

Sorbonne Université/ China Scholarship Council program 2021

Thesis proposal

Title of the research project: Marine polychaete symbionts as a source of new natural products – Ecological aspects and biosynthesis

Keywords: natural product chemistry, symbiosis, marine polychaete, biosynthesis, chemical ecology

Joint supervision: yes (Kevin CALABRO)

Joint PhD (cotutelle): no

Thesis supervisor: Cédric HUBAS

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Institution: National Museum of Natural History

Doctoral school: ED 227 « Sciences de la nature et de l'Homme: évolution et écologie »

Research laboratory: BOREA Biologie des Organismes et des Ecosystèmes Aquatiques

Address of the laboratory: MNHN Station marine Concarneau, Station de biologie marine, Place de la Croix, 29900 Concarneau, France

Name of the laboratory director: Tarik Meziane

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Subject description (2 pages max):

1) Study context

Marine invertebrates-derived microorganisms have been recognized as an outstanding source of diverse and bioactive specialized metabolites, with sponge and tunicate-derived microbes being the most studied producers of these exceptional natural products. In a biodiscovery strategy, most of bioprospecting studies target these two phyla for their environmental niche leaving others out and poorly studied.¹

In this context, we plan to identify, isolate and culture the microorganisms living in a host-symbiont system with annelid worms. A metabarcoding approach will be initiated to identify several families of bacteria and fungi that will be then cultured in our facilities. A chemical investigation will be performed on annelid-derived microorganism grown in different environmental conditions to reveal cryptic metabolites as underlined in the “One Strain Many Compounds” (OSMAC) strategy.² The second objective of this work will be to understand the ecological roles of the isolated compounds within a bi- (annelid/microbe) and a tripartite (annelid/annelid/microbe) marine system.

The CPNFB team (MCAM unit of MNHN) is particularly well equipped to undertake the natural product chemistry aspect. 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 analytical laboratory instruments (polarimeter, electronic circular dichroism, preparative and analytical HPLC). As previously mentioned, the laboratory includes a fungal and a bacterial culture service (incubators).

The SOMAQUA team (BOREA unit of MNHN, Concarneau Marine Station) possess all the facilities to perform both chemical ecology and biosynthesis studies with state-of-the-art culture systems such as fully equipped aquaria, mesocosms and a seawater pumping system.

2) Details of the proposal

Eupolyornia nebulosa is a widely spread terebellid worm usually living buried in tube-shape sand structures yet there is an increasing number of reports of it being enrolled in a host-symbiont association with sponges and other polychaetes. Even if toxic and deterrent properties have been proposed for *E. nebulosa* as anti-predatory strategies, no secondary metabolite has been correlated to this reported behavior so far.³

A recent work in our group allowed the isolation and structure elucidation of a novel family of natural products, the nebulosins.⁴ These metabolites consist in a cysteine amino acid condensed on a tetraketide unit forming a rare and highly branched thiolane ring. Nebulosins contain structural features that suggest a possible microbial origin, namely a nonproteinogenic amino acid (D-cysteine) and a mixed polyketide-amino acid pathway. Additionally, these natural products seem to play an important ecological role as they were also found in another marine polychaete *Polynoe scolopendrina*, living in a commensal relationship with *Eupolyornia nebulosa* in the same tube-shape sand structure.

In the course of its PhD, the successful candidate will connect anatomic structures of the annelid to potential producers of cysteine-containing natural products such as nebulosins by stable isotope tracing using nanoSIMS (National Ion MicroProbe Facility of MNHN).⁵ Employing a metabarcoding approach, the candidate will then compare the microbial content in the anatomic compartment of the commensal polychaetes, in which nebulosins were localized. Within an OSMAC strategy, the candidate will chemically screen each isolated microbial strain for the discovery of new natural products using a Molecular Networking approach (MCAM). The candidate will also assess the ecological role of these compounds including nebulosins through

developed aquarium experiments. Finally, the candidate will study the biosynthesis of nebulosins by feeding experiments with ^{13}C -labeled precursors and compound specific isotope analysis.⁶

First year: Stable isotope probing experiments to look for the nebulosin-producers using nanoSIMS. Microbial metabarcoding in the selected compartment of the two commensal polychaetes. Microbial Isolation and optimization of cultures. Writing at least one article. Present one poster in a conference.

Second year: Chemical screening of all cultured strains using a Molecular Networking approach. Isolation and structure elucidation (NMR spectroscopy and mass spectrometry) of the secondary metabolites. Preparation of the aquarium experiments for both the ecological and biosynthesis studies. Writing at least one publication. Present one oral communication in a conference.

Third year: Aquarium experiments and subsequent data treatment. Writing the PhD thesis and writing article(s).

3) References

1. Carroll, A. R.; Copp, B. R.; Davis, R. A.; Keyzers, R. A.; Prinsep, M. R., Marine natural products. *Nat. Prod. Rep.* **2019**, *36*, 122-173.
2. Romano, S.; Jackson, S. A.; Patry, S.; Dobson, A. D. W., Extending the “One Strain Many Compounds” Principle to Marine Organisms. *Mar. Drugs.* **2018**, *16*, 244-272.
3. Martin, D.; Le Nourichel, C.; Uriz, M. J.; Bhaud, M.; Duchêne, J. C., Ontogenic Shifts in Chemical Defenses of the Northwest Mediterranean Sea *Eupolymnia nebulosa* (Polychaeta, Terebellidae). *Bull. Mar. Sci.* **2000**, *67*, 287-298.
4. **Calabro, K**; Jennings, L. K.; Lasserre, P.; Doohan, R.; Rodrigues, D.; Reyes, F.; Ramos, Thomas, O. P., Nebulosins: Trisubstituted Thiolane Natural Products from the Northeastern Atlantic Annelid *Eupolymnia nebulosa*. *J. Org. Chem.* **2020**, *85*, 14026-14041.
5. **Hubas, C.**; Boeuf, D.; Jesus, B.; Thiney, N.; Bozec, Y.; Jeanthon, C., A Nanoscale Study of Carbon and Nitrogen Fluxes in Mats of Purple Sulfur Bacteria: Implications for Carbon Cycling at the Surface of Coastal Sediments. *Front. Microbio.* **2017**, *8*, 1995-2008.
6. Cruz, S.; LeKieffre, C.; Cartaxana, P.; **Hubas, C.**; Thiney, N.; Jakobsen, S.; Escrig, S.; et al. Functional Kleptoplasts Intermediate Incorporation of Carbon and Nitrogen in Cells of the Sacoglossa Sea Slug *Elysia Viridis*. *Sci. Rep.* **2020**, *10*, 10548-10559.

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

The applicant should have knowledge and/or experience in:

- Natural product chemistry: isolation and structure elucidation using mass spectrometry and NMR spectroscopy
- Culture of marine fungi and bacteria
- Biosynthesis of natural products
- Chemical ecology

The applicant should have a first publication record expressing its ability to write scientific publications and a good command of scientific English.

Finally, the applicant should have demonstrated high proficiency in lab work in the field of natural product chemistry and/or microbiology.

Contacts:

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