17èmes Journées Scientifiques du RFMF

Réseau Francophone de Métabolomique et Fluxomique

Université Paris Cité

Campus Saint-Germain-des-Prés

45 rue des Saints-Pères, 75006 Paris

10 – 13 juin 2025

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Informations pratiques

Lieu de la conférence

Université Paris Cité
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45 rue des Saints-Pères, 75006 Paris

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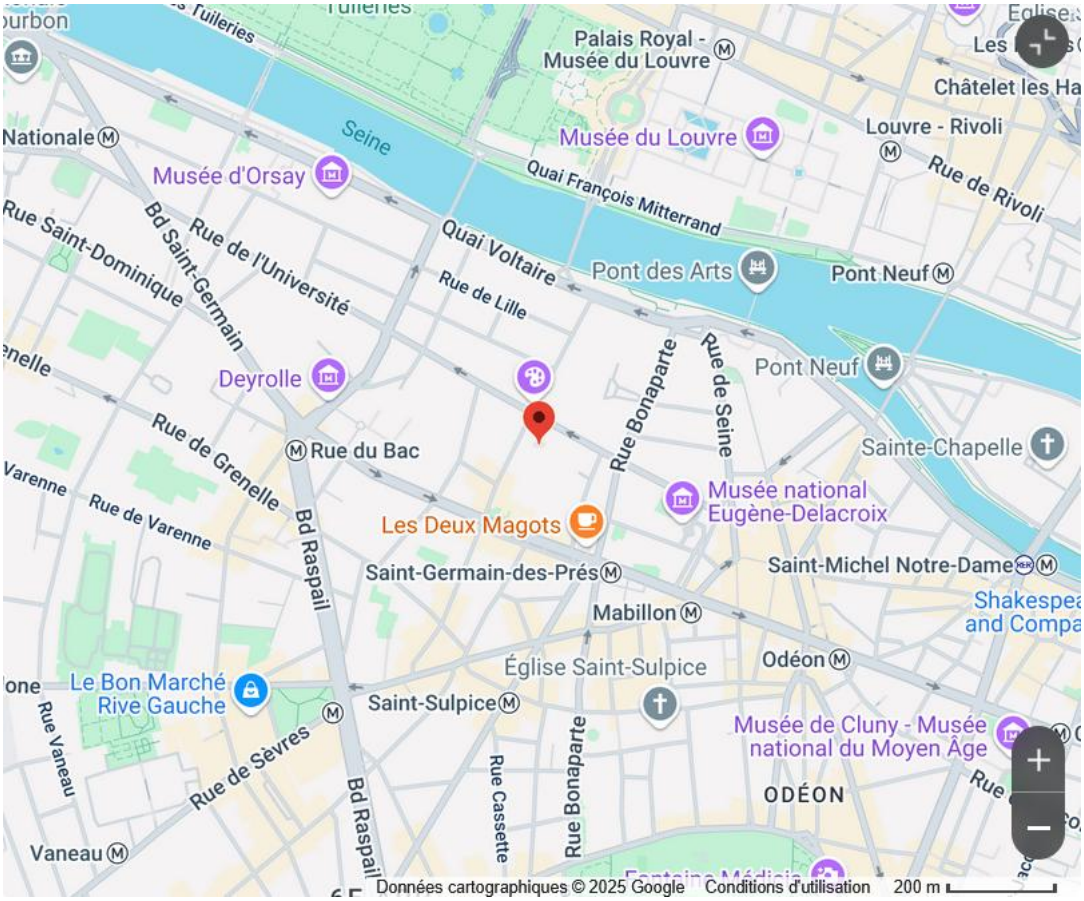
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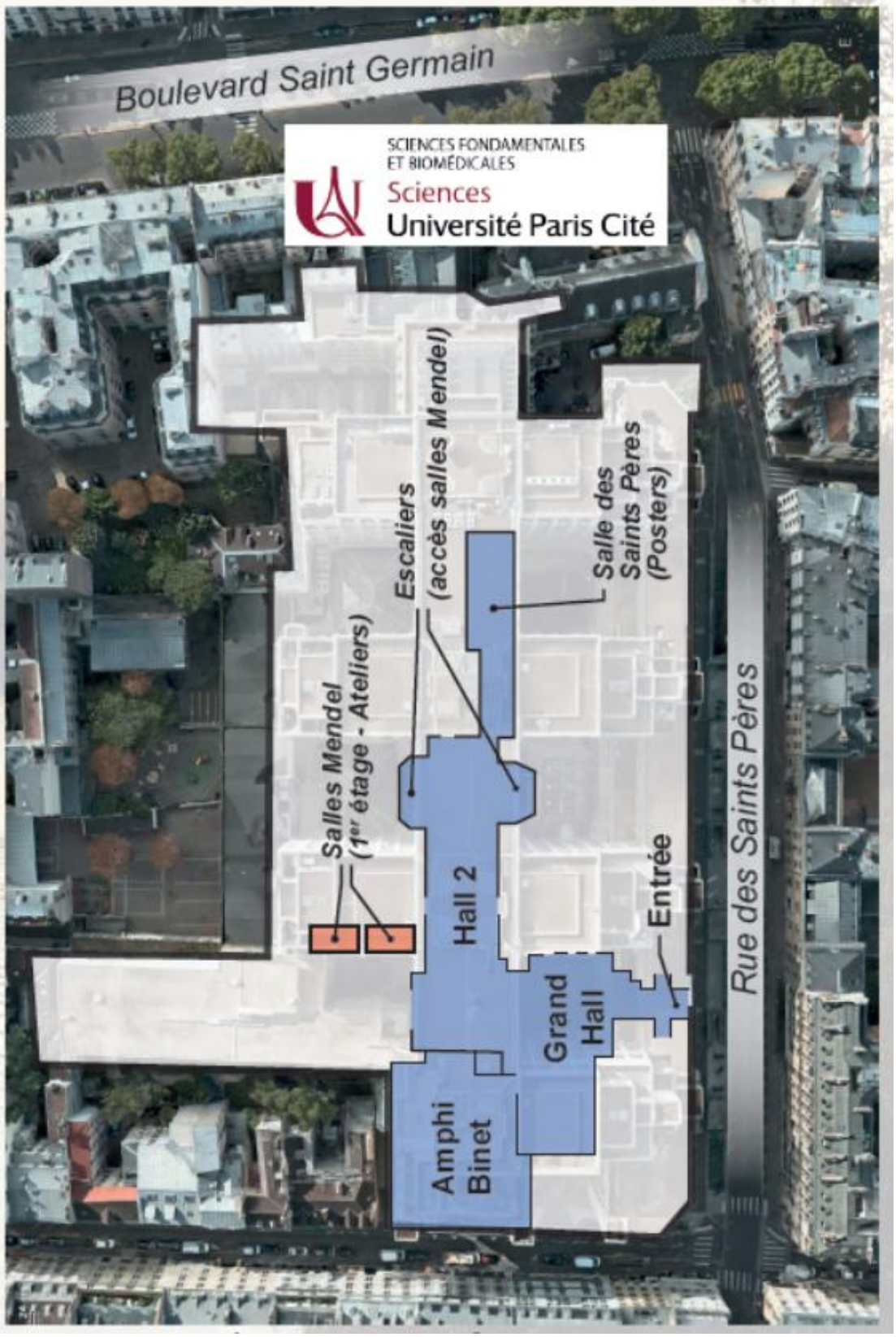

