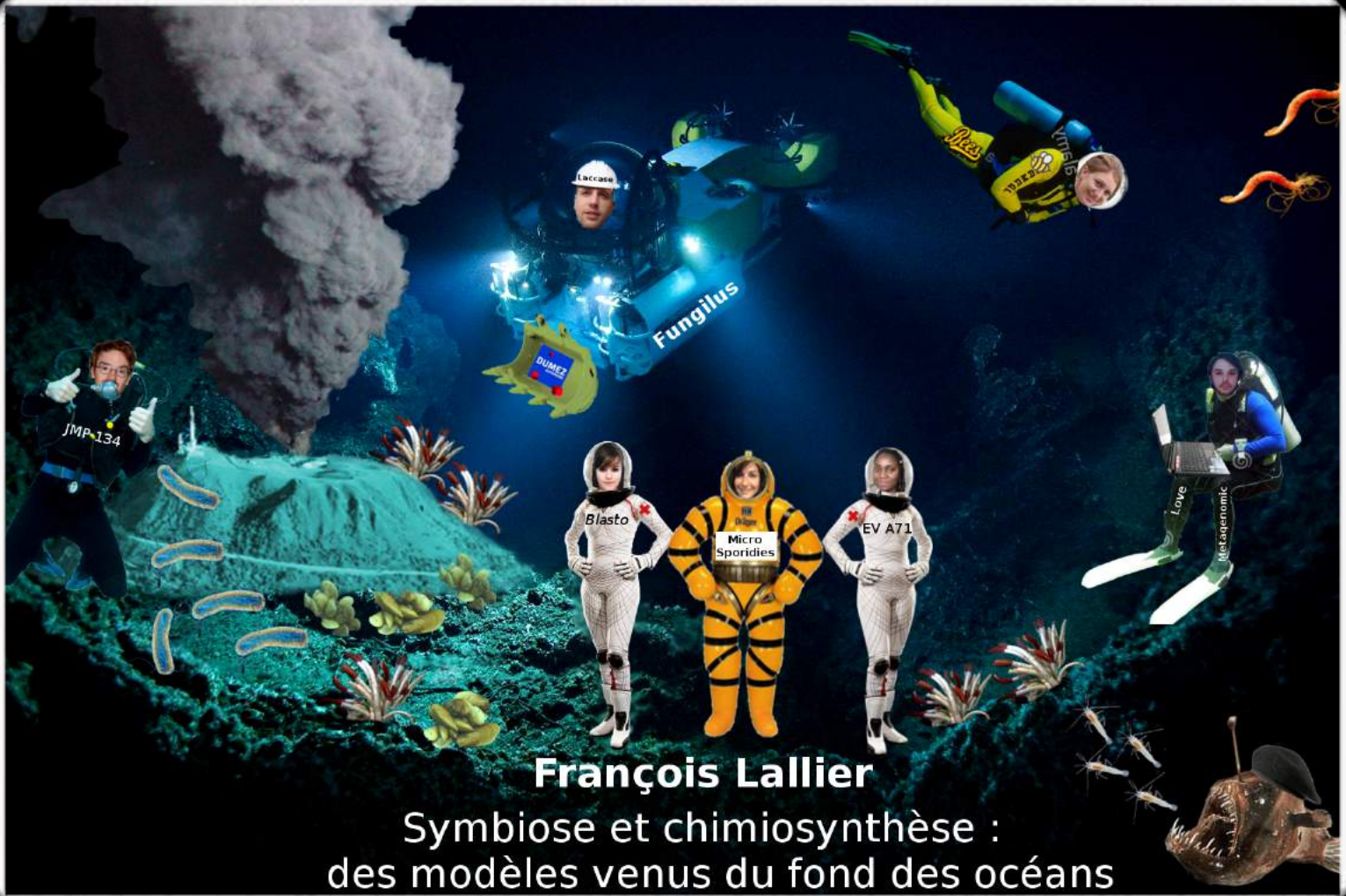


6^e Journée des doctorants du LMGE



Mardi 13 mars 2018

Amphithéâtre de recherche, bâtiment physique

Campus Universitaire les Cézeaux

**Laboratoire Microorganismes, Génome, Environnement, LMGE
UMR6023**

Université Clermont Auvergne, UCA



6^e Journée des doctorants du LMGE

Mardi 13 mars 2018 - Amphithéâtre de recherche

Pour la 6^e année consécutive, une journée est organisée par et pour les doctorants du Laboratoire Microorganismes : Génome et Environnement.

Outre le fait que cette journée soit un moment de rencontre et de convivialité, elle permet à des doctorants de présenter leurs recherches quel que soit l'état d'avancement de leurs travaux.

Elle permet à la fois de formaliser leurs questionnements et réflexions ainsi que d'échanger avec l'ensemble des membres des différentes équipes du laboratoire.

Pour débiter cette rencontre, nous aurons le plaisir d'accueillir le Pr. François LALLIER, Directeur du laboratoire Adaptation et Diversité en Milieu Marin, à l'Université Pierre et Marie Curie (Paris, 6), dont les travaux portent sur les écosystèmes des sources hydrothermales et plus particulièrement sur le mutualisme entre microorganismes et animaux des fonds marins. Il nous présentera « Symbiose et chimiosynthèse : des modèles venus du fond des océans ».

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Programme

8h45	<i>Ouverture de la 6^e JDD du LMGE</i>
8h50-10h	Symbiose et chimiosynthèse : des modèles venus du fond des océans <i>par Pr. François LALLIER - Station biologique de Roscoff</i>
10h-10h30	<i>Pause - café</i>
