MAST VISTING FELLOWSHIP

Project Summary

Paris, 22/11/2016

Visiting Fellow

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National Museum of Natural History

Team “Chemistry of Fungal Natural Products”

UMR 7245 MNHN/CNRS

Paris, France

Host Institution

Dr Claire Gachon

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Date of visit

January 2016-July 2016

Introduction

Whilst the human and plant microbiomes are currently intensively explored, and their biotechnological potential as a source of pharmaceuticals and biocontrol agent is well established, the structure and functions of the microbiome is only beginning to be characterized in marine organisms, including algae. Prof. Prado a natural product chemist with a particular interest in beneficial fungal and bacterial endophytes of plants and algae went to SAMS to pursue her collaboration with Dr Gachon which aims to characterize ecological impact of endophytes associated to seaweed and to develop disease management techniques for the seaweed aquaculture. Thus, this MAST visiting fellowship sponsored the visit of S. Prado during 6 month to the research group of Claire Gachon and was used for SP’s subsistence (travel, accommodation) while CNRS and NERC budgets were devoted toward lab consumables.

The aim of the project was 1) to develop a robust experimental ‘kelp-endophyte’ system, where clonal endophytic fungal strains will be reinoculated on lab-reared kelp sporophyte 2) to finalize peer-reviewed manuscript on existing data, alongside exploring their potential for IP protection in the context of disease management in algal aquaculture.

Results

1) Co-inoculation endophyte-sporophytes of endophyte

Previously more than 120 fungal strains have been isolated and taxonomically determined from kelps in SP's team. The fungus *Paradendriphiella arenaria* appeared as a good candidate for co-inoculation experiments because i) it has been exclusively described on marine environments and ii) it was both isolated from *Saccharina latissima* and *Laminaria digitata*.

In the other hand, axenic sporophytes could not be obtained in the course of SP's visit. Sporophytes were kindly provided by Phil Kerrisson for co-inoculation experiments. Bacterial microbiote was controlled by addition of antibiotics.

As represented Figure 1, co-inoculation of two different strains of *P. arenaria* (SL467 T and SL540T) did not alter fitness of the *S. latissima's* sporophytes when co-cutivations were performed during 15 days in presence or absence of antibiotics. Same results were observed on *L. digitata's* sporophytes (data not shown). It should be noted that after 2 weeks we encountered difficulties to maintain sporophytes in lab culture conditions and thus could not perform experiments longer.