Programme social

📅 Mercredi 11 juin

• 19h00 – 21h00 : 🍷 Apéritif d’accueil

Lieu : à confirmer, à proximité du campus Saint-Germain-des-Prés.

📅 Jeudi 12 juin

• 20h00 : 🎉 Dîner de gala

Lieu : Duplex Club

Adresse : 2 bis Avenue Foch, 75116 Paris

Accès : Métro Charles de Gaulle – Étoile (lignes 1, 2, 6 et RER A)

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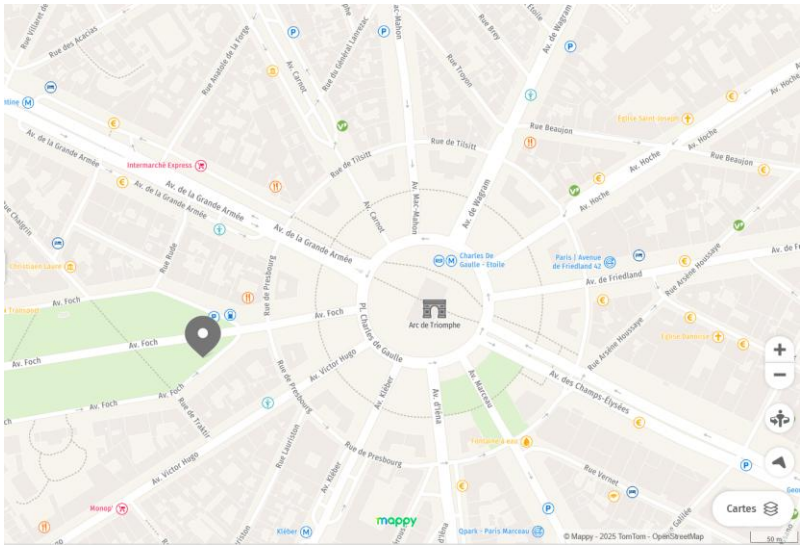

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Programme détaillé

Mardi 10 juin

Ateliers du RFMF

11:30 - 13:45 - Atelier Junior
Intervenants / Salle Mendel A

14:00 - 16:00 - Atelier RMN Biomédicale
G. Bertho, C. Lucas Torres / Salle Mendel B

14:00 - 16:00 - Base de Données MS2
Intervenants / Salle Mendel A

16:00 - 18:00 - Thermo Fisher
Intervenants / Salle Mendel A

16:00 - 18:00 - LECO
Intervenants / Salle Mendel B

Mercredi 11 juin

Chaire : S. Aros

***9:00* - A. Le Gouellec, D.Touboul, G. Bertho**
Mot de bienvenue

***9:30* - Conférence plénière : M. Witting**
Enhanced metabolite and lipid identification: usage of RT, CCS and alternative fragmentation methods

***10:15* - F. Puig Castelli**
Metabolome Richness, its Determinants and its Relevance to Obesity

***10:35* - Pause**

Chaire : M. Letertre, T. Palama

***11:00* - Agilent & Présentations Flash**
F.I. Hocini • C. Terra • C. Touaibi, E. Peti-Jean • C. Saccaram • A. Castaldi • L. Barreda

***11:30* - Thermo Fisher**

***11:50* - L. Mas-Normand**
Metabolomics as a tool for revealing the secrets of cultural heritage objects

***12:10* - Déjeuner & Session Posters**

Chaire : T. Hebra, C. Mauve

***14:00* - F. Courant**
Approche multi-omique pour décrypter les effets moléculaires de la fluoxétine chez Mytilus galloprovincialis

