UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

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

Research Topic ID: XL – 1.16

Proponent: Prof. Valeria Mapelli

Project Title: Development of microbial cell factories for production of carotenoids for edible electronic applications.

Scientific background and 'open issues'

The unconventional field of edible electronics takes inspiration from the previous interdisciplinary "green" approaches under-taken by the scientific community toward a safe and sustainable future. By 1970s green engineering gained momentum, promoting the idea of designing processes and products in a way that reduces impact on the environment and minimizes risks to human health. Going past the traditional model of electronic devices, edible electronics envisions a technology which is not only environmentally friendly, cost-effective, energy-efficient, but also safe for ingestion, and degradable within the body after performing its function. The ultimate goal of this new approach is to exploit the inherent electronic properties of food, food-derived or edible synthetic materials for development of ingestible functional electronic devices and systems (1). With respect to basic electrical properties, a number of natural molecule can be classified either as conductors or semiconductors. Semiconductors are key to electronic functionalities, as they determine the operation of active devices, such as charge transport, light emission and photon to charge conversion. In contrast to conventional materials, the central pillar for edible semiconductors is not only high electronic performance, but also a challenging balance between inherent safety for ingestion and sufficient electronic stability to perform a specific function over a limited period of time (1). To date, the majority of potentially edible semiconductors are constituted by pigments and dyes that are naturally derived from animals, foods, plants, and minerals.

Carotenoids are naturally found coloured pigments which are of semiconducting nature. The structural diversity of carotenoids and their molecular nature make them ideal candidates for devices requiring photo-sensitization and controlled electrical conduction (2). Carotenoids can be produced by microorganisms spanning from bacteria species to yeast and algal species (3). Each microbial species can produce an array of carotenoid molecules at different levels, always producing a predominant carotenoid and a number of minor forms. The different chemical features of each carotenoid molecular species result in small but relevant differences in the functionalities of these molecules. Therefore, depending on the desired activity, specific molecules can be selected over others for specific applications.

Objectives

The general objectives of the project are:

- To evaluate, based on the chemical features, the carotenoid molecule with highest potential as semiconductor.
- To synthesize selected carotenoids via microbial fermentation processes
- To test the potential of the synthesized carotenoids for their use as semiconductors.

The specific objective of the project are:

- To identify one or more microbial carotenoids producers and characterize the carotenoid production profile by identifying the carotenoids moieties naturally produced.
- To test and evaluate the potential of the specific carotenoid molecules produced for their performance as semiconductors.
- To engineer the natural microbial producer(s), in order to maximize the synthesis of the carotenoid molecule(s) featured as best semiconductors.
- To engineer a microbial strain that is not capable of natural carotenoid synthesis, but is typically used in established cell factories, in order to produce the selected carotenoid molecule(s).

Methodologies

The design of the proposed research project is based on the preliminary identification of natural carotenoid molecules that have a good potential to be applied as semiconductors. An analytical LC-based method to detect and quantify the carotenoid compounds of interest will be developed to be used throughout the entire project.
Natural microbial producers of the carotenoids of interest will be identified and the carotenoid profile of these microorganisms will be defined both qualitatively and quantitatively.
Where possible, the natural carotenoid producer(s) will be genetically engineered with the aim of maximizing the titer of the specific carotenoid of interest. In addition, a cell factory based on the use and engineering of a microorganism that has known potential to be successfully used in industrial scale bioprocesses will be pursued. The bioprocess will be developed, fully characterized and optimized in controlled bioreactors that are part of the fermentation platform present in the proposing research group.
The collaboration with research group(s) with expertise in the development and characterization of edible electronics will be continuous throughout the project as it will allow to monitor the applicability of the microbially produced carotenoids and will provide critical information for further development and optimization of the bioprocess.

Collaboration / Co-tutoring opportunities

External collaboration is envisioned with research groups active in the characterization of the semiconductor properties of natural molecules and their application as edible semiconductors: the Center for Nano Science and Technology @PoliMi, Istituto Italiano di Tecnologia is a potential collaborator thanks to the expertise in developing edible electronics.

Project's Sustainability & Mobility

- *Competences in the proposing research group*

The lab hosting the project has long time experience in physiological characterization and genetic engineering of microbial strains, and in the development of fermentation processes in controlled bioreactors. In addition, experience in working with natural microbial producers of carotenoid is present in the research group, along with the analytical methods necessary for carotenoid species identification.

- *Relevant publications within the research group*

Enzymatic Hydrolysate of Cinnamon Waste Material as Feedstock for the Microbial Production of Carotenoids. *Int J Environ Res Public Health*. 2021 Jan 28;18(3):1146. doi: 10.3390/ijerph18031146.

Camelina sativa meal hydrolysate as sustainable biomass for the production of carotenoids by Rhodosporidium toruloides. *Biotechnol Biofuels*. 2020 Mar 12;13:47. doi: 10.1186/s13068-020-01682-3.

- *Mobility – Hosting research group(s)*

VTT Technical Research Center of Finland, Research unit Production Host Engineering, where a universal method for gene expression in novel yeast species has been developed. In addition, or in alternative, the research group led by Prof. Petri-Jaan Lahtvee, at the Division of Food and biotechnology, Department of Chemistry and Biotechnology, Tallin Techical University (Tallin, Estonia) is a potential hosting institution for the PhD student mobility. In particular, this research group has experience with production of carotenoids in "non-conventional" yeast species.

References

[1] Sharova A.S. *et al. Adv. Mater. Technol*. 2021, 6, 2000757
[2] Singh A. and Mukherjee T. *Mater. Adv*., 2022, 3, 1341
[3] Joshi K. *et al. Process Biochemistry* 128 (2023) 190–205
[4] Yeast Metabolic Engineering: Methods and Protocols, Methods in Molecular Biology, Vol. 2513,