Biodiscovery of new antibiotics through the heterologous expression of entire fungal biosynthetic gene clusters (BGCs) in genetically tractable hosts

Biodiscovery of new natural compounds has always been of interest of the biotech industry due to their enormous potential to be used in the treatment of plant, animal, and human diseases; as functional or bioactive ingredients for food and cosmetic industries; and, most importantly, as a persistent source of new chemical scaffolds for the development of innovative therapeutic agents and drug leads[1].

A great diversity of these bioactive metabolites is produced by microorganisms, especially fungi[1,2]. Although microbial secondary metabolites have long been an important source of new bioactive compounds, the rediscovery of compounds that have already been characterised has stagnated enthusiasm for this route of drug discovery. In recent years, however, advances in genetic tools, screening strategies, isolation and synthesis of natural products have led to a revitalisation of biodiscovery efforts that source microbial secondary metabolism for new bioactive candidates[2,3]. Within this context, the development of analytical tools in metabolomics has had a marked impact on our ability to identify novel biologically active molecules[4].

The innovative focus of this project is on the avant-garde genome sequence-independent methods for heterologous expression of intact BGCs coupled to an advanced high-throughput metabolomic-based bioactive compound screening platform. Sequence-independent methods for heterologous expression of entire BGCs have only been possible recently with the latest advances in molecular biology tools[3]. These methods make use of expression libraries on sheared genomes obtained from environmental DNA or from pure cultures[3].

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 biodiscovery of industrially relevant biologically active molecules as a central research and technology axis. This internship is proposed to be developed in a 6-months project focusing on the heterologous expression of intact fungal secondary metabolite (BGCs) in *Aspergillus nidulans*.

The student will:

1) Establish reliable protocols for extraction and isolation of high-quality high-molecular weight DNAs from pure cultures and environmental samples

2) Establish an unbiased method for DNA fragmentation aiming to have better control of size distribution and maintain the randomness of fragmentation

3) Set up and optimise a cloning strategy using fungal artificial chromosomes (FACs)

4) and if time allows, Optimise FAC transformation of a clean filamentous fungus chassis for production of bioactive secondary metabolites

At the end of the 6-months internship the student will have acquired knowledge in microbial cell culture (fungi and bacteria) and molecular biology, as well as basic skills in fungal biology and genetics.

The interested students should contact Dr Silas VILLAS-BOAS (e-mail: silas.villas-boas@list.lu). More information about the supervisor can be found at: https://orcid.org/0000-0003-0148-5882

References

1. Brakhage & Schroeckh. 2011. Fungal secondary metabolites – Strategies to activate silent gene clusters. *Fungal Genetics and Biology* 48: 15 – 22. DOI: 10.1016/j.fgb.2010.04.004
2. Lam K. S. 2007. New aspects of natural products in drug discovery. *Trends in Microbiology* 15: 279 – 289. DOI: 10.1016/j.tim.2007.04.001
3. Lin et al. 2020. Bioprospecting through cloning of whole natural product biosynthetic gene clusters. *Frontiers in Bioengineering and Biotechnology* 8: 526. DOI: 10.3389/fbioe.2020.00526
4. Klinkenberg et al. 2011. Two-dimensional LC-MS fractionating and cross-matching of mass spectrometric data for rational identification of bioactive compounds in crude extracts. *Journal of Separation Science* 34: 3359 – 3363. DOI 10.1002/jssc.201100468