Indo-French Center for the Promotion of Advanced Research (IFCPAR / CEFIPRA)

Franco-Indian workshop in Functional Metagenomics

- from ecosystem processes to biotechnology -

Université Claude Bernard Lyon 1, Campus LyonTech la Doua, Villeurbanne - France

6 – 8 February 2019

Franco-Indian workshop in Functional Metagenomics, from ecosystem processes to biotechnology

Scientific committee

- Roland MARMEISSE, CNRS-UCBL Lyon
- Pierre PEYRET, UCA-INRA Clermont-Ferrand
- Sudhakara M. REDDY, Thapar Institute of Engineering & Technology Patiala

Organizing committee

- Danis ABROUK
- Dominique BOULANGER
- Marie-André LAVITAL
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- Betty BIGAÏ
- Laurence FRAISSINET-TACHET
- Patricia LUIS
- Stéphane MICHALAK
- Laurent VALLON

With the participation of:

- **IFCPAR / CEFIPRA**
  http://cefipra.org/

- **Université Claude Bernard Lyon 1 (UCB)**
  https://www.univ-lyon1.fr/

- **Agence Nationale de la Recherche (ANR) – Project "PeroxiDiv"**
  http://www.agence-nationale-recherche.fr/

- **Ecologie Microbienne Lyon (UMR UCB-CNRS-INRA)**
  http://www.ecologiemicrobiennelyon.fr/

- **Thapar Institute of Engineering & Technology, Patiala, India**
  http://www.thapar.edu

Venue

Université Claude Bernard Lyon 1, Campus LyonTech la Doua, 43 Boulevard du 11 Novembre 1918, Villeurbanne – France

Salle Guilliermont – "l'Herbier" Building

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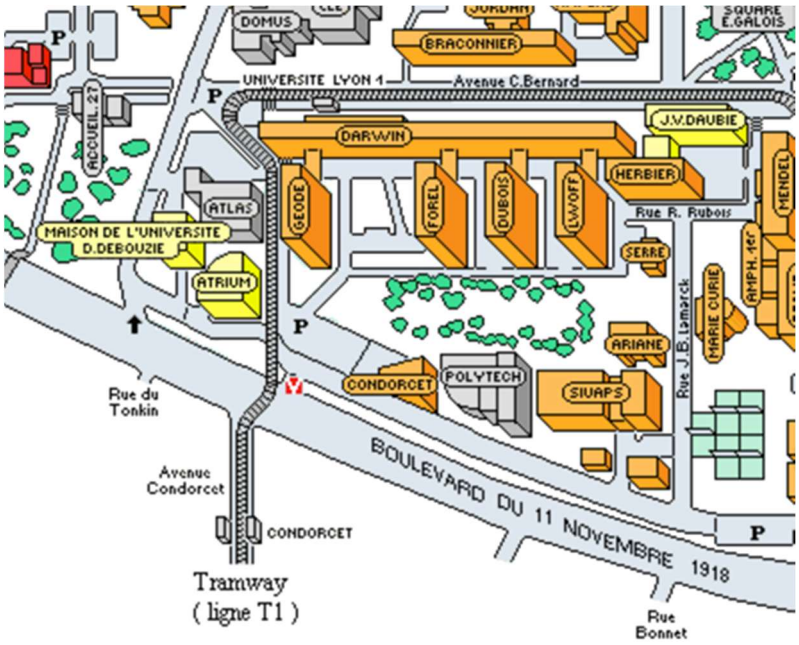

Scientific program

Wednesday February 6th

- **8:30 – 10:00 am Registration & Coffee**
- **10:00 – 10:30 Welcome address**
- **10:30 – Session 1: Functional screening of metagenomes (Chair: R. Sharma, H. Blotière)**
  - 10:30 Gabrielle POTOCKI-VERONESE - *High-throughput functional metagenomics for enzyme discovery*
  - **11:00 Chaitanya JOSHI - Rumen and gut microbiome studies in livestock and** *poultry*
  - 11:30 Patricia LUIS - *Exploring the functional diversity of sugar transporters in fungal communities using an environmental genomics approach based on the functional complementation of a sugar transport-deficient S. cerevisiae mutant by soil cDNA libraries.*
- **12:00 Lunch Break**
- **14:00 Session 1 (continue)**
  - 14:00 Denis FAURE - *Functional metagenomics extended the diversity of quorum-quenching enzymes.*
- **14:30 Session 2: Microbial biotechnology from and for environment (Chair: R. Marmeisse, P. Sar)**
  - 14:30 Laurence FRAISSINET-TACHET, Rajiv YADAV, Sudhakar M REDDY - *Environmental metatranscriptomics: A source for isolation of novel genes involved in metal tolerance (an illustration of a CFIPRA-funded collaborative project)*
  - 15:45 Rakesh SHARMA, *Metagenomics for Industrial and Environmental Biotechnology*
- **16:15 Coffee Break**
- **16:45 Session 2 (continue)**
  - 16:45 Prashant S PHALE - *Evolution and Compartmentalization of Carbaryl Degradation Pathway in Pseudomonas sp. strain C5pp*
- **17:15 Session 3 Bioinformatics for metagenomics mining (Chair: M. Krishnamohan, P. Peyret)**
  - 17:15 Alessandra CARBONE - *Many probabilistic models are better than one: a new look into Metagenomic Data and functional annotation*
  - 17:45 Sharmila S MANDE - *Human microbiome and health*
  - 17:45 Eric PELLETIER - *Ocean 'omics: A path to large scale analysis of marine organisms*
  - 18:15 Vincent LOMBARD - *Metagenomic analysis of carbohydrate-active enzymes*

