UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XL CICLO

Research Topic ID: XL – 1.3

Proponent: Prof. Stefania Brocca

Project Title: Biotechnological approaches for valorizing carbohydrate- and protein-rich biomasses from Italian industrial supply chains

Scientific background and 'open issues'

The transformation of waste biomasses into new bio-based materials and the development of sustainable processes are the basis for upcycling and circular economy processes. The development of new sustainable processes involves biocatalysts, the use of green solvents, and the utilization of waste biomasses [1, 2].

In this context, lipases and non-conventional media play an important role in the synthesis of new bio-based materials. Lipases are particularly attractive due to their chemo-, regio-selectivity, and ability to catalyze condensation reactions, often requiring mild reaction conditions [3]. Recombinant lipases are commonly produced in two main hosts, namely in *Escherichia coli* or *Pichia pastoris—Komagataella phaffii* cells. The recombinant production in *E. coli* requires the use of complex media (e.g. Luria-Bertani), and inducers, such as pure lactose or IPTG [4]. The replacement of conventional nutrients and inducers with waste biomasses paves the way to improve the sustainability of enzyme production, and the competitiveness of biocatalytic processes.

Non-conventional media such as ionic liquids, deep eutectics and bio-based solvents are safer solvents than traditional ones, and promotes condensation reactions, reducing the environmental impact of the processes [5]. Non-conventional media can have several effects on lipase activity and stability, spanning from enzyme activation to enzyme inhibition or denaturation [5, 6]. The effects of bio-based solvents on enzyme structure and activity is still unknown.

Objectives

The scientific aims of this project are to use waste biomasses for the recombinant production and immobilisation of industrially relevant lipases. Secondarily, recombinant lipases will be analysed to assess their stability/activity in bio-based solvents and their ability to synthesise sugar-based polyesters, in the perspective of designing new environmentally friendly plastic materials.

The potential biomasses to be employed in this project include cheese whey permeate, crude glycerol, starch wastes, and silk-based materials. Lipases from *Candida antarctica*, *Geotrichum kaustophilus* and *Geobacillus zalihae* are potential target lipases to be recombinantly produced and used for the synthesis of sugar-based polyesters, in the presence of bio-based solvents. This project may have the potentiality to assess the role of

waste biomasses in recombinant production of lipases and other industrially relevant enzymes, and to assess novel conditions for biocatalysis.

Methodologies

The experimental design involves setting up cultures to identify the optimal growth medium formulation maximising the use of waste biomasses, the cell growth and the recombinant enzyme production. Purification techniques will be used to obtain the pure enzyme, which will then be immobilised on non-canonical matrices derived from waste biomasses such as starch and silk-materials.

Biophysical techniques (e.g. circular dichroism and Fourier transform infrared spectroscopies) and activity assays using model substrates will be used to test the effects of bio-based solvents on the structure and the activity of the selected lipase(s). These experiments will be performed both on free and immobilised lipases using a panel of different bio-based solvents such as cyrene and cygnet

The synthesis of sugar-based polyesters will be carried out using the most promising lipase formulations and biobased solvents. Bio-based monomers, such as mucic acid, adipic acid, and glux-diol, will be used. These monomers have been synthesised in the laboratory of Prof. Cipolla, by chemically modifying glucose and galactose from the hydrolysis of lactose contained in cheese whey permeate.

Collaboration / Co-tutoring opportunities

This multidisciplinary project could involve the following collaborators:

- Dr. Luca Brambilla (BtBs), for his expertise in microbial cultures in bioreactors
- Prof. Antonino Natalello (BtBs), for his expertise in structural characterization of proteins
- Prof. Laura Cipolla (BtBs), for her experience in carbohydrates chemistry
- Dr. Alessandro Pellis, Università degli Studi di Genova, Dipartimento di Chimica e Chimica industriale, for his experience in biocatalysis
- Dr. Francesco Secundo, CNR, Istituto di Scienze e Tecnologie Chimiche "Giulio Natta" (Milano), for his experience in synthetic reactions catalyzed by lipases
- Prof. Daniela Ubiali, Università degli Studi di Pavia, Dipartimento di Scienze del Farmaco, for her expertise in biocatalysis.

Project's Sustainability & Mobility

The laboratory has extensive experience in enzymology and recombinant enzyme production in various hosts, including *E. coli* and *P. pastoris* cells. The employment of lipases in biocatalytic reactions, the assessment of their structural and catalytic properties will take advantage through collaborations with both in-house and external researchers.

Pertinent research articles published by the proposer:

- de Divitiis M, Ami D, Pessina A, Palmioli A, Sciandrone B, Airoldi C, Regonesi ME, Brambilla L, Lotti M, Natalello A, **Brocca S***, Mangiagalli M. Cheese-whey permeate improves the fitness of Escherichia coli cells during recombinant protein production. Biotechnol Biofuels Bioprod. 2023;16(1):30. doi: 10.1186/s13068-023-02281-8.

- Mangiagalli M, Carvalho H, Natalello A, Ferrario V, Pennati ML, Barbiroli A, Lotti M, Pleiss J*, **Brocca S***. Diverse effects of aqueous polar co-solvents on Candida antarctica lipase B. Int J Biol Macromol. 2020 May 1;150:930-940. doi: 10.1016/j.ijbiomac.2020.02.145.

Possible foreign institution for ordinary mobility:

- Enzymicals AG, Greifswald (Germany) (https://enzymicals.com/)
- Alessandro Pellis, Università degli Studi di Genova, Dipartimento di Chimica e Chimica industriale.

References

[1] Ravindran R, Jaiswal AK. Microbial Enzyme Production Using Lignocellulosic Food Industry Wastes as Feedstock: A Review. Bioengineering (Basel). 2016 Nov 16;3(4):30. doi: 10.3390/bioengineering3040030.
[2] Bell, E.L., Finnigan, W., France, S.P., Green, A.P., Hayes, M.A., Hepworth, L.J., Lovelock, S.L., Niikura, H., Osuna, S., Romero, E., Ryan, K.S., Turner, N.J., Flitsch, S.L., 2021. Biocatalysis. Nat. Rev. Methods Primer 1, 46. https://doi.org/10.1038/s43586-021-00044-z
[3] Ortiz, C., Ferreira, M. L., Barbosa, O., dos Santos, J. C. S., Rodrigues, R. C., Berenguer-Murcia, Á., Briand, L. E., & Fernandez-Lafuente, R. (2019). Novozym 435: the “perfect” lipase immobilized biocatalyst? Catalysis Science and Technology, 9, 2380–2420. https://doi.org/10.1039/C9CY00415G
[4] Rosano, G.L., Morales, E.S., Ceccarelli, E.A., 2019. New tools for recombinant protein production in Escherichia coli : A 5-year update. Protein Sci. 28, 1412–1422. https://doi.org/10.1002/pro.3668
[5] van Schie, M. M. C. H., Spöring, J. - D., Bocola, M., Domínguez de María, P., & Rother, D. (2021). Applied biocatalysis beyond just buffers – From aqueous to unconventional media. Options and guidelines. Green Chemical, 23, 3191–3206. https://doi.org/10.1039/D1GC00561H.
[6] Mangiagalli M, Carvalho H, Natalello A, Ferrario V, Pennati ML, Barbiroli A, Lotti M, Pleiss J, Brocca S. Diverse effects of aqueous polar co-solvents on Candida antarctica lipase B. Int J Biol Macromol. 2020 May 1;150:930-940. doi: 10.1016/j.ijbiomac.2020.02.145.