10h30-11h	Décryptage des mécanismes d'adhésion des microsporidies à leurs cellules-hôtes <i>par Nastasia PRYBYLSKI - IHP</i>
11h-11h30	Emergence, diffusion, evolution the 2015 variant of enterovirus A71 associated with neurologic manifestations <i>par Stéphanie TOMBA - EPIE</i>
11h30-12h	Impact of aqueous multi-contamination on the pure and formulated pesticide and/or antibiotics contamination <i>par Clément DESCARPENTRIES - CMES</i>
12h-14h	<i>Repas</i>
14h-14h30	Leaf litter decomposition in lotic Ecosystems in a global change context: role of heterotrophic microbial communities <i>par Florent ROSSI - CMES / IRTA</i>
14h30-15h	Blastocystis infection induces intestinal dysbiosis associated visceral hypersensitivity <i>par Manon DEFAYE - IHP</i>
15h-15h30	<i>Pause - café</i>
15h30-16h	Microbial genome reconstruction from metagenomic data <i>par Kévin GRAVOUIL - MEB</i>
16h-16h30	Impact of genetic background, eusocial behaviors and abiotic factors on spatio-temporal patterns of parasites in West-European honeybees <i>par Iris EOUZAN - IHP</i>
16h30	<i>Clôture de la 6^e JDD du LMGE</i>

Symbiose et chimiosynthèse : des modèles venus du fond des océans

Francois LALLER

*Professeur Sorbonne Université, Station biologique de Roscoff
Laboratoire Adaptation et Diversité en Milieu Marin (AD2M), UMR 7144*

Peu après leur découverte il y a 40 ans, les principales espèces colonisant les sources hydrothermales profondes se sont révélées être en (endo)symbiose avec des bactéries dont le métabolisme chimioautotrophe assurait leur croissance phénoménale. C'est le cas du ver tubicole *Riftia pachyptila*, et de la moule *Bathymodiolus azoricus*, les deux espèces sur lesquelles je me focaliserai lors de cet exposé.