Thursday February 7th

- **8:30 – Session 4: The human microbiome (Chair: S.S. Mande, D. Bouchon)**
  - 8:30 Yogesh S. SHOUCHE - *Human Microbiome: Indian Perspective*
  - 9:00 Hervé BLOTIERE - *A Functional Metagenomic approach to decipher microbiotia / intestinal barrier cross-talk*
  - 9:30 Krishna M MEDICHERLA - *Microbiome diversity analysis in subclinical and clinical Rheumatic Heart Disease in school going children*
  - 10:00 Bhabatosh DAS - *Role of the gut microbiome of healthy population in the emergence of resistant enteric pathogens*
- **10:30 Coffee Break**
- **11:00 Round table 1: Research funding in France/India, research opportunities in metagenomics (Chair: D. Faure, Y. S. Souche)**
- **12:15 Lunch Break**
- **14:00 Visit of the Herbarium (+ group photo)**
- **15:00 – Session 5: Microbial ecosystem functioning (Chair: S.M. Reddy, E. Pelletier)**
  - 15:00 Tom DELMONT - *Nitrogen-fixing populations of Planctomycetes and Proteobacteria are abundant in surface ocean metagenomes*
  - 15:30 Pinaki SAR - *Metagenomic roadmap to deep biosphere within the crystalline earth crust*
  - 16:00 Didier BOUCHON - *Lignocellulose degradation at the holobiont level: teamwork in a keystone soil invertebrate*
- **16:30 Coffee Break**
  - 17:00 Stéphane UROZ - *Functional characterization of the soil microbiome in relation with soil properties*
  - 17:30 Cécile LEPERE - *"Omic" approaches to decipher microbial eukaryotes function in freshwater lake ecosystems*
  - 18:00 Christoph KEUSCHNIG - *An example study on the potential of co-occurrence networks in meta-transcriptomic studies*

Friday February 8th

- **9:00 – Session 6: Novel approaches in metagenomics (Chair: C. Lepère, C. Jochi)**
  - 9:00 Thomas BENEYTON / Jean-Christophe BARET - *Droplet-based microfluidics for functional metagenomics*
  - 9:30 Pierre PEYRET – *Sequence capture by hybridization*
  - 10:00 Nicolas CHEMIDLIN PREVOST-BOURE - *The biogeography of soil microorganisms*
- **10:30 Coffee Break**
- **11:00 Round table 2: Metagenomics 4.0, the future of functional metagenomics (Chair: R. Marmeisse, S.M. Reddy)**
- **12:15 Lunch and departure**

PARTICIPANTS

Jean-Christophe BARET

PhD, Professeur
CNRS, Univ. Bordeaux, Centre de Recherche Paul Pascal

Thomas BENEYTON

PhD, CNRS Research Engineer
CNRS, Univ. Bordeaux, Centre de Recherche Paul Pascal

Droplet-based microfluidics for functional metagenomics

Droplet-based microfluidics allows for the production and manipulation of monodisperse water-in-oil droplets at kHz frequencies. Typically, picoliter droplets are used as independent microreactors to perform biological assays at the single cell level. Droplets can be made, injected, incubated and sorted based on fluorescence[5] to build high-throughput screening (HTS) platforms for the screening of libraries based on enzymatic activities [3,4]. In a single experiment, millions of microorganisms are encapsulated (single cell per droplet) and individually sorted based on their enzymatic activities with significantly reduced time and cost footprints compared to robotic microtiter plate-based systems.

We present the development of droplet-based microfluidics for the single-cell analysis and screening of metagenomics libraries. First, we demonstrate the screening of uncultured bacteria communities extracted from soil sample [2]. Over 10^5 cells were screened based on cellobiohydrolase activity in less than 20 min and selected population show very different taxonomic diversity than when screened for growth. We also present a microfluidic platform for the HTS of heterologous enzymes secreted in the yeast *Yarrowia lipolytica*. [1] These platforms are available for the screening of metagenomics libraries to speed up the exploration of microbial functional diversity. In the future microfluidics presents tools that appear to be extremely promising in the context of metagenomics and metatranscriptomics analysis.

Hervé BLOTTIERE

PhD, Director of Research INRA
Head of the FInE/Blottière lab at Micalis Institute,
Scientific Director at MetaGenoPolis
INRA, AgroParisTech, Université Paris-Saclay

A Functional Metagenomic approach to decipher microbiotia / intestinal barrier cross-talk

Since the "microbiota revolution", the human gut microbiota is now considered essential in the control of key physiological functions for its human host, in particular considering the barrier function of the intestine and the maturation of the immune system. However, the mechanisms by which this key organ contributes to human physiology are still poorly understood. Indeed, it is a complex ecosystem composed of hundreds of different species, and the vast majority of microbes are either not yet cultivated or difficult to cultivate. To decipher the mechanisms of interaction between commensal bacteria and intestinal epithelial cells (IEC), we have developed a high-throughput innovative functional metagenomics approach (Lakhdari et al, 2010, de Wouters et al, 2014). Using human IECs stably transfected with a reporter gene (luciferase) under the control of the important gene promoter or key transcription factor binding elements (NF-κB, PPARγ, AP1, AhR), we have developed a high throughput screening method. This method was used to screen metagenomic libraries containing large DNA fragments (~ 40 kb) of the human gut microbiota, but also cultured commensal strains. We have shown that short-chain fatty acids are important regulators of gene expression and identified bioactive metagenomic clones modulating key signaling pathways in human IEC. Sequencing, annotation and transposition mutagenesis experiments allowed the identification genes involved in these effects. So far, about thirty metagenomic clones have been selected. For one clone derived from a *Bacteroides vulgatus*, we identified 2 loci involved in the activation of NF-κB. Another clone, derived from a Firmicutes, was selected for its ability to stimulate the NF-κB and AP1 pathways, as well as the secretion of IL-8 and TSLP. Two other clones derived from Firmicutes allowed to the identification of a new pathway for NF-κB activation by commensal bacteria, involving TIFA and ALPK1. The strategy is also used to decipher how commensal or probiotic bacteria can exert their benefic effects on host cells. In conclusion, this innovative approach of functional metagenomics leads to the identification of new bacterial genes and metabolites involved in the dialogue with the intestinal epithelium with functional consequences for the host.