***14:20* - V. Eparvier**
Mutualistic Fungal Defense in Meta-holobiont "termite nest": the Role of Scedosporium boydii and Its metabolome

***14:40* - L. Zi**
Developing High-Throughput Metametabolomics in Freshwater Periphyton to Enhance Chemical Risk Assessment

***15:00* - LECO**

***15:20* - Pause & Session Poster**

Chaire : M. Corso, M. Anouar

***16:30* - Conférence plénière : A. Dellagi**
Arbuscular Mycorrhizal Fungus can alleviate Maize Nitrogen deficiency stress: multi-omics approach and metabolic modelling

***17:15* - C. Cloteau**
Caractérisation des empreintes métaboliques et protéiques associées au variant LIPC-E97G impliqué dans l'hypocholestérolémie combinée

***17:35* - F. Bonnet-Serranaud**
Steroid imaging in adrenocortical tissue using chemical derivatization and MALDI-FTICR

***17:55* - Assemblée Générale du RFMF**

***19-00* - Cocktail de Bienvenue**
Exposition : 20 ans du RFMF

Jeudi 12 Juin

Chaire : G. Bertho, N. Giraud, G. Hajjar

***8:45* - Mot de Bienvenue de l'Université Paris Cité**

Maximilien Cazayou
Doyen de la Faculté des Sciences
Frédéric Charbonnier
Directeur de l'UFR Sciences Fondamentales et Biomédicales

***9:00* - Conférence plénière : J. Kirwan**

Measurements, metabolites, microbiome: interplay of factors in monitoring health and disease

***09:45* - Présentations Flash**

C. Tangeten • C. Remy • C. Guillier • M. Zonnequin • L. Gutierrez • M. soussi-Therond • S. Elfoutat • C. Bocca

***10:15* - Pause & Session Poster**

***11:00* - 20 ans du RFMF**

Chaire : J. Dairou, L. Mervant

***11:30* - J. Omar**

Sub-Cellular NMR Metabolomics of Adherent Mammalian Cells

***11:50* - X. Chen**

Pure Shift NMR with Solvent Suppression: A Robust and General Method for Determining Quantitative Metabolic Profiles in Biofluids

***12:10* - Déjeuner & Session Posters**

Chaire : J. Monteiro, S. Zirah

***14:00* - T. Hebra**
Metabolomics enables enzyme discovery at the angiosperm scale: test case of norcoclaurine synthase.

***14:20* - R. Wisson**
Biosynthesis and diffusion kinetic of metabolites produced by a micro-organism associated with termites by Mass Spectrometry Imaging

***14:40* - A.S. Valadon**
Exploring the Diversity, Evolution and Genetic Determinism of Specialized Metabolites in Pea (Pisum spp.) Seed Coat and Embryo

***15:00* - Conférence plénière : E. Poupon**
Substances naturelles et métabolisme souterrain

***15:45* - Pause & Session Poster**

Chaire : V. Eparvier, N. Lacrampe

***16:45* - F. Mehl**
MetaNetX: Enhancing metabolomics data integration through comprehensive reconciliation

***17:05* - P. Couacault**
Non-targeted metabolomics workflow for blood-microsampling devices

***17:25* - Membre d'honneur : P. Giraudeau**

***18:00* - Atelier Mentorat**

***20:00* - Dîner de Gala**

Vendredi 13 Juin

Chaire : C. Lucas-Torres, A. Rocher, D. Touboul

***9:00* - R. Kouakou**
IsoDesign: software for optimizing the isotopic composition of labeled substrates in ^{13}C-fluxomics experiments

***9:20* - N. Creusot**
Environmental drivers of metabolic and taxonomic temporal dynamics of freshwater biofilms and their role in the sensitivity to the chemical stress

***9:40* - J. Boccard**
Improving Model Interpretability in Metabolomics by Assessing Variable Importance Stability via Resampling

***10:00* - J. Gauvreau**
Annotating 1D and fast 2D NMR spectra of complex mixtures in a metabolomics and lipidomics workflow

***10:20* - Pause & Session Poster**

Chaire : C. Cloteau, A. Wong

***10:50* - Conférence plénière : E. Holmes**
Metabolic Profiling in Health and Nutrition

***11:35* - Prix de Thèse Rolin-Portais : T. Brunet**

***12:00* - Cloture**

Conférences invitées

Michael Witting (1 Metabolomics and Proteomics Core, Helmholtz Zentrum München, Neuherberg, Germany / 2 Chair of Analytical Food Chemistry, TUM School of Life Sciences, Technical University of Munich, Freising-Weihenstephan, Germany)

Enhanced metabolite and lipid identification: usage of RT, CCS and alternative fragmentation methods

Metabolite and lipid identification represents still a major bottleneck in non-targeted analysis, despite years of research. This is due to the vast amount of different methods, separation and fragmentation techniques as well as the structural differences between metabolites and lipids, compared to DNA, RNA and proteins. For correct identification, multiple layers of information need to be combined, since only correctly identified metabolites and lipids allow correct biological interpretation.

In this talk approaches for the integration of mass spectrometric data with retention times (RTs) and collisional cross sections (CCS) are presented and how they can be used for metabolite identification. Lastly, electron-induced dissociation, which has been recently commercially available has the potential to transform metabolite and lipid identification, by delivering additional structural information.