Je me suis d'abord intéressé aux adaptations physiologiques de l'hôte-animal : comment un animal peut-il fournir à ses symbiotes les molécules nécessaires à leur métabolisme particulier ? A savoir, dans le cas de *Riftia*, du dioxyde de carbone, du sulfure d'hydrogène et de l'oxygène. De 1994 à 2007, en collaboration avec l'équipe de Jim Childress, nous avons affiné notre compréhension de la physiologie du transport de ces molécules. Mais plusieurs étapes nous échappaient et nous nous sommes alors tournés vers des approches plus globales dans l'espoir de les révéler.

Riftia n'étant pas un modèle se prêtant facilement à l'expérimentation, nous nous sommes concentrés depuis 2006 sur la moule *Bathymodiolus azoricus*. Cette espèce abrite non pas une, mais deux souches de symbiotes dans ses branchies, une thiotrophe et une méthanotrophe. Et leur symbiose est très plastique : selon les conditions environnementales l'abondance et la proportion relative des deux symbiotes peut varier. Mais qui contrôle qui ? La moule peut-elle réguler le développement des populations de ces deux bactéries ? Pour répondre à cela, restait à réaliser des expérimentations par 2000m de fond et à développer les outils moléculaires capables de nous révéler les acteurs du dialogue nécessaire entre ces partenaires. Un génome, des transcriptomes, des puces ARN et quelques plongées profondes plus tard, les résultats de ces 3 dernières années commencent à mettre au jour des pistes intéressantes : reconnaissance moléculaire, défenses immunitaires et apoptose...



Décryptage des mécanismes d'adhésion des microsporidies à leurs cellules-hôtes

Nastasia PRYBYLSKI - 1ère année
Encadrant : Hicham EL ALAOU
Équipe Interaction Hôtes-Parasites (IHP)

Décryptage des mécanismes d'adhésion des microsporidies à leurs cellules-hôtes

Les microsporidies sont des parasites eucaryotes unicellulaires apparentés aux champignons. Chez l'Homme, les microsporidies sont considérées comme des pathogènes émergents. De plus, plusieurs espèces parasitent des animaux d'intérêt économique, entraînant par conséquent d'importantes pertes.

L'infection nécessite une étape d'adhésion préalable des microsporidies à la surface des cellules-hôtes. Plusieurs études ont démontré que cette adhésion était médiée par des glycanes sulfatés. Ainsi, plusieurs analogues de glycanes ont montré leurs propriétés antimicrosporidiennes notamment par leur capacité à interférer avec l'adhésion. Ce projet de thèse vise ainsi à (i) caractériser le mécanisme d'action des analogues de glycanes, (ii) caractériser les protéines de surface des spores microsporidiennes impliquées dans l'adhésion aux cellules-hôtes et (iii) identifier les glycanes ciblés par les parasites. Ce travail sera réalisé sur *Encephalitozoon cuniculi* et *Anncaliia algerae*, deux modèles de microsporidies cultivé au laboratoire.

Plusieurs analogues de glycanes seront évalués pour leur capacité d'interaction aux protéines parasitaires de surface. Ces protéines seront sélectionnées par une approche bio-informatique en utilisant les algorithmes de prédiction de localisation pariétale (SignalP, WoLF PSORT) et leur localisation sera validée expérimentalement. Des études d'interaction de ces protéines avec les analogues de glycanes seront réalisées notamment par la méthode de titration calorimétrique isotherme (ITC pour Isothermal Titration Calorimetry). Des approches par ARN interférence (ARNi) permettront de confirmer le rôle de ces protéines parasitaires dans l'adhésion. Ce projet permettra ainsi de mieux comprendre les processus d'adhésion des microsporidies à leurs cellules-hôtes.