Didier BOUCHON

PhD, Professor

Université de Poitiers
Laboratoire Ecologie et Biologie des Interactions - UMR CNRS 7267
Equipe Ecologie Evolution Symbiose

Lignocellulose degradation at the holobiont level: teamwork in a keystone soil invertebrate

Lignocellulose is the main component of plants and is composed of cellulose, lignin and hemicellulose that requires the collective action of diverse Carbohydrate-Active enZymes (called CAZymes). Many invertebrates express some lignocellulose-degrading enzymes, but in most of them efficient degradation of lignocellulose is only possible thanks to mutualistic associations with endosymbionts.

Due to their important role in the decomposition of organic matter, terrestrial isopods are recognised as keystone species in terrestrial ecosystems. Past studies have shown that they can digest cellulose and are able to produce some endogenous cellulases (Kostanjsek et al. 2010). Although marine isopods like *Limnoria quadripunctata* secrete all the enzymes necessary for cellulose digestion in the absence of gut microbes (King et al. 2010), terrestrial isopods would not be able to digest cellulose without the help of their microbiota (Bouchon et al. 2016). Similar to termites, it has been suspected that several hepatopancreatic symbionts may be involved in the lignocellulose degradation in terrestrial isopods completing the CAZyme repertoire of their hosts (Zimmer et al. 2002).

To test this hypothesis, transcriptomic and metagenomic approaches have been used in the pill bug *Armadillidium vulgare*. We identified the CAZyme repertoire from both the microbiome and the isopod host. Depending on CAZyme families, complementary as well as redundancy between host and microbiome repertoires were recorded. Tissue specific expression of some representative of the host CAZymes were shown. Experimental diet manipulations showed that the expression of these CAZymes were modified in correlation with the modification of the microbiote. Our results provide an insight into the role of the microbiome in the evolution of terrestrial isopods and their adaptive radiation in terrestrial habitat.

Alessandra CARBONE

PhD/ Distinguished Professor

- **Distinguished Professor - Sorbonne Université**
- **Director of the Department of Computational and Quantitative Biology (LCQB), CNRS-SU UMR7238**
- **Team leader 'Analytical Genomics' at LCQB.**

Many probabilistic models are better than one: a new look into Metagenomic Data and functional annotation

New computational paradigms have been recently used to identify and classify very divergent protein sequences in genomes and metagenomes. This opens the way to much finer annotations implying an unprecedented zooming into the metabolic processes of different environments. We shall present the main ideas of the computational method and its impact in the understanding of ecological and evolutionary information about ecosystem function.

Nicolas CHEMIDLIN PRÉVOST-BOURÉ

PhD, Lecturer in soil biology, microbial ecology

UMR 1347 Agroécologie, AgroSup Dijon – INRA -Université de Bourgogne Franche-Comté, Dijon

The biogeography of soil microorganisms

Microorganisms are extremely abundant and diverse in soils, supporting soil functioning either quantitatively or qualitatively; but are highly sensitive to anthropic activities. Nevertheless, documenting the spatial scaling and the determinism of of soil microorganisms' distribution at multiple scales remains necessary to better understand their ecology and support sustainable use of soils.

Over ten years, the soil microbial communities were investigated from the scale of France (2200 sites, RMQS) to the scale of an agricultural landscape (289 sites, Fénay) to better understand their characterize their distribution and the underlying environmental filters by means of high-throughput sequencing techniques. Conclusions were congruent between scales, soil bacterial communities being distributed according to heterogeneous biogeographical patterns and shaped mainly by soil texture, pH, Carbon content, and land-use (occupation or agricultural practices). These constitute the first extensive referential demonstrated operational and allowed developing models to evaluate the impact of agricultural practices on soils microbial communities and support actions for the sustainable use of soils.

Bhabatosh DAS

PhD, Associate Professor

Translational Health Science and Technology Institute

Postal Address: NCR Biotech Science Cluster, Gurgaon-Faridabad Expressway, Faridabad, Haryana, India, PIN: 121001

Role of the Gut Microbiome of Healthy Population in the Emergence of Resistant Enteric Pathogens

Human bodies carry trillions of microscopic organisms from all three domains of life. Among all the body sites the gastrointestinal tract (GIT) harbors highest bacterial load and microbial diversity. The resident microbial species encode key functionalities that play crucial role in host metabolic functions, synthesis of macro- and micronutrients, modulating efficacy and toxicity of xenobiotics, development of innate and adaptive immune systems, and resistance against colonization and invasion of enteric pathogens.

We recently investigated the gut microbiome of rural and urban healthy Indians living in sea level and high altitude areas and identified Firmicute and Bacteroidetes dominated gut microbial ecology in all the healthy Indians. Shotgun metagenomic analysis revealed differential abundance of microbial metabolic functions and xenobiotic degradation pathways in different population. Further analysis of the genome of commensal gut microbiota disclosed presence of multiple antibiotic resistance genes in the dominant commensal microbiota. The resistance genes present in the genome of commensal bacteria are linked with mobile genetic elements and have the ability to disseminate to the enteric pathogens in optimal laboratory conditions.