Alia Dellagi (IJPB, INRAE Centre Ile-de-France de Versailles-Saclay, France)

Arbuscular Mycorrhizal Fungus can alleviate Maize Nitrogen deficiency stress: multi-omics approach and metabolic modelling

Bérengère Decouard, Niaz Bahar Chowdhury, Aurélien Saou, Martine Rigault, Mohamad Yassine, Isabelle Quilleré, Thomas Sapir, Anne Marmagne, Christine Paysant le Roux, Alexandra Launay-Avon, Florence Guerard, Caroline Mauve, Bertrand Gakière, Céline Lévy-Leduc, Pierre Barbillon, Pierre-Emmanuel Courty, Daniel Wipf, Bertrand Hirel, Rajib Saha, Alia Dellagi

Our work addresses the long-term challenge of optimizing the use of Arbuscular Mycorrhizal Fungi in association with maize to reduce nitrogen (N) fertilizers use. To this aim, we used the arbuscular mycorrhizal fungus Rhizophagus irregularis. We investigated the reorganization of metabolome and transcriptome during the symbiosis in both partners under different N nutrition levels, using the two genetically distant maize inbred lines B73 and Lo3 that displayed significant yield increase following fungal inoculation. R. irregularis can help maize maintaining yield with five-time less N supply. We unravelled the metabolic implications of this symbiotic relationship under limiting N by integrating the transcriptomic data into a maize multi-organ genome-scale metabolic model called iZMA6517 through a stoichiometric

approach. The iZMA6517 model proved trustful for predicting plant biomass and allowed us to determine the maize metabolic bottleneck reactions boosted by the symbiosis using Metabolic Bottleneck Analysis. This has predicted that pyrimidine metabolism may be a key player in the symbiosis which was supported by increased pyrimidine levels in leaves of mycorrhizal Lo3 plants under low N condition. We identified maize and fungal genes potentially involved in efficient symbiosis under low N by a multi-omics approach. The metabolic processes taking place in this interaction were partially different for each maize line highlighting natural variation in symbiosis mechanisms. We showed that combining multi-omics approaches with mathematical metabolic modelling can allow the discovery of novel metabolic mechanisms associated with mycorrhizal symbiosis, without prior assumptions.

Jennifer Kirwan (Berlin Instiute of Health, Germany)

Improving the study of inflammatory disease with metabolomics and quality management

Inflammation is a contributory factor in multiple diseases states. Understanding how inflammatory processes are regulated thus holds significant promise for better precision medicine. Metabolomics has consistently proven itself to be a complementary technology to better understand these processes.

Using examples of both infectious and non-communicable diseases, this talk will highlight how metabolomics can unlock the diagnostic and prognostic potential of the microbiome-metabolite-immune regulation axis, especially with respect to the key role of tryptophan metabolites in this chain. Since biological insight relies on technically robust and reproducible data, critical aspects of pre and post analytical processing will be discussed, including light sensitivity on molecules of biological importance and unruly behaviour of compounds in the mass spectrometer. I will also introduce our in-house tool *MSLineaR* for easy filtering of non-linear ions and which can be incorporated into your data clean up strategies.

Erwan Poupon (BIOCIS, Université Paris-Saclay ,France)

"Substances naturelles et métabolisme souterrain"

À l'heure des sciences « omiques » et des sciences des données, la chimie des substances naturelles connait des mutations qui bousculent les paradigmes. Loin d'un vision dogmatique, notamment en ce qui concerne les activités biologiques, nous essaierons de donner un panorama actuel permettant de comprendre à la fois la diversité chimique et, parfois, l'incroyable complexité moléculaire des « métabolites spécialisés » avec en ligne de mire la notion de « métabolisme souterrain ». Des exemples d'émergence de complexité moléculaire particulièrement étudiés dans notre équipe illustreront le propos.

In the era of "omics" and data science, natural product chemistry is undergoing transformations that challenge established paradigms. Far from a dogmatic perspective, particularly with regard to biological activities, we aim to provide a current overview that could explain both the chemical diversity and, at times, the remarkable molecular complexity of "specialized metabolites," with a focus on the concept of "underground metabolism." Examples of emerging molecular complexity, which have been extensively studied by our team, will illustrate the talk.

Elaine Holmes (Murdoch University, Australia & Imperial College London, UK)

Metabolic Profiling in Health and Nutrition.

Metabolic profiling of biofluids such as urine, plasma or fecal water in combination with multivariate statistical modeling tools, provides a window for investigating the impact of disease on human health. High-resolution spectroscopic methods (NMR spectroscopy, CE-MS, LC-MS, GC-MS etc) are used to generate information-dense metabolic profiles that carry information relating both to genetic and environmental influences, including contributions from the diet, xenobiotics and gut microbiome. Since every individual has a unique combination of gene-environment interactions, their disease risks, pathogenesis and response to therapeutic interventions will also be unique. Clinical studies have shown that inter-individual differences in either host or microbial metabolism can impact on patient responses to therapeutics or surgical interventions (pharmacometabonomics) and that these baseline or pretreatment profiles can be used prognostically to predict drug metabolism, efficacy or toxicity. Metabolic phenotyping approaches allow the construction of a framework for stratifying patients and improving patient care with respect to improved clinical outcomes and reduced expenditure. Here, the wider role of metabolic profiling, in the context of biomonitoring applications, will be discussed with a focus on new translational technologies and data modelling tools exemplified by a range of clinical case studies.