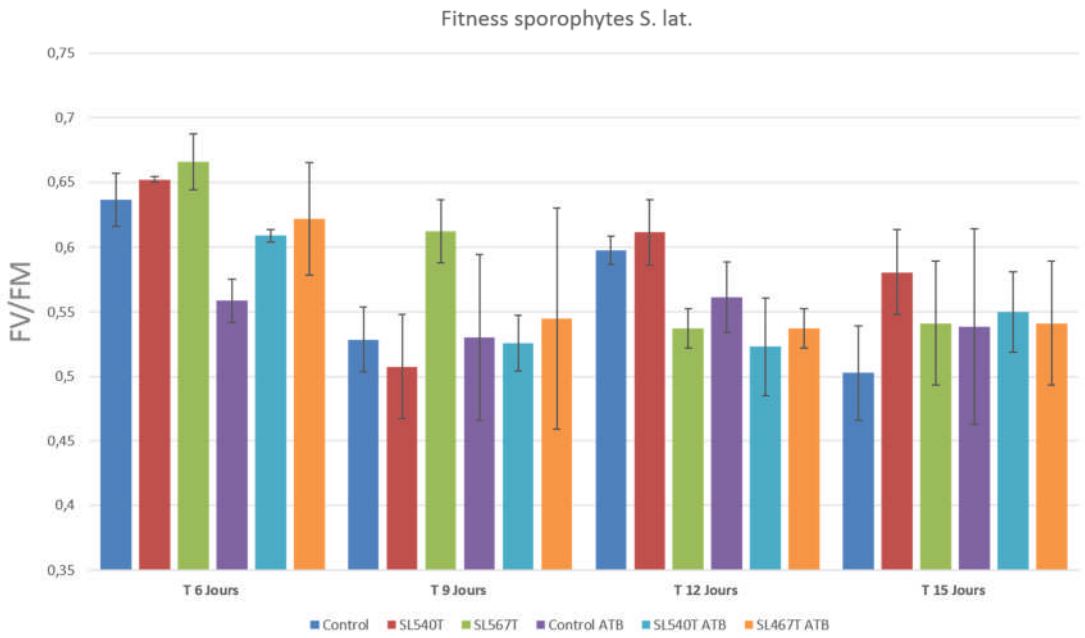

Figure 1. Photosynthetic activity (FV/FM) of sporophytes co-incubated with the fungus *P. arenaria* (strains SL 540T and SL 467T) in presence or absence of antibiotics (ATB) (n=3)

Fungal penetration within sporophytes were observed by fluorescent microscopy with WGA/FITC staining. No clear penetration of fungi was observed in filtered sea-water (FSW) at 6 days and 15 days (fig. 2A), while apressorium (organ of penetration) were detected when spores germination is facilitate (presence of malt extract) (Fig 2B).
However, penetrations were also observed at 32 days of co-incubation when physiological state of the sporophytes was degrading as represented figure 2C. These results were in accordance with observations made within dead sporophytes tissues, in which appressoriums were observed (Fig. 2D).

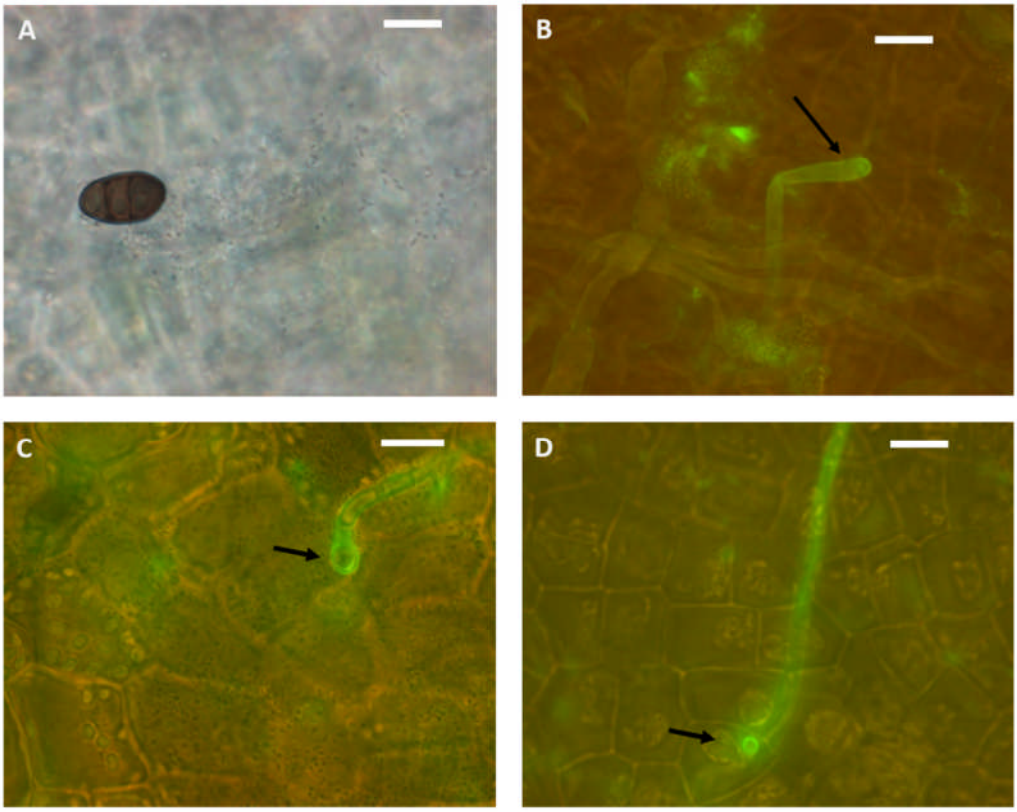

Figure 2: Observation fungal apressorium by fluorescent microscopy on sporophytes inoculated with *P. arenaria* A) After 6 days in FSW. B) After 6 days in Malt Extract. C) After 6 days with dead sporophytes D) After 32 days. Arrow indicate appressorium. Scale (20 µm).

2) Chemical mediation involved in fungus-algae interaction.