Tom DELMONT

PhD, Postdoc

Genoscope, CEA, France

Nitrogen-fixing populations of Planctomycetes and Proteobacteria are abundant in surface ocean metagenomes

Nitrogen fixation in the surface ocean impacts global marine nitrogen bio-availability and thus microbial primary productivity. Until now, cyanobacterial populations have been viewed as the main suppliers of bio-available nitrogen in this habitat. While PCR amplicon surveys targeting the nitrogenase reductase gene have revealed the existence of diverse non-cyanobacterial diazotrophic populations, subsequent quantitative PCR surveys suggest that they generally occur in low abundance. Here, we used state-of-the-art metagenomic assembly and binning strategies to recover nearly one thousand non-redundant microbial population genomes from the TARA Oceans metagenomes. Among these, we provide the first genomic evidence for non-cyanobacterial diazotrophs inhabiting surface waters of the open ocean, which correspond to lineages within the Proteobacteria and, most strikingly, the Planctomycetes. Members of the latter phylum are prevalent in aquatic systems, but have never been linked to nitrogen fixation previously. Moreover, we demonstrate that the discovered diazotrophs were not only widespread but also remarkably abundant (up to 0.3% of metagenomic reads for a single population) in both the Pacific Ocean and the Atlantic Ocean northwest.

Denis FAURE

PhD/ CNRS research director

- **CNRS research director at the Institute for Integrative Biology of the Cell (I2BC), University Paris-Saclay: Deputy-chief of the Microbiology department at I2BC; Team leader 'Plant-bacteria interactions ' at I2BC**
- **Director of the French Network on environmental genomics (CNRS-INRA-MNHN)**

Functional metagenomics extended the diversity of quorum-quenching enzymes.

N-acylhomoserine lactones (NAHLs) are diffusible signals used by many Proteobacteria to correlate gene expression to cell density via a regulatory pathway called quorum-sensing (QS). Aside from the enzymes implicated in biosynthesis of NAHLs, others are able to cleave or modify NAHLs, hence to disrupt QS-signaling. They have been identified in bacteria and eukaryotes, and are collectively called NAHLases or quorum-quenching enzymes. In this work, we used functional metagenomics for discovering new genes coding for quorum-quenching enzymes. I will also present the actions of the French network on environmental genomics (GDR GE).

Laurence FRAISSINET-TACHET

PhD, Lecturer

Lyon1 University - Microbial Ecology UMR CNRS 5557 INRA 1418

From the screening of soil eukaryotic metatransciptomes to the functional characterization of new metallothioneins

The objective of this study was to isolate and characterize new environmental genes expressed directly in soils by eukaryotic microorganisms and involved in metal resistance mechanisms. Then we have implemented the functional metatranscriptomic approach, based on the direct extraction of eukaryotic polyadenylated mRNAs from soil samples followed by their conversion into cDNAs cloned in a yeast expression vector. These environmental cDNA libraries were used to complement a Cd-sensitive yeast mutant and resistant genes were recovered and analysed. Fifty percent of these genes were completely new, without homologs in databases. Some of these last genes encode cysteine-rich proteins that we have called CRPs. These protein sequences present specific features that relate them to metallothioneins (MTs). They, however, also display unusual features. CRP expressions in E. coli, purifications and ESI-TOF-MS assays revealed that CRPs are able to chelate Cd, Zn and Cu and belong to a completely new MT family. These results highlight the power of the functional metatranscriptomic approach which allows the discovery of new metal resistance genes.

Chaitanya JOSHI

PhD, Professor

Director Gujarat Biotechnology Research Centre

Gujarat Biotechnology Research Centre**,** 6th Floor, M.S.Building Gandhinagar-382011, India

Rumen and gut microbiome studies in livestock and poultry

Rumen is fermentative vat harboring plethora of microbiome carrying out useful function of fermentative digestion of fodder and making important nutrients available to the host. Its diversity consist of all domains namely Bacteria, Archaea and Eukaryotes as well as viruses. Their abundance and homeostasis is maintained in the healthy ruminant. Feed being the substrate for these microorganisms, their abundance and diversity changes in response to different feed in the diet of ruminants.

Major focus of our study is diet dependent changes in different breeds of cattle, buffalo and camel located in different geographical regions of Gujarat. The analysis through metagenomics aimed at diversity and gene content of these microbiome , whereas metatranscriptomics analysis aimed at identifying active microbes and functionally active genes.

We also identified glycosyl hydrolase family genes and cloned and expressed and characterized. We also isolated facultative bacteria and fungi as well as anerobic bacteria from rumen. We reconstructed microbial genomes from the metagenomes sequences as well.

Further we investigated Interaction of gut microbes and host transcriptome modulates physiology and metabolism of full-sibs broilers with genetic variation. As well as gut microbial abundance and diversity in global commercial broilers and local breed of poultry-Kadaknath and correlation of genotype (using HD chip) of birds with microbial diversity (using metagenomics).