RFMF
Réseau Francophone de Métabolomique et Fluxomique
17èmes Journées Scientifiques du RFMF
10-13 Juin 2025, Paris

Résumés

Oral 1 - O1

Metabolome Richness, its Determinants and its Relevance to Obesity

Francesc Puig Castellví*[1], Man-Yi Jia[2], Romina Pacheco Tapia[1], Ema Mocsonokyova[2], Zhaojie Wang[1], Ines Castro Dionicio[1], Mickael Chevalier[1], Yuan Wang[2], Consortium Metacardis[2], Philippe Froguel[1], Sofia Kirke Forslund-Startceva[3], Sara Vieira-Silva[4], Ulrike Loeber[3], Kanta Chechi[2], Petros Andrikopoulos[2], Stanislav Dusko Ehrlich[5], Oluf Pedersen[6], Karine Clement[7], and Marc-Emmanuel Dumas†[1]

[1]EGENODIA U1283 INSERM UMR8199 CNRS, Institut Pasteur de Lille, CHU de Lille, Université de Lille. – Metabolic functional (epi)genomics and molecular mechanisms involved in type 2 diabetes and related diseases - UMR 8199 - UMR 1283 – France

[2]Division of Systems Medicine, Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London – Royaume-Uni

[3]Experimental and Clinical Research Center, a cooperation of Charité Universitätsmedizin and the Max-Delbrück Center – Allemagne

[4]Institute of Molecular Biology (IMB), Mainz – Allemagne

[5]Department of Clinical and Movement Neurosciences, University College London – Royaume-Uni

[6]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen – Danemark

[7]Service de Nutrition, hôpital de la Pitié-Salpêtrière, AP–HP, Sorbonne Université, Inserm – Unité Nutrition et Obésités, approches systémiques, Nutriomique – France

Résumé

Obesity is influenced by lifestyle, dietary patterns, physical activity and gut microbiome alterations. In particular, the Bact2 enterotype, a distinct microbiome ecology that has been linked to obesity, is characterized by a proinflammatory functional profile and poor gene richness.
Here, we introduce the concept of metabolome richness and show that the loss in microbiome richness in obese individuals mirrors a reduced number of metabolites in blood plasma in a METACARDIS study subset, which we replicated in FLORINASH. To uncover the determinants of metabolome richness, we applied gradient-boosting decision trees models to predict metabolome richness using demographic, clinical, nutritional, and metagenomics data. Feature attribution analysis using SHAP suggested that the inter-individual variability of metabolome richness was mostly influenced by age, diet, kidney function, and the gut microbiome. Correlation analyses also revealed that metabolome richness is primarily driven by host-, diet-, and microbial-metabolites.

We then stratified metabolome richness by origin (i.e., diet, drug), and tested associations with lifestyle, clinical and metagenomic profiles, identifying significant contributions from

*Intervenant

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pulse intake, kidney function and KEGG pathways. We further examined the metabolites' structural similarity using clustering of their chemical fingerprints data and we observed that the metabolites disappearing in obesity shared structural similarities, with the metabolites most strongly associated with metabolome richness were predominantly microbial and dietary-related.

Altogether, our results suggest that metabolome richness depicts the loss of a constellation of dietary and microbial protective metabolites in severe obesity, mirroring the collapse in microbiome gene richness, suggesting a gradual loss in bio- and chemodiversity in obesity.

Oral 2 - O2

Metabolomics as a tool for revealing the secrets of cultural heritage objects

Lindsay Mas-Normand*[1], Marine Chambaud[1], Olivier Dangles[2], Carole Mathe De Souza[1], and Gérald Culioli†[1]

[1]Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE) – UMR CNRS 7263, IRD 237, Aix Marseille Université, Avignon Université – France

[2]Sécurité et Qualité des Produits d'Origine Végétale (SQPOV) – UMR 408, INRAE, Avignon Université – France

Résumé

Natural dyes are an important part of our heritage. They are present in a variety of materials, fabrics and antique objects, such as clothes, carpets and tapestries. The development of analytical tools and protocols specifically designed for such compounds could lead to a better understanding of the historical and geographical context of an object's manufacture, and provide crucial information for optimizing its conservation-restoration.
Most natural dyes are obtained from dye plants. Of all the colors in nature's palette, yellow is the one that can be produced from the widest range of plant species, which can contain chemical families as varied as flavonoids, anthraquinones and/or alkaloids.

The aim of this work was first to study, through a specifically dedicated untargeted LC-MS-based metabolomic approach, the impact of the fabric extraction protocol on chemical compounds produced by three common yellow dye plants: common barberry (*Berberis vulgaris*), European buckthorn (*Rhamnus cathartica*) and weld (*Reseda luteola*). Then, the most suitable method was applied to extract the dyes present in old carpets and thus determine their botanical origin. Finally, the same metabolomics workflow was used to evaluate the impact of dyeing conditions (plant maturity, temperature of the dye bath) on the stability of *R. cathartica* dye molecules.
In short, the use of metabolomics and annotation tools such as molecular networks, which is still anecdotal in the field of heritage chemistry and archaeometry, has shown its full potential in the course of this work and should be exploited in even greater depth in the future.