Previous work initiated by Martina Strittmatter (Post-doc SAMS) and Kady Du (Master 2 Erasmus SAMS/MNHN) highlighted the negative effect of healthy sporophytes on spores germination *in situ*. As represented Fig. 3A, contact with algae led to a lower rate of germination by comparison to spores away from algal tissues (Fig.3B). In addition, physiological state of the sporophytes impact also this rate of germination. As represented Fig.3C a significant lower rate of germination was observed in presence of healthy sporophytes by comparison to non-healthy ones. These resulted suggested the production of algal compounds inhibiting the germination.

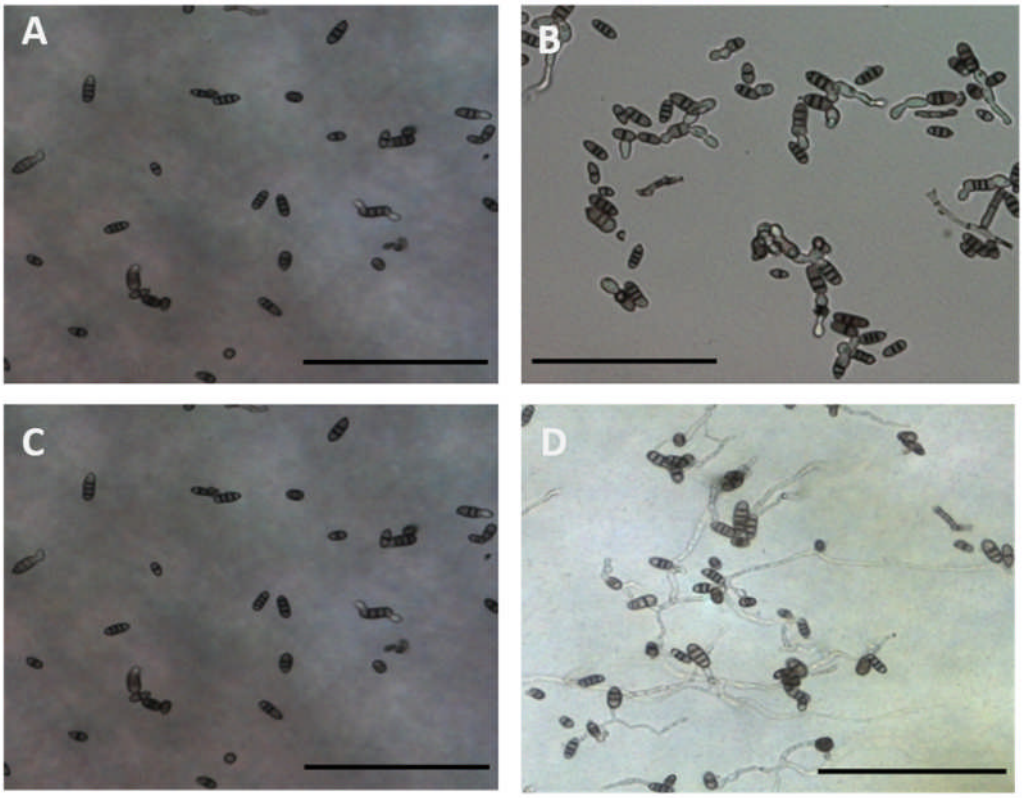

Figure 3. Spore's germination depending of the presence and fitness of sporophytes. A) Spores in contact with the algae. B) Spores away from the sporophyte's tissue. C) Spores in contact with healthy sporophytes. D) Spores in contact with non-healthy sporophytes. Scale (100 µm)

To confirm putative chemical mediation involved on the fungal spore's inhibition, the same experiments were performed in presence of supernatant obtained from sporophytes cultivation. No significant difference was observed by comparison to the control (fig 3A versus fig 3C).

As inductive compounds only produced in contact with fungi could be responsible of this inhibition, spores were cultivated with supernanant previously obtained from co-culture of sporophytes and fungi. Again, no spore's inhibition was observed (Fig 3B) suggesting that molecules from the algal cell wall could be more involved in the inhibition than diffusible compounds.