Christoph KEUSCHNIG

PhD, Post-Doc Researcher

Ecole Centrale de Lyon/ Centrale Innovation
Environmental Microbial Genomics Group
Laboratoire Ampere, UMR CNRS 5005

An example study on the potential of co-occurrence networks in meta-transcriptomic studies

Previous experiments on N_2O producing fungal cultures showed that N_2O production kinetics is lower in fungi than in bacteria by a factor of at least 10^3. However, soil-based studies implicate fungi in a dominant role during N_2O emissions even though we cannot use enzyme kinetics to explain the quantity of N_2O found produced by fungi. We hypothesized that nitrogen cycling prokaryotic communities in soil depend on the presence of fungi. Three types of organic matter (paper pulp, sewage sludge and green compost) were added to the same agricultural soil in microcosms and soil was sampled at eight time points over 30 days of incubation. Fungicides were added as controls to inhibit fungal metabolic activity. Metatranscriptomes of total RNA (prokaryotic and eukaryotic transcripts) and poly-A-tail isolated mRNA (eukaryotic transcripts) and metagenomes were sequenced by NGS and analyzed. N_2O, CH_4 and CO_2 were also quantified in the microcosm headspace. N_2O production depended on the presence of fungi, whereas CH_4 was produced independent of fungal inhibition. Organic matter degradation signatures were primarily found in eukaryotic derived transcripts, whereas nitrogen-cycling related transcripts were found to be of prokaryotic origin. Co-occurrence networks gave candidates of potentially closely related fungi and bacteria for further species targeted studies.

Cécile LEPERE

PhD, Associate professor

Laboratoire microorganismes: génome et environnement (LMGE), CNRS, Clermont Auvergne University, IUF (institut Universitaire de France)

"Omic" approaches to decipher microbial eukaryotes function in freshwater lake ecosystems

Even though microbial eukaryotes are central for the functioning of aquatic ecosystems, they still constitute one of the least characterized components of the biosphere, particularly in lacustrine ecosystems, partly due to their lack of morphological characteristics and difficulty to cultivate them. The overall goal of this project is to enhance our knowledge on their ecological functions in the ecosystems through metatranscriptomics and metagenomics. We focus on two key trophic modes, which the importance has been highlighted these last years: parasitism and mixotrophy. This study is performed on a freshwater model lake: lake Pavin (France), which is a meromictic lake characterized by the presence of a permanent anoxic zone. The sampling occurs at 4 different seasons, in the oxic and anoxic zones and by day and night.

Vincent LOMBARD

PhD, Research Engineer (IR1)

Architecture & Fonction des Macromolécules Biologiques (AFMB); CNRS / Aix Marseille University

Metagenomic analysis of carbohydrate-active enzymes

Over the past 20 years, the Carbohydrate-Active enZyme (CAZy) database has become the reference classification system for enzymes that acts on glycans. This system relies on daily manual annotations augmented by deep human expertise on the families created and stored in the database (exclusively after experimental characterization). While the CAZy website only displays the classification of Genbank sequences, we also participate to the annotation of carbohydrate-active enzymes in genomic and metagenomic projects. The collaborators' data are privately annotated and analyzed and are not displayed on the CAZy website. The analysis starts from either raw data, or assembled contigs or gene predictions but the annotation quality increases with the degree of assembly. Our past collaborations focused on various ecosystems: mammalian or insect microbiota, oceanic samples, volcanic ponds, forest soils, ancient muds... The CAZy database system also performed the analysis of gene catalogs such as those derived from human, cow, pig and mouse microbiota. These analyses enlighten our understanding of carbon/energy acquisition by organisms and microbial communities and guide enzyme discovery.

Patricia LUIS

PhD, Lecturer

Institution/affiliations : Université Lyon 1 – UMR CNRS 5557 / UMR INRA 1418 Ecologie Microbienne

Exploring the functional diversity of sugar transporters in fungal communities using an environmental genomics approach based on the functional complementation of a sugar transport-deficient *S. cerevisiae* mutant by soil cDNA libraries.

Plant litter decomposition is an essential process in global terrestrial carbon cycling. Mechanisms involved in this degradation process are under the control of soil saprotrophic fungi, which secrete numerous hydrolytic enzymes to access to their main source of carbon contained in complex polymers such as cellulose and hemicelluloses. Such polymer hydrolysis produces a large variety of monosaccharides that enter the fungal cells to be further degraded and used as energy sources. In the literature, most studies dealing with the breakdown of plant biomass concentrate on the enzymatic hydrolysis of plant polymers and largely ignore the downstream events of sugar assimilation by microbial cells. As a consequence, despite the reasonable expectation that carbohydrate assimilation mechanisms developed by fungal cells are as important as the hydrolysis steps for the comprehension of litter decomposition and carbon cycling in soils, the diversity of sugar transporters expressed in soil has never been studied. The present work focuses on the functional diversity of sugar porters expressed by soil fungi in two mono-specific forests and the nature of monosaccharides transported according to the tree species considered (Beech vs Spruce) as we hypothesized that fungal communities selected by different tree species may express sugar porters with different preference for monosaccharide uptake.

Sharmila Shekhar MANDE

PhD, Chief Scientist, TCS Research
Tata Consultancy Services Ltd

Hadapsar Industrial Estate, Pune 411013

Human microbiome and health

The microbial communities inhabiting our body (called 'human microbiome') outnumber our own cells and play critical roles in maintaining our health. An imbalance in this microbial community has been associated with several diseases and metabolic disorders. Therefore, it is essential to understand the composition of microbiome in healthy human in order to delineate the host-microbiome interactions. Our studies have shown that gut microbiome changes with nutritional status as well as lifestyle differences (rural/urban, ethnicity, diet).

The mutual associations within the co-inhabiting microbes are also important in determining health status of individuals. We have performed comprehensive analyses of variations in bacterial communities and have utilized them to identify (a) metabolic pathway based biomarkers for monitoring the status of gut health, and (b) microbial community based biomarkers that can accurately predict the risk of preterm birth in early stage of pregnancy.