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Oral 3 - O3

Approche multi-omique pour décrypter les effets moléculaires de la fluoxétine chez Mytilus galloprovincialis

Etienne Lemaire[1], Elena Gomez[1], Julien Boccard[2], Jean Armengaud[3], Eider Bilbao[4], and Frédérique Courant*†[1]

[1]Hydrosciences Montpellier – Institut de Recherche pour le Développement, Centre National de la Recherche Scientifique, Université de Montpellier – France

[2]Biomedical and Metabolomics Analysis, School of Pharmaceutical Sciences, University of Geneva – Suisse

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[4]CBET Research Group, Dept. Zoology and Animal Cell Biology, Faculty of Science and Technology and Research Centre for Experimental Marine Biology and Biotechnology, PiE, University of the Basque Country UPV/EHU, Basque Country – Espagne

Résumé

Les pollutions induites par les activités humaines exposent continuellement l'environnement aquatique à des " contaminants émergents ", parmi lesquels les substances pharmaceutiques. Le rejet direct des eaux usées en milieu marin et la faible efficacité des traitements conventionnels accentuent les risques pour les organismes aquatiques. Ce travail visait à étudier les effets de la fluoxétine (FLX), un antidépresseur largement prescrit, chez la moule méditerranéenne *Mytilus galloprovincialis*, organisme sentinelle reconnu en écotoxicologie marine.

Durant ce projet, des moules prélevées dans un étang près de Montpellier ont été exposées (ou non) à 3,1 μg/L de FLX pendant 28 jours. Aux jours 2, 7, 14 et 28, les glandes digestives ont été prélevées afin i) d'identifier les produits de biotransformation de la FLX par analyses chimiques non ciblées (LC-HRMS) et ii) conduire des approches métabolomiques, protéomiques et transcriptomiques sur ces échantillons appariés afin de caractériser les effets moléculaires induits par la FLX. Un objectif majeur de cette étude était d'évaluer la faisabilité d'obtenir des biomarqueurs robustes, i.e. issus de voies confirmées simultanément sur les trois niveaux moléculaires, et présentant une large fenêtre de détection pour une surveillance ultérieure sur le terrain.

Les résultats ont révélé plusieurs produits de biotransformation de la FLX, dont certains non répertoriés dans la littérature scientifique actuelle. Par ailleurs, le mécanisme d'action connu de la FLX via la modulation de la voie sérotoninergique a été confirmé, accompagné d'une modulation de la voie dopaminergique. La discussion portera principalement sur la pertinence et l'adéquation de ces marqueurs en contexte environnemental.

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Oral 4 - O4

Mutualistic Fungal Defense in Meta-holobiont "termite nest": the Role of Scedosporium boydii and Its metabolome

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Résumé

The intricate symbiotic relationships between insects and microorganisms play a fundamental role in ecological stability and host fitness. Among social insects, termites exhibit a remarkable association with a diverse microbiome that supports key biological functions, including digestion, immunity, and pathogen defense.

This study investigates the symbiotic interaction between termites and fungal strains of the genus Scedosporium, focusing on their potential role in nest protection. Our results demonstrate that Scedosporium boydii produces antimicrobial compounds, in particular tyroscherin and its derivatives, which effectively inhibit the entomopathogenic fungus Beauveria bassiana. Metabolomic analyses by LC-MS/MS and MS-imaging revealed complex chemical interactions within the termite ecosystem, with tyroscherin (and its analogues) being widely distributed in the colony and produced only during biotic interactions.

We were able to demonstrate by genomics and metabolomics that S. boydii can be detected at different levels of the meta-holobiont reinforcing its strong ecological association with these insects. These findings underscore the significance of microbial symbioses in termite ecology and highlight the protective role of mutualistic fungi in mitigating biotic threats to termite colonies.

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Oral 5 - O5

Developing High-Throughput Metametabolomics in Freshwater Periphyton to Enhance Chemical Risk Assessment

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Résumé

Facing increasing chemical pollution of aquatic ecosystems, there is an emerging need to improve the risk assessment on natural microbiomes playing key role in ecosystems functions and services. To such an end, applying metabolomics appears relevant but remain limited by low-throughput screening. This study aims to develop a high throughput (HT) workflow integrating miniaturized exposure setups, automated sample preparation, data acquisition and data analysis by using aquatic periphyton to enhance chemical risk assessment. Our results showed that 1 mg of periphyton provides sufficient metabolomics intensity while testing glass discs in microplates (48, 24, 12-wells) showed similar periphyton growth (_˜1 mg) after 14 days, supporting the use of 48-well discs for further studies. Automated metabolite extraction was as efficient as current method. A standardized data analysis pipeline is under developing to determine community metabolism sensitivity threshold based on meta-metabolome dose response (DRomics) and annotation. To validate the workflow, we tested two azole fungicides. The overall metabolome showed no clear dose-response, aligning with our hypothesis as the target fungi are not abundant in periphyton. After 24 h exposure, half of the metabolites reached benchmark dose (BMD1SD) at 0.01-0.1 mg L-1, much lower than at 72 h (5-10 mg L-1), indicating active metabolism in early exposure and later recovery, potentially explaining periphyton tolerance to azoles. The most sensitive metabolites were amino acids and peptides. This workflow will be applied to screen pharmaceuticals, bisphenols and mycotoxins, addressing regulatory gaps in evaluating chemical toxicity on aquatic microbial communities with potential impact on ecosystem functions.

