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Sorbonne Université/ China Scholarship Council program 2020

Thesis proposal

Title of the research project: Chemo-genomics approach for the discovery of new secondary metabolites from endophytic fungi

Keywords: chemistry of natural products, secondary metabolites, genome mining, biosynthesis, endophytes, bioactivities.

Joint supervision: Yes/ Caroline Kunz

Joint PhD (cotutelle): no

Thesis supervisor: Soizic Prado

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Institution: National Museum of Natural History

Doctoral school (N°+name): ED 227 « Sciences de la nature et de l'Homme : évolution et écologie »

Research laboratory: Unit Molecules of Communication and Adaptation of the Microorganisms. National Museum of natural History (MNHN)/National Center of the Scientific Research (CNRS). UMR 7245.

Address of the laboratory: National Museum of natural History. 63 rue Buffon 75005 Paris

Name of the laboratory director: Philippe Grellier

Email address of the laboratory director: philippe.grellier@mnhn.fr

Subject description (2 pages max):

1) Study context

Filamentous fungi are prolific reservoirs of biologically active secondary metabolites (SMs). These compounds have been of major interest due to their therapeutic applications, such as penicillin, cyclosporine or lovastatin, while many SMs also represent deleterious toxins occasionally retrieved in foodstuffs. Thus, isolation and characterization of secondary metabolites from fungi have covered a wide range of areas in bio-organic chemistry, biology and ecology, as demonstrated by numerous publications in these fields every year. For this PhD project, we plan to isolate and characterize fungal secondary metabolites from relevant strains selected by a genome mining approach. This modern method allows predicting the presence of original biosynthetic pathways through genome analysis and then searching for the corresponding natural products. The correlation between the

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production of the metabolites and the genes involved in the biosynthesis will be validated by gene inactivation. Epigenetic fungal mutants will be also construct to potentiate the production of secondary metabolites. In addition, all metabolites isolated will be evaluated for their bioactivities mostly against phytopathogens.

The CPNFB team is particularly well equipped to undertake such research dealing with microbiology, molecular biology and organic synthesis. The laboratory has access to two NMR spectrometers (400/600 MHz cryoprobe), two LC-ESI-QTOF and UPLC-MS mass spectrometers as well as common analytic tools (preparative and analytical HPLC). Moreover, the laboratory includes a fungal culture service.

Further information and a list of publications of the team is available on the team webpage: http://mcam.mnhn.fr/fr/cpnf-chimie-des-produits-naturels-fongiques-449

2) Details of the proposal

Hirsutellone and related compounds on this series have raised the attention of natural product chemists and biochemists due to significant antibiotic activities and striking structural complexity. Recent work in the lab revealed their biosynthesis pathways and the PKS-NRPS biosynthetic genes governing the production of these compounds. Research of fungal genomes harboring sequences related to PKS-NRPS gene clusters have been screened for the selection of fungal species (*e.g.* genome mining). However, many biosynthetic gene clusters are cryptic and are not expressed under laboratory conditions. Accordingly, specific attempts to wake up such dormant clusters will be undertaken by using epigenetic engineering, microbial co-culturing or the "One Strain Many Compounds" (OSMAC) strategy.

These species will thus be chemically studied for the presence of PKS-NRP products. This selection will be combined with a molecular networking approach and should allow to specifically isolate related compounds. All compounds will be chemically described by using NMR and MS spectrometries. A systematic biological evaluation (phytotoxicity, impact on seed germination, antimicrobial activity etc..) of the pure isolated compounds will be undertaken.

In the course of the PhD thesis, the candidate will perform the chemical exploration of the selected strains and characterize the produced secondary metabolites. The candidate will be also in charge of the construction of mutants and of the biological evaluations of the selected metabolites.

The PhD program will be as follows:

First year: strain selections by genome mining. Optimization of fungal cultures and comparing metabolic profiles by HPLC/MS and MS-MS analysis. Starting the molecular biology for the epigenetic mutants construction.

Second year: Isolation and structural determination by spectroscopic analyses (NMR, MS) of the secondary metabolites. Continuation of the work undertaken in molecular biology.

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Third year: Continuation of the work undertaken in chemistry. Biological evaluations on relevant targets, and writing the PhD thesis. The PhD should be defended as soon as possible once the thesis is written. Eventually, during the 4th year, the student will be able to complete some work and to write up articles for high-ranked journals.

3) References

1) Vallet, M.; Vanbellingen, Q. P.; Fu, T.; Le Caer, J.-P.; Della-Negra, S.; Touboul, D.; Duncan, K. R.; Nay, B.; Brunelle, A.; Prado, S., An Integrative Approach to Decipher the Chemical Antagonism between the Competing Endophytes Paraconiothyrium variabile and Bacillus subtilis. *Journal of Natural Products* **2017,** *80* (11), 2863-2873

2) Amand, S.; Vallet, M.; Guedon, L.; Genta-Jouve, G.; Wien, F.; Mann, S.; Dupont, J.; Prado, S.; Nay, B., A Reactive Eremophilane and Its Antibacterial 2(1H)-Naphthalenone Rearrangement Product, Witnesses of a Microbial Chemical Warfare. *Organic Letters* **2017,** *19* (15), 4038-4041.

3)Vallet, M.; Strittmatter, M.; Murua, P.; Lacoste, S.; Dupont, J.; Hubas, C.; Genta-Jouve, G.; Gachon, C. M. M.; Kim, G. H.; Prado, S., Chemically-Mediated Interactions Between Macroalgae, Their Fungal Endophytes, and Protistan Pathogens. *Frontiers in Microbiology* **2018,** *9*.

4)El-Demerdash, A.; Genta-Jouve, G.; Barenstrauch, M.; Kunz, C.; Baudouin, E.; Prado, S., Highly oxygenated isoprenylated cyclohexanoids from the fungus *Parastagonospora nodorum* SN15. *Phytochemistry* **2019,** *166*.

5) Tourneroche, A.; Lami, R.; Hubas, C.; Blanchet, E.; Vallet, M.; Escoubeyrou, K.; Paris, A.; Prado, S., Bacterial-Fungal Interactions in the Kelp Endomicrobiota Drive Autoinducer-2 Quorum Sensing. *Frontiers in Microbiology* **2019,** *10*.

4°) Profile of the Applicant (skills/diploma...)

The applicant should have :

-Experience in chemistry of natural products: isolation and characterization of molecules by MS, NMR and metabolomics. A particular interest in the biosynthetic pathways of natural products will be highly appreciated.

- Good interpersonal skills, ability to work in a team. Ability to write scientific publications and very good command of scientific English.

- Excellent English communication skills

Contacts:

Thesis supervisor

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