Apart from utilizing differential taxonomic abundances between healthy and disease states as disease markers, it is also important to quantify the changes in inter-microbial associations. In order to quantify rewiring and community changes in microbial association networks in healthy and disease states, we have developed a method (called 'NetShift') for analyzing 'case-control' microbiome datasets. Netshift enables quantification of salient changes between two distinct states (e.g. disease and healthy) and can identify taxonomic groups that possibly act as 'drivers' for the diseased state. I will discuss in detail the above mentioned aspects during my talk.

Roland MARMEISSE

PhD, CNRS senior research scientist (Research Director)

Ecologie Microbienne, CNRS/INRA/University Lyon 1 joint Research Department

Screening eukaryotic microbial communities for genes of relevance in ecology and biotechnology

Microbial eukaryotes (fungi, "protists") play key roles in different ecological processes such as organic matter degradation or nutrient cycling in soils. Several of these processes are tightly linked to the activities of enzymes or transmembrane transporters produced by these microorganisms. These enzymes and transporters are also of major interest in biotechnology for the production of biofuels, in biorefinery or for the synthesis of high added value chemicals. We have developed specific strategies to mine microbial eukaryotic communities for these genes of interest using environmental genomic approaches. They are essentially based on the direct extraction of eukaryotic poly-A mRNA from environmental samples followed by their conversion into cDNAs that can be screened by expression in yeast. Over the years, we have used this approach to select novel transporter genes, enzymes implicated in plant organic matter degradation and genes encoding novel proteins participating to metal homeostasis.

Krishna Mohan MEDICHERLA

PhD, Head, Biotechnology and Bioinformatics and Professor, Biotechnology, BISR, Jaipur & Professor, Department of Bioengineering, Birla Institute of Technology, Mesra, Ranchi.

Microbiome diversity analysis in subclinical and clinical Rheumatic Heart Disease in school going children

Sore throat caused by "Group A Streptococcus" (GAS) may also lead to serious complications like acute rheumatic fever (ARF) with subsequent rheumatic heart disease (RHD). Natural throat microbiome is thought to be beneficial to the host by priming the immune system and providing colonization resistance to harmful microbes. Recently several echocardiographic studies have shown high prevalence of subclinical RHD in asymptomatic school going children. Therefore, we attempted to identify and understand the microbiome diversity in Subclinical RHD children and compare them with that from age matched control group and also clinically confirmed RHD group. Out of 520 children (mean age 11.89±1.85, age ranging 10-15 years) examined, 320 were from high socioeconomic status (mean age 12.11±1.96, age ranging 10-15 years) and 200 were from low socioeconomic status (mean age 11.67±1.24, age ranging 10-15 years). We found high prevalence of 60 per thousand subclinical GAS infections in low socioeconomic group as compared to 6.25 per thousand in high socioeconomic group. This finding is consistent with our finding of high prevalence of subclinical RHD in school going (38.59 per thousand) by Echocardiography using World Heart Federation criteria. Microbiome (n=42) analysis revealed differences in the subclinical group that is closer to RHD group. Role of antibiotic usage on microbiome changes is being studied.

Eric PELLETIER

PhD

CEA / Genoscope

Ocean 'omics: A path to large scale analysis of marine organisms

Albeit it's crucial importance to most of the main planetary geochemical cycles (O, C, N, S, P), and rooting the oceanic food web, the marine plankton compartment remains poorly described. Covering viruses, prokaryotes and eukaryotes, autotrophs and heterotrophs, unicellulars and pluricellulars, planktonic populations are highly complex and fluctuent, and can be highly impacted by environmental changes.

The *Tara* ocean project aims to build a holistic view of this key organisms, using a large panel of approaches, from satellite observation to numerical oceanic models via high throughput imaging, multi-omics and microscopy.

In this talk, focus will be made on the usage of most recent metagenomics and metatranscriptomics approaches to describe structure and function of planktonic eukaryotes on a large scale, from genes to populations.

Pierre PEYRET

Professor/ Director MEDIS

MEDIS / University Clermont Auvergne - INRA

Gene capture by hybridization

Microorganisms comprise the majority of living organisms on our planet. Most of them, identified by indirect molecular approaches, belong to microbial dark matter. For many years, exploration of the composition of microbial communities has been performed through the PCR-based study of the small subunit rRNA gene due to its high conservation across the domains of life. The application of this method has resulted in the discovery of many unexpected evolutionary lineages. The advent of metagenomic and metatranscriptomic approaches has highlighted the metabolic capabilities of microbial communities. Successive sequencing improvements combined with dedicated bioinformatics tools have contributed to the exponential acquisition of data. Thus, linking functions back to the species has revolutionized our understanding of how ecosystem function is sustained by the microbial world. However, the sequencing depth required to provide a comprehensive view of microbial communities and the sequence data treatment remain particularly challenging for numerous ecosystems. New strategies of sequence capture by hybridization have been developed, to reveal the missed microbial diversity and functions.

Prashant Sitakant PHALE

PhD, Professor

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Evolution and Compartmentalization of Carbaryl Degradation Pathway in *Pseudomonas* sp. strain C5pp.