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Oral 6 - O6

Caractérisation des empreintes métaboliques et protéiques associées au variant LIPC-E97G impliqué dans l'hypocholestérolémie combinée

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Résumé

Le variant génétique E97G de *LIPC,* codant la lipase hépatique, est le second gène, avec l'ANGPTL3, responsable de l'hypocholestérolémie combinée. Ce variant augmente l'activité phospholipase de l'enzyme sans altérer l'hydrolyse des triglycérides, et favorise la clairance des lipoprotéines. Notre étude vise à explorer l'impact métabolique de *LIPC*-E97G par à une approche multi-omiques chez la souris.
Des souris athérogéniques (n = 16 par groupe) exprimant la forme humaine sauvage ou E97G de *LIPC* ont été nourries avec un régime riche en lipides. Les analyses métabolomiques, lipidomiques et protéomiques ont été réalisées par LC-HRMS sur les échantillons de foie et de plasma. Les jeux de données ont été intégrés au moyen d'une analyse multi-blocs Consensus-OPLS-DA afin d'identifier de nouvelles voies métaboliques.

Les analyses ont révélés des phénotypes caractéristiques de *LIPC*-E97G avec un modèle statistique permettant la bonne prédiction des échantillons plasmatiques ($R^2Y = 0.99$, $Q^2Y = 0.88$). L'approche multi-blocs a révélé des différences phénotypiques attribuables aux concentrations lipidiques et protéiques, et notamment une réduction marquée des phosphatidylcholines, des niveaux d'apoE, d'apoB et de paraoxanase 1 et une augmentation des niveaux d'apoA-V. Les analyses n'ont pas révélé d'impact majeur du variant sur les profils hépatiques. L'approche multi-omique intégrée a permis de confirmer l'impact du variant *LIPC*-E97G sur des voies métaboliques déjà identifiées et d'en révéler de nouvelles à explorer. Cette approche a montré que *LIPC*-E97G impacte principalement les concentrations circulantes en lipides et protéines. Les mécanismes métaboliques du variant après traitement par un inhibiteur d'ANGPTL3 est en cours.

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Oral 7 - O7

Steroid imaging in adrenocortical tissue using chemical derivatization and MALDI-FTICR

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Résumé

Steroidogenesis in the adrenal cortex relies on a cascade of enzymatic reactions taking cholesterol as initial substrate and leading to the synthesis of mineralocorticoids in zona glomerulosa, glucocorticoids in zona fasciculata and androgens in zona reticularis. This process is often altered in adrenocortical tumors leading to symptomatic steroid excess. Little is known about the spatial distribution of bioactive steroids and their precursors inside adrenal tissue. Our goal was thus to use MALDI-FTICR to describe the spatial distribution of steroids in adrenal tissue.

Due to very low ionization efficiency, in-situ ketone derivatization with Girard reagents (including Girard T, Girard P and deuterated Girard P reagents to differentiate some signals subject to interferences) was performed on both normal and tumoral adrenal tissue sections after spraying the solution using the iMLayer from Shimadzu. HCCA was employed as the matrix and acquisitions were performed in the positive ion mode on a MALDI-FTICR instrument (Bruker SolariX XR 9.4 T).

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For the first time, the spatial distribution of precursors (pregnenolone, progesterone, 17-hydroxypregnenolone, 17-hydroxyprogesterone), gluco-/mineralo-corticoids (isomers corticosterone/11-desoxycortisol, cortisol) and androgens (androstenedione, isomers DHEA/testosterone, 11-hydroxyandrostenedione) was determined on human tissue sections at a spatial resolution of 30 μm (2-3 cells resolution).
Therefore, the complete metabolomic pathways were accessible in situ by MALDI imaging and compared between control and tumors enlightening significant changes in steroid production and fluxes in adrenal tissue not accessible by any other biological imaging techniques.

Oral 8 - O8

Sub-Cellular NMR Metabolomics of Adherent Mammalian Cells

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Résumé

Subcellular compartmentalization in eukaryotic cells plays a critical regulatory role. It organizes metabolic enzymes and substrates within distinct organelles, thereby enhancing the efficiency and control of metabolic pathways. Such an organization generates organelle-specific microenvironments with distinct cofactors and metabolite pools, enabling tuned metabolic regulation. Despite the high potential of spatially resolved metabolomics for revealing detailed metabolic regulation, most cell-based metabolomic studies employ a whole-cell approach due to the limitations of conventional cell fractionation methods in reliably quantifying compartment-specific metabolites. In this work, we developed and optimized a global cell-organelle isolation strategy using the hepatic cancer cell line HepG2, combined with high-resolution Nuclear Magnetic Resonance (NMR) spectroscopy, to decipher subcellular metabolic profiles across mitochondrial, nuclear, and cytosolic fractions. NMR analysis employing 1H NOESY and CPMG experiments demonstrated a unique metabolic signature for each isolated fraction, markedly distinct from the whole-cell HepG2 profile. A thorough comparison between the conventional fractionation method differential centrifugation (DC), and immunopurification (IP) of tagged mitochondria was conducted. Parameters such as processing time, yield, isolation buffer, extraction buffer, and purity were evaluated during optimization. Notably, immunopurified mitochondria exhibited a higher yield, reduced buffer interference, and a broader detection of key intermediates (31 mitochondrial metabolites, including coenzyme A, ATP derivatives, essential intermediates of the TCA cycle, and oxidative phosphorylation) compared to 21 metabolites detected using the DC method. These findings establish a robust platform for subcellular NMR metabolomics, offering new insights into compartment-specific metabolic patterns and laying the foundation for applications in cancer research and real-time organelle functional studies.

