarly, there is no precise data on the effect of yeast growth conditions on the chemical β(1,3/1,6)-glucan structure. The aim of the research was to determine the influence of various carbon and nitrogen sources on β(1,3/1,6)-glucan synthesis in fodder yeast *Cyberlindnera jadinii* DSM 2361, immunolabelling of this polymer in cells from applied growth conditions, and structural characterization of isolated β-glucan. Results showed that the yeast biomass composition varied with the growth medium, especially regarding glucan content. The highest levels of β(1,3/1,6)-glucan were achieved in cells cultivated using glycerol and deproteinated potato juice water as a nutrient source. Differences were also noted in the susceptibility of β(1,3)-glucan within the cells to specific antibodies. Cells grown in this medium showed the strongest antibody binding, while those in a medium with glycerol and yeast extract exhibited reduced susceptibility. Obtained results confirmed differences in the structure of the β(1,3/1,6)-glucan polymers considering side-chain length and branching frequency, as well as in the quantity of β(1,3)- and β(1,6)-chains, however, no visible relationship was observed between the structural characteristics of the isolated polymers and its susceptibility to immunolabeling in whole cells. Other outer surface components and molecules could mask, shield, protect, or hide epitopes from antibodies. β(1,3)-Glucan was more intensely recognized by monoclonal antibody in cells with lower trehalose and glycogen content. This suggests the need to cultivate yeast biomass under appropriate conditions to fulfil possible therapeutic functions. However, our *in vitro* findings should be confirmed in further studies using tissue or animal models.

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PO41. How does the cultivation of *Wickerhamomyces anomalus* on brewers' hops hydrolysate stimulate changes in the structural composition of mannoproteins that are important for the emulsifying properties of these cell wall components?

Anna Bzducha-Wróbel[1]*, Elisabete Coelho[2], Katarzyna Rybak[3], Monika Janowicz[3]

[1]*Katedra Biotechnologii i Mikrobiologii Żywności, Instytut Nauk o Żywności, Szkoła Główna Gospodarstwa Wiejskiego w Warszawie, Poland.* [2]*Department of Chemistry, University of Aveiro, Portugal,* [3]*Katedra Inżynierii Żywności i Organizacji Produkcji, Instytut Nauk o Żywności, Szkoła Główna Gospodarstwa Wiejskiego w Warszawie, Poland.*

*** Corresponding author:** anna_bzducha_wrobel@sggw.edu.pl

Keywords: *Wickerhamomyces anomalus*, mannoproteins structure, brewers' hops hydrolysate, emulsifying properties

Abstract

Yeast-derived mannoproteins (MP) are complex protein-sugar molecules that form the cell walls of these microorganisms. They can serve as a natural, vegan food additive with prebiotic, emulsifying, thickening, or stabilizing properties. The functional properties of yeast-derived MP are influenced by their composition and chemical structure, which depend on factors such as yeast species, culture conditions, and the method used to isolate these glycoproteins. The aim of this research was to assess how cultivating *Wickerhamomyces anomalus* CCY 38-1-13 yeast in substrates based on brewer's hop hydrolysate affects the chemical composition and structure of mannoproteins, as well as their emulsifying activity. The biomass of *W. anomalus* was obtained after cultivation in YPG medium (control), a medium prepared from a mixture of acidic and enzymatic hydrolysates of brewery hops (KEO medium), and a medium based on the enzymatic hydrolysate of residues after acidic pretreatment of the waste (EO medium). The results confirmed that the cultivation conditions for the yeast affect the protein and sugar content in the tested mannoprotein preparations. Furthermore, the preparations differed in the composition of individual glycosidic bonds in the saccharide fraction, including those that determine the degree of mannan branching. Additionally, we observed varying phosphorus content among the tested preparations. The particle size distribution in the obtained 50% oil-in-water emulsions depended on the type and concentration of the mannoproteins. Those isolated from biomass cultivated on brewer's hop hydrolysate exhibited better emulsifying properties than those from the control medium. After the designated storage period, the most stable emulsions contained 2.5% of the EO preparation in the aqueous phase.

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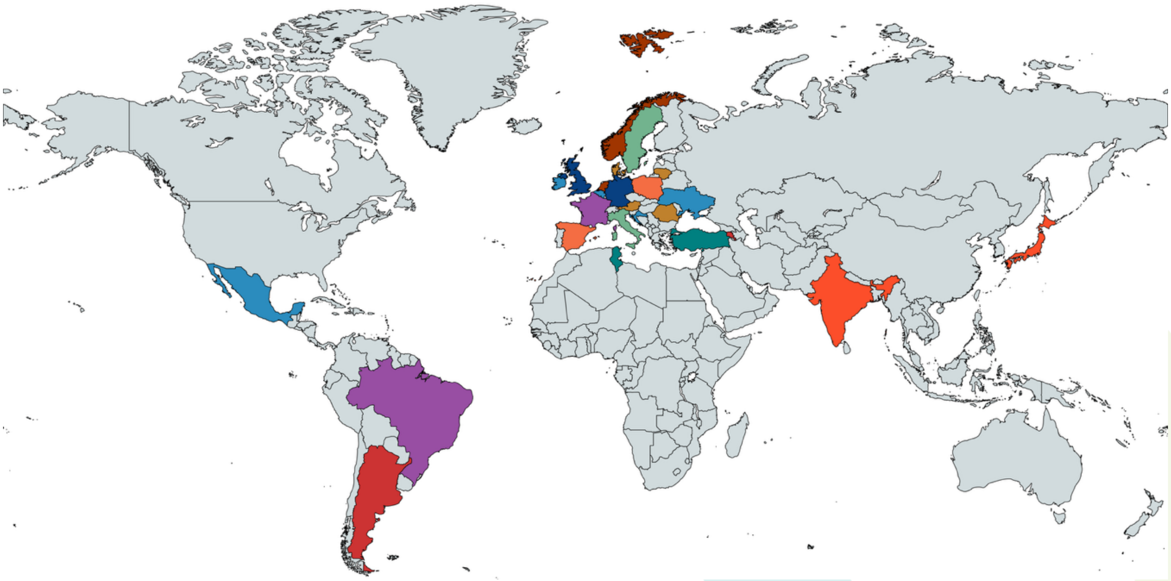

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