Carbaryl (1-naphthyl-*N*-methylcarbamate), a carbamate family pesticide, acts by inhibiting the enzyme acetylcholinesterase competitively, thus impairing the insect's nervous system. *Pseudomonas* sp. strain C5pp, a soil isolate, mineralizes carbaryl efficiently *via* 1-naphthol, salicylate and gentisate into central carbon pathway. Biochemical studies indicate that, the pathway is organized into 'upper' (carbaryl to salicylate), 'middle' (salicylate to gentisate) and 'lower' (gentisate to central carbon intermediates) segments. The draft genome analyses and comparattive genomics confirms the presence of three distinct operons for the degradation. The annotation of upper pathway operon revealed the presence of new genes encoding carbaryl hydrolase (CH), a member of new family of esterase and new gene encoding 1-naphthol 2-hydroxylase (1-NH). 1NH shared a common ancestry with 2,4-dichlorophenol monooxygenase, suggesting the evolution of new active-site. The 'upper' pathway genes encoding CH and 1NH were reported to be a part of an integron, while the 'middle' and 'lower' pathways were present as two distinct class-I composite transposons, suggesting the horizontal gene transfer events in this isolate. Genes encoding CH and 1-NH with novel features have probably evolved under the positive selection pressure of carbaryl in the environment. CH which catalyzes the first key step of carbaryl degradation pathway is localized in the periplasm and rest of the enzymes responsible for the metabolism were found to present in the cytoplasm of the strain C5pp. The strain was found to utilize carbaryl as the sole carbon as well as nitrogen source. Methylamine generated by the hydrolysis of carbaryl is also transported across the inner membrane and utilized as the sole source of the nitrogen. Thus, the compartmentalization of degradation pathway such as translocating CH to the periplasm and evolution of a new cytoplasmic enzyme like 1NH might be a strategy for the successful adaptation by the isolate to survive in the carbaryl contaminated environment. This also helps strain to overcome the toxicity of the intermediate metabolite, i.e 1-naphthol produced during the degradation of carbaryl.

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High-throughput functional metagenomics for enzyme discovery

Glycans are widely distributed in nature. Produced by almost all organisms, they are involved in numerous cellular processes, such as energy supply and storage, cell structuration, protein maturation and signalling, and cell recognition. They also represent a reliable source of carbon for microbes, which have developed complex strategies to face their structural diversity and to harvest them.
In order to boost their identification and characterization, a functional metagenomic approach was developed, based on various high-throughput and ultra-high-throughput activity-based screening strategies. The functional potential of Gbp of metagenomic DNA from various origins was explored, revealing dozens of novel enzyme families and functions.
Integration of biochemical, structural, meta-omic and omic data allowed us to decipher, from the molecular to the ecosystemic scale, novel mechanisms of plant, microbial and mammal glycan metabolization, which appear as new targets to control host-microbe interactions. They also constitute efficient biotechnological tools for biorefineries and synthetic biology.

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Environmental metatranscriptomics: A source for isolation of novel genes involved in metal tolerance

Eukaryotic microorganisms living in heavy metal polluted soil represent a rich source of genes that control the metal homeostasis. But, these studies are adversely affected by the inability of many micro-eukaryotes to grow in pure culture. Functional metatranscriptomics may serve as a powerful approach in these conditions and can be applied to discover novel genes involved in metal tolerance. In the present investigation, RNA was isolated from Cd contaminated site, size fractionated, cDNAs were synthesized and 3 size fractionated cDNA libraries were constructed in a yeast expression plasmid. The libraries were screened for metal tolerance genes by using metal sensitive phenotype of *S. cerevisiae* mutants such as *ycf1*$^{\Delta}$ for Cd, *DTY4* for Cu, *zrc1*$^{\Delta}$ for Zn and *Cot1*$^{\Delta}$ for Co. Highly resistant clones were sequenced and annotated. Few discovered genes were already known for their role in metal resistant while many were showing similarity to genes that had not been studied for their role in metal resistance. Other genes had showed no matches in the database. The screened genes can be used to elaborate new pathways involved in metal homeostasis and can be manipulated in selected organism for desired function. Present study results suggested that metatranscriptomic approach adopted in this study to identify genes in polluted soils could be applied to different areas or environments to identify genes involved in organic degradation or in bioremediation.

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Metagenomic roadmap to deep biosphere within the crystalline earth crust

Metagenomic exploration of the deep (3000 m below surface) biosphere underneath hot, oligotrophic, igneous continental crust of late Cretaceous to Archean age provides new insights in to the diversity-distribution of microbial life in such extremities, their interaction with elements and helps to gauge the environmental limits of life. Together with geochemical demarcation, continental crust at varied depth harbors distinct microbial assemblages with characteristic biogeochemical functions. Close association of autotrophic carbon fixers (Anaerolineae, Cyanobacteria); inorganic N, S, Fe oxidizers (Nitrospira, Spirochitae, Acidimicrobia); denitrifying, sulfate reducing members (Firmicutes and Deltaproteobacteria), and methanogenic archaea highlights the microbial guilds and their metabolic tie ups. Shotgun metagenomics reveals that the synergy in utilization of geogenic products (CH4, H2, CO2, HCO3, SO42-, PO43-) could be the strategic response of the deep microbial community harboring chemolithotrophs, autotrophs and fermentative microbial groups to sustain within the deep continental crust.

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Metagenomics for Industrial and Environmental Biotechnology

Microorganisms inhabit various niches and face different abiotic stress like high salt, toxic heavy metals and oxidative stress. They possess different stress tolerance machineries to overcome these stresses. 'Unculturable' bacteria present in different environments are expected to possess unique stress tolerance mechanisms. Identification of stress tolerance genes from 'unculturable' bacteria and their characterization will help in elucidation of these mechanisms in different microbial communities. We screened metagenomic libraries and isolated salt tolerant clones. Three salt tolerant clones were chosen for further characterization. These clones were able to grow on inhibitory concentration of NaCl, KCl and LiCl. The Identified genes showed low homology match with the known sequences in the databases indicating their origin from yet uncharacterized organisms. Their possible function and role in stress tolerance mechanisms was predicted using in-silico approach.