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Oral 9 - O9

Pure Shift NMR with Solvent Suppression: A Robust and General Method for Determining Quantitative Metabolic Profiles in Biofluids

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Résumé

Ultrahigh-resolution pure shift NMR has recently been shown as a promising approach for providing quantitative metabolic profiles that can be used to study the metabolic footprint left by cancer cells in their aqueous growth medium. In this approach, a library of reference 1H pure shift spectra with water suppression was implemented to determine metabolite concen- trations from the NOESY-presat-PSYCHE-SAPPHIRE spectrum recorded on the extracellular medium.(1) This achievement clearly called for a generalization of a quantification method relying on ultrahigh-resolution data to other biological samples of interest (urine, plasma, tissue extracts, etc.), which requires evaluating the robustness of the analytical workflow. We have first addressed the influence of sample preparation on the quality of metabolite quantification. The quantification performed on a model mixture of metabolites prepared under different conditions shows good linearity, trueness, and precision, which highlights the high reproducibility of the proposed analytical protocol regardless of the physicochemical conditions in the sample. Second, we have successfully implemented this quantification protocol to determine metabolite levels in real urine and plasma samples, thereby paving the way for the use of the library of pure shift reference spectra for accurate and quantitative metabolic profiling of a broad range of aqueous samples.(2) (1) X. Chen, C. Caradeuc, A. Montagne, V. Baud, G. Bertho, C. Lucas-Torres and N. Giraud, Anal. Chem., 2022, 94, 14974–14984.
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Oral 10 - O10

Metabolomics enables enzyme discovery at the angiosperm scale: test case of norcoclaurine synthase.

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Résumé

Using untargeted metabolomics, molecular networking, and data mining from open-source repositories, we discovered 5 classes of alkaloids in Piper fimbriulatum, notably identifying a broad molecular diversity of previously unreported benzylisoquinoline alkaloids and their aporphine derivatives. As benzylisoquinoline alkaloids are precursors of pharmacologically important metabolites (e.g., morphine, berberine), we investigated their biosynthesis. The first enzymatic step, catalyzed by norcoclaurine synthase (NCS), has only been reported in Ranunculales, a distant order from Piperales.
Traditional homology-based searches failed to retrieve candidates (sequences with $< 60\%$ identity) in Piper transcriptomes. Instead, we applied a in-house multi-omics pipeline (PiperNET, integrating transcriptomics and metabolomics). With this approach, we successfully identified and characterized three novel NCSs with low sequence identity (_˜35%) to previously described enzymes. These represent the first NCSs characterized outside of the order Ranunculales.
We then decided to expand our finding at the angiosperm (flowering plant) scale. We hypothesize that metabolomics data-driven discovery can significantly enhance identification of enzymes even with low sequence similarity (_˜30%) in genomics or transcriptomics datasets, at scale. We first performed a MS/MS query to public metabolomics data repositories. Surprisingly, we detected fragmentation spectra characteristic of benzylisoquinoline alkaloids in four plant orders (Malvales, Oxalidales, Apiales, Zingiberales) where benzylisoquinoline alkaloids were never reported before. This strongly hints that these plants possess enzymatic machinery to synthesise these compounds.

Therefore, we retrieve 54 candidates from low similarity (_˜30%) NCS sequences within transcriptomes from plants of the four newly identified benzylisoquinoline alkaloids-containing orders. We are currently testing 16 candidates experimentally by heterologous expression in Saccharomyces cerevisiae.

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Oral 11 - O11

Biosynthesis and diffusion kinetic of metabolites produced by a micro-organism associated with termites by Mass Spectrometry Imaging

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Résumé

Termites are eusocial insects live in nests with large numbers of individuals, in an environment conducive to the development of pathogens (1). However, the termites have an ecological success thanks in part to their association with micro-organisms that protect them against biotic stresses. It has been shown that termites of French Guïana are often found associated with microscopic fungi strains of the species *Scedosporium boydii*. Those strains produce several molecules with biological activities, such as the antifungal compound tyroscherin. Those molecules seem to be involved in the biotic defence of the termites against pathogens, as shown by the appearance of an inhibition zone in confrontation culture with a generalist entomopathogen, *Beauveria bassiana* (2,3). To better understand the chemical interaction between *S. boydii* and *B. bassiana*, production, excretion and diffusion in culture media of the molecules in co-cultures were studied during seven days by MALDI-FT-ICR spectrometry. A transfer of each culture was made by pressing an Indium-Tin oxide slide on the confrontation culture. Those slides were sprayed with α-cyano-4-hydroxycinnamic acid matrix and analysed in 9,4T Solarix XR MALDI-FT-ICR mass spectrometer. Analysis of the data revealed the production of compounds of interest on day 3-4 of the confrontation. Indeed, tyroscherin and one of its analogues are observed to be excreted in the inhibition zone, supporting the role of the activity of these molecules against the entomopathogen.

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Oral 12 - O12

Exploring the Diversity, Evolution and Genetic Determinism of Specialized Metabolites in Pea (Pisum spp.) Seed Coat and Embryo

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Résumé

Pea (*Pisum sativum* L.) is a widely cultivated legume species valued for its high seed protein content, making it an important food and feed resource. Seed specialized metabolites (SM) protect seeds against environmental stresses and offer health benefits but can also negatively impact seed quality due to antinutritional or toxic properties. Managing this dual nature is crucial for crop improvement.
Despite their importance, little is known about the genes and enzymes involved in SM biosynthesis and modification in pea seeds. To fill this gap, we performed untargeted metabolomic analyses (LC-MