

Production and quantification of betalains using *Hylocereus* spp. plants and cell cultures

Plants are among the organisms with the richest and most complex metabolic diversity. The total content of secondary metabolites within the plant kingdom is indeed estimated to be around 1 million [1]. Plants synthesise a diverse panel of specialised metabolites (a.k.a. phytochemicals) that are the product of their secondary metabolism. Secondary metabolism is usually considered non-essential for plants' growth and development, but important for the interaction with the environment [1]. Currently, the properties and bioactivities of only a small percentage of this repertoire of molecules are known and used for applications in e.g. the agrochemical, medical, nutraceutical and cosmetic sectors. Among the most interesting practical applications, it is worth mentioning anti-oxidant/radical-scavenging activities, as well as pesticidal and anti-microbial properties [2].

The advantage of using plants as sources of phytochemicals is given by their renewable nature and their versatility in bioprocess engineering: plant tissues, under conditions requiring specific balances of phyto-hormones, can be induced to de-differentiate to a stem cell-like state (the cells proliferate into a callus) and these cells can be cultivated in liquid media as suspensions. Plant cell suspension cultures are widely used as "phytofactories" for the production of valuable compounds (one emblematic example is paclitaxel produced by *Taxus* spp. cultures) and their biomass/metabolite yield can be optimised via cultivation in bioreactors of different volumes.

The Luxembourg Institute of Science and Technology-LIST has recently established the GreenTech Innovation Centre-GTIC (<https://www.list.lu/en/institute/centres/greentech-innovation-centre/>), a biotechnology-inspired open innovation facility that has set the valorisation of plants as a central research and technology axis. The GTIC hosts a collection of plant cell cultures for different applications. It is here proposed to develop a 6-months project focusing on *Hylocereus* spp. (pitaya, or dragon fruit) plants and cell cultures with the goal of establishing a bioprocess for the production of betalains in bioreactors. Betalains are pigments found in specific plant orders [3] and are of high interest for food- and cosmetic use.

The student will:

- 1) perform an initial bibliographic survey of the literature data available on the optimisation of betalains' extraction and production using cell cultures;
- 2) optimise the extraction of betalains (betacyanins) using different solvents and quantify their content in several pitaya varieties available at LIST;
- 3) optimise the growth conditions of pitaya cell cultures and the production of betacyanins by using different parameters, namely the use of elicitors, as well as different temperatures and bioreactors' configuration (wave-tank and stirred-tank bioreactors, different impellers' types);
- 4) perform a molecular investigation on the cell cultures grown using the optimised conditions (gene expression analysis).

At the end of the 6-months internship the student will have acquired knowledge in plant tissue culture and molecular biology, as well as basic skills in plant biochemistry and bioprocess engineering.

The interested students should contact Dr Gea Guerriero (e-mail: gea.guerriero@list.lu)

References

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2. Guerriero, G.; et al. Production of Plant Secondary Metabolites: Examples, Tips and Suggestions for Biotechnologists. *Genes (Basel)* **2018**, *9*, doi:10.3390/genes9060309.
3. Polturak, G.; Aharoni, A. "La Vie En Rose": Biosynthesis, Sources, and Applications of Betalain Pigments. *Molecular Plant* **2018**, *11*, 7–22, doi:10.1016/j.molp.2017.10.008.