Arsenic is a major environmental contaminant in various part of the world including Bangladesh and West Bengal in India. Arsenic is toxic to bacteria, plants and animals. Arsenic detoxification systems have been identified from various organisms including bacteria and fungi to understand arsenic detoxification strategies and to develop bioremediation processes. We focused our study to access pathways for arsenic resistance from unculturable bacteria. Arsenic tolerant clones were selected from metagenomic libraries. Clones tolerate up to five fold higher amount of sodium arsenate compared to control and showed higher tolerance to arsenite. Unique arsenic resistant clones were completely sequenced and sequence analysis revealed genes similar to putative arsenate reductase, arsenite efflux pump and membrane proteins.

We are applying NGS based metagenome sequencing to understand microbial community dynamics in open waste water streams, their metabolic potential and to understand impact of drain water mixing on microbiome of the river. We have also identified pathogens and antibiotic resistance genes in waste water drain samples.

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Human Microbiome: Indian Perspective

The human gut microbiota is "the ecological community of commensal, symbiotic and pathogenic microorganisms that literally share our gastrointestinal tract". Dominated by eubacteria, the metabolic activities performed by the gut microbiome is often as complex as an organ and hence it is now being appreciated and studied in much detail. Increasing evidence suggests that the human gut microbiota changes according to diet, age, lifestyle, climate and geography, genetic make-up, early microbial exposure and health status. Studying the Indian population is relevant given the known dietary and geographical variety, unique family structure and ethnic diversity.

In traditional Indian familial system, where three generations can be studied for changes in the gut microflora with age, it has been shown that the gut microbiota changes according to age within individuals of the same family and a shift in the *Firmicutes/Bacteroidetes* ratio with age is observed, which is different than previously reported in European population. With the incoming wave of lifestyle changes observed now in India and given the availability of sugar-rich diet, the population is at high risk of developing obesity and diabetes. Preliminary work indicates prominence of genus *Bacteroides* amongst obese Indian individuals with an elevated fecal SFCA (Short-Chain Fatty Acids) levels. In the case of Diabetes, a consolidated disbiosis of not just eubacterial but also of archaeal and eukaryotic components is seen in the gut microbiota of newly-diagnosed and known-diagnosed diabetic individuals as compared to healthy individuals.

The selection of gut microbiota is also influenced by the genetic make-up of individuals and their birth mode as seen in a study which compared gut bacterial community of Indian and Finnish children with the host genotype, which revealed that FUT-2 gene polymorphism and birth mode does indeed affect the eventual gut microbial profiles of children. This selection is driven by specific bacterial groups such as *Prevotella*, *Megasphaera* for Indian subjects and *Blautia* and *Bifidobacterium* for Finnish subjects. Comparative analysis of gut microbiota of healthy Indian subjects with other populations highlights that the gut microbiomes of Indians is different from that of other Western populations and even cluster separately from Asian populations. The distinctive feature of the healthy Indian gut microbiome is the predominance of genus *Prevotella* and *Megasphaera.*

Taken together, the relevance of studying the Indian microbiome is justified given its unique microbiome features and further studies are necessitated to understand the determinants shaping the Indian microbiome. This will be helpful to develop microbial consortia for prebiotic and probiotic application and devise population specific microbiome therapies.

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Functional characterization of the soil microbiome in relation with soil properties

In temperate regions, forests are usually developed on nutrient-poor soils and rarely amended in comparison to agrosystems, making them low input ecosystems. In such conditions nutrient access and recycling are key processes. In this context, our aim is to understand how the microbial communities are structured at both taxonomic and functional levels according to the resource availability and in different reactive interfaces (i.e., soil, rhizosphere, mineralosphere) occurring in the soil environment to better determine their relative role in nutrient cycling and tree nutrition. To do it, we are combining culture-dependent and –independent approaches as well as soil sciences (geochemistry, mineralogy). Recently, we focused on the soil and/or tree rhizosphere microbiomes to determine how the soil microbiome adapt to different levels of fertility and which microorganisms are preferentially enriched in the different interfaces considered, especially the tree rhizosphere. We deciphered the taxonomic composition using 16sRNA gene amplicon sequencing and the functional potentials of the microbial communities using functional screening of culturable bacteria, microarray, shotgun sequencing and fosmid libraries. All these data are cross-compared to increase our understanding of the soil/plant/microbes interactions.

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Heavy-metal tolerant eukaryotic gene resources from polluted sites

The molecular mechanisms associated with the interaction of microorganisms to their environments have always been a subject of great interest. Some microorganisms have capability to withstand the stressful environmental conditions including high concentration of toxic heavy metals. Heavy metal metabolism and homeostasis has been studied in few model organisms and has led to the identification of several cellular and biochemical response mechanisms. It is now a well-established fact that a vast majority of microbial species is uncultivable under standard laboratory conditions. Although this statement has initially been made for bacteria, it also holds true for eukaryotic microbes which encompass fungi and the poorly studied unicellular eukaryotes collectively referred as protists. These uncultivated eukaryotic microorganisms present a rich source of novel genes involved in heavy metal metabolism and homeostasis. Discovering these genes and the mechanisms in which they are involved will elaborate our understanding towards the interaction of microorganisms to their environment. Moreover, selected genes can be used to engineer suitable organisms that can be exploited in bioremediation to reclaim polluted ecosystems.