Mots clés : microsporidies / adhésion / protéines pariétales / glycosaminoglycanes



Emergence, diffusion, evolution the 2015 variant of enterovirus A71 associated with neurologic manifestations

Stéphanie Tomba - 2e année

Encadrant : Jean-Luc BAILLY et Audrey MIRAND

Équipe Épidémiologie et Physiopathologie des Infections à Entérovirus (EPIE)

Introduction. Two outbreaks of enterovirus (EV) infections associated with neurologic manifestations were reported in 2016, in Spain and France. The EV type A71 (EV-A71) was identified in 49 to 87% of cases. Most virus EV-A71 strains were genetically related to a subgenogroup C1 variant, which was reported for the first time in Germany in 2015. This new EV was reported to be recombinant but the evolutionary origins are unknown.

Aim. To describe the diffusion of the EV-A71 variant in France in 2016 and the evolutionary origins.

Materials and methods. The viral VP1 sequences (n=29) were determined in 21 patients admitted to different hospitals in France, 3 children with HFMD, and 5 children with asymptomatic infections. The geographic diffusion and the temporal origin of the virus were analysed with phylogenetic analysis. The evolutionary origin was studied by comparing five complete EV-A71 variant genomes to 842 reference genomes.

Results. In our study, the EV-A71 was associated with the following manifestations: cardiorespiratory distress, n=1; encephalitis, n=2; cerebellitis, n=2; acute meningitis, n=2; fever (neonates), n=9; and HFMD, n=5; the virus was also reported in the faeces of 5 children under 3 years. The 29 infection cases were caused by a same virus strain, phylogenetically related to that reported in Germany. Molecular dating suggested that virus spread began in 2013 in Europe. This virus harboured one amino acid change (VP3 capsid protein) near a receptor site and was subject to multiple recombination events with other EV types reported in China, Russia, and Turkmenistan.

Conclusion. The variant strain is a recombinant and circulating form of EVA71 and the spread was associated with severe neurological manifestations. Global surveillance needs to be reinforced to determine whether intercontinental dissemination of this virus may be associated with long-term risk of severe diseases in children.

Keywords: Infectious disease; Molecular epidemiology; Virus evolution; Picornavirus



Impact of aqueous multi-contamination on the pure and formulated pesticide and/or antibiotics contamination

Clément DESCARPENTRIES - 1ère année

Encadrant : I. BATISSON, P. BESSE-HOGGAN

Équipe Communautés Microbiennes : Écotoxicologie, Santé (CMES)

The combined use of various pesticides, applied each at low doses, is now generalized in conventional agriculture in order to improve their efficiency but also to reduce the application costs. As a consequence, a multi-contamination is observed in soil and water due not only to the presence of the different parent compounds but also of many degradation products. Furthermore, land application of livestock manure, as a soil fertility amendment, may directly release veterinary antibiotics into the field and aquifers, where they can persist. Up to date, the effects of pesticide and antibiotic multi-contaminations on the fate of each individual molecule have rarely been taken into account. Moreover, in ecosystems, the toxicity of mixtures of those molecules, in terms of additive, synergistic or antagonistic effects, remains unclear.

The first research axis of my thesis project is to study the effect of a mixture of pure or formulated pesticides (nicosulfuron, S-metolachlor and mesotrione, commonly used in corn production) in the presence of two widely used sulfonamide veterinary antibiotics detected on their respective biodegradation and toxicity. Specific objectives are to: 1/ isolate microorganisms that are able to transform those pesticides in order to identify and characterize (by HPLC, LC-MS and NMR) the different metabolites formed during biodegradation, 2/ investigate the effects of molecule mixtures on their biodegradation, in terms of kinetics and metabolic pathways, 3/ evaluate the toxicity of mixtures of pure and formulated molecules on the growth of selected microorganisms.

The second research axis of my thesis will focus on an innovating bioremediation system aiming at reducing soil and water contamination. This system consists in spreading a pesticide simultaneously with a microorganism able to mineralize it. The difficulty will be to find the optimal conditions for microbial degradation of pesticides after their specific action and before their transport to environmental compartments.

Keywords: biodegradation, pesticide formulations, antibiotics, cocktail effect, bioremediation



Leaf litter decomposition in lotic Ecosystems in a global change context: role of heterotrophic microbial communities

Florent ROSSI - 3^e année

Encadrant : Joan Artigas et Clarisse Mallet

Équipe Communautés Microbiennes : Écotoxicologie, Santé (CMES)

Équipe Interactions dans les Réseaux Trophiques Aquatiques (IRTA)

Leaf-litter decomposition is a key mechanism in headwater streams, allowing the transfer of nutrients and energy into the entire food web. However, many streams receive a large variety of chemical compounds, including pesticides and pharmaceuticals that may exercise a high pressure on aquatic organisms, such as heterotrophic microbial communities and their organic matter decomposition activity.

In this thesis, different experimental approaches were used to assess the effects of a complex and realistic chemical contamination, including nutrients and xenobiotics, on the structure, biomass and activity (decomposition ability and related extracellular enzyme activities) of microbial communities (fungi and bacteria) colonizing *Alnus* litter, and their response to cope with this stress.

Firstly, a field experiment was performed on six streams, presenting different gradients of contamination over four seasons. Results showed that nutrients (especially nitrogen) were the main parameter controlling microbial activity, partially buffering the effects of xenobiotics on *Alnus* microbial decomposition. To go further, a microcosm experiment was performed, testing the effects of single and combined effects of nutrients (mesotrophic versus eutrophic) and pesticides (tebuconazole and s-metolachlor, alone or in mixture) on leaf-microbial decomposition. The interaction between high nutrients and xenobiotics led to more active microbial communities for leaf decomposition and extracellular ligninolytic activities (laccase and phenol oxidase), that can be explained by changes in community structure. Similarly, when testing the ability of these communities for xenobiotics degradation using culture approaches, higher herbicide dissipation (nicosulfuron) was observed in communities previously exposed to high nutrients and xenobiotics. Despite their adaptability, an almost full recovery in these communities was observed (structure and activity) after stress removal, as shown by a translocation experiment performed in microcosm.

Overall, chemical multi-contamination may affect leaf-associated microbial communities, but their high plasticity allow them to cope with such stress to ensure their function of organic matter decomposition in contaminated streams.



Blastocystis infection induces intestinal dysbiosis associated visceral hypersensitivity

Manon DEFAYE - 3e année

Encadrant : Frédéric DELBAC et Frédéric CARVALHO

Équipe Interaction Hôtes-Parasites (IHP)

Blastocystis is the most common parasite colonizing the human intestinal tract. Its clinical significance remains controversial despite recent epidemiological studies reporting higher prevalence in subjects suffering from Irritable Bowel Syndrome (IBS). IBS is a functional intestinal chronic disorder affecting about 11% of the population and is associated to a significant impairment of the patients' quality of life. This multifactorial disease is often associated with an increased intestinal permeability, a colonic hypersensitivity, an intestinal dysbiosis and deregulation of brain-gut axis. Currently, few studies have focused on the physiopathology of *Blastocystis* infection. However, *Blastocystis* infection has been correlated with visceral pain and in our previous studies with a decrease in beneficial bacteria. The aim of this study was to investigate in a rat model the impact of *Blastocystis* infection on colonic sensitivity, intestinal mucosa integrity, microbiota composition and consequences on animal behavior.

Juvenile Wistar male rats (4 week-old) were orally inoculated with *Blastocystis* subtype 4 (ST4) purified cysts. *Blastocystis* ST4 infection was associated to a colonic hypersensitivity at 4 weeks post-infection, without detectable intestinal permeability changes, inflammatory mediator secretion or histological modifications. However, this colonic hypersensitivity was associated to anxiety and depression-like behavior. In addition, 16SrDNA sequencing revealed microbiota differences between infected and non-infected animals. Analysis of SCFA production in feces revealed a significant decrease of acetate and propionate. Our results suggest that *Blastocystis* could contribute to the establishment of a visceral hypersensitivity contributing to behavior changes. Microbiota dysbiosis is also associated with *Blastocystis* infection, similar to those observed in IBS patients. Thus, *Blastocystis* infection could play a role in the triggering or contribute to symptoms of IBS patients' subgroup.



Microbial genome reconstruction from metagenomic data

Kévin GRAVOUIL - 3e année

Encadrant : Didier Debroas, Eric Peyretailade et Marie Pailloux

Équipe Génomique environnementale et bioinformatique (MEB)

Metagenomics has made the study of non-cultivable micro-organisms possible so we can can apprehend the biological functioning of any type of ecosystem. The inherent difficulties in this approach (sampling bias, sequencing bias, rare biosphere, etc.) do not allow an exhaustive inventory of genomes and genes, neither linking community structure to biological functions. To overcome this limitation, several strategies have been developed to *de novo* reconstruct genomes from metagenomes, notably through the use of *binning*. In this approach, we consider sequences with similar characteristics (in terms of composition and/or abundance) would belong to the same genome, without having to know this genome *a priori*.

An evaluation of different binning methods was conducted based on simulated metagenomes. The reliability of the methods depends partly on the taxonomic group to which binned sequences belong, but no taxonomic group are completely "insensitive" to binning. Moreover, the solutions proposed by the different methods are sometimes very different from each other. Thus, a consensus clustering approach would ideally improve the proportion of correctly binned sequences from 84% to 96% on average. However, binning methods are all based on the same information (composition and abundance of sequences), which limits the diversity of approaches required for consensus clustering. Thus, we have developed a new binning strategy which combine an new sequence model and an adaptive clustering approach. Multiple existing sequence model were integrated into a single model. This sequence model feeds an iterative clustering algorithm where only the most discriminant features of the model are considered at each iteration. Hence, clusters from the same dataset can be defined according to different criteria.

Keywords : Bioinformatics, metagenomic binning, sequence modeling, machine learning



Impact of genetic background, eusocial behaviors and abiotic factors on spatio-temporal patterns of parasites in West-European honeybees

Iris EOUZAN - 3e année

Encadrant : David BIRON et Télésphore SIME-NGANDO

Équipe Biodiversité microbiennes et adaptations fonctionnelles (BioADAPT)

European honeybees are agronomic interest species. Thereby, many imports of subspecies and non- native hybrids to the territory have taken place in the recent decades. These massive imports have caused a sharp decline in endemic populations that have been present for several millions of years and are well adapted to local conditions (e.g. climate, pests, and resources). This is particularly problematic in the current context of global honeybee decline, where adaptation is essential to enable bees to cope with pests and pesticides. My thesis is part of a project for the conservation of endemic honeybees of Western Europe (*Apis mellifera mellifera* and *A. m. iberiensis*), and is based on studies conducted on seven conservatories: four in France, one in Spain and two in Portugal. The aim of my thesis is to carry out spatio-temporal monitoring of parasites using three tools: metagenomics (16S) for midgut microbiome, flux cytometry for quantification of bacterial and virus loads in honeybee head and midgut, and PCR analysis for the most harmful viruses. First, we were able to demonstrate the bees' ability to maintain all year long 60% relative humidity in the hive. In a second time, we got many data concerning the main microorganisms (i.e. viruses, bacteria and microsporidia) present in our bees. According to these data, the virus communities are very different between the conservatories. We also found differences in the gut microbiomes: the same species were found in all the conservatories but varying in abundance from one conservatory to another and from one month to another. The analysis of other data such as colony weight, rain or brood production, is in progress. This project will identify the factors that positively or negatively impact the presence of honeybee parasites and propose concrete solutions to beekeepers in order to optimize the diseases' management.

Keywords: Honeybee, parasites, metagenomics, virus, microbiome





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Mardi 13 mars 2018

Amphithéâtre de Recherche

Bâtiment Physique

Campus Universitaire des Cézeaux

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