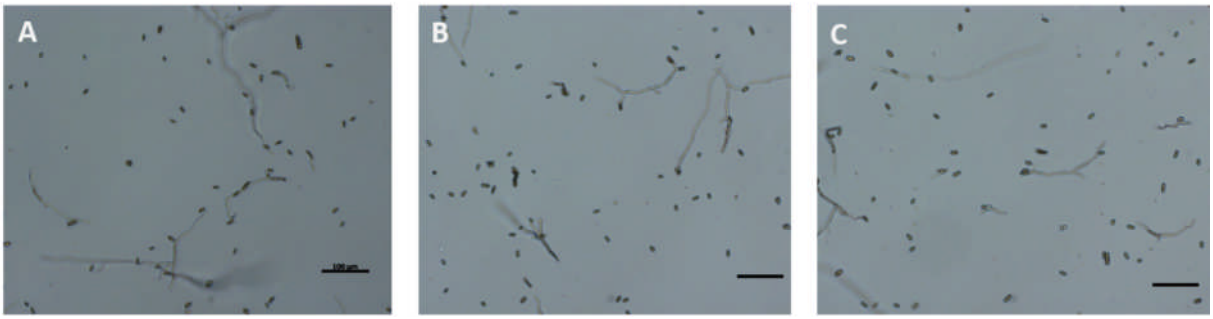

Figure 4. Spores of *P. arenaria* in presence. A) Supernatant of sporophytes, B) supernatant of the co-culture fungi/sporophytes. C) Control. Scale (100 µm)

Statistical analysis of the rate of germination quantification, depending of the distance from algal tissues and the fitness of the algae, are undergoing and should lead soon to a

publication. Results suggested also the impact of the spores density on the rate of germination. This aspect will also be quantified.

3) IP protection and generation of new data

Previous work initiated by CG and SP highlighted the impact of molecules produced by endophytic fungi against infection by pathogens of commercially-important algae. These results were valorized by an international PCT patent and the visiting fellowship was also the occasion to finish redaction of this patent (PCT/FR2016/050137) which was filed on 22/01/2016. Complementary experiments were also performed and allowed to refine the active concentration range as well as to better understand mechanism of action for active fungal mediators.

Significance and future prospects

Structure and function of the microbiote is only beginning to be characterized in marine organisms. Whilst main results focused on bacterial microbiote, few works have investigated the ecological roles of fungi associated to algae and even less on endophytes.

Whilst endophytic fungi are commonly described as beneficial for the host-plant a completely different scenario is suspected for algae.

Indeed, these results suggest that endophytic fungus associated to host-algae do not have a positive impact on their host but behave more as saprophytic or latent pathogenic than mutualistic fungi. Such impact could thus be particularly detrimental for algal aquaculture.

However, these results also highlighted that host-algae developed defense against *P. arenaria* by limiting their germinate rate according to a mechanism not yet elucidated. In addition, contact inhibition rather than diffusible compounds is suspected.

Deciphering such chemical communication as well understanding parameters able to turn the tide toward fungal pathogeny appear as a challenging but urgent need in a context of algal cultivation and disease management. Obviously, these aspects will be the subject of further collaboration between the two institutes using complementary and integrative approaches.

Networking with Scottish researchers

In addition to the specific collaboration with CG, the MAST fellowship allowed the establishment of a new collaboration with Dr Rainer Ebel (University of Aberdeen, School of Natural and Computing Sciences). In this context, SP is invited to be examiners of Rosemary Iosioma PhD defense in January and Dr Rainer Ebel has been invited to give a talk at the MNHN. It is thus expected that the collaboration initiated herein can be continued and expanded in the future.

This MAST fellowship also allowed a collaboration with Katherine Ducan (Post doctorant at SAMS till July 2016 and presently in the University of Strathclyde, Glasgow). Fruitful conversations concerning dereplication by molecular networking led to the preparation of a manuscript (“An integrative approach to decipher the chemical communication between competing endophytes *Paraconiothyrium variabile* and *Bacillus subtilis”*) which should be submitted soon to *Journal of Natural Products.*

Acknowledgments

I am grateful to MASTs for giving me the great chance to work in Claire Gachon's group on such a challenging and interesting project. I appreciated all of the kind support from staff members and students in SAMS. Aside from a patent, we have already started to draft a manuscript describing the results obtained in this project. As requested, the SAMS funds will be acknowledged in any publication derived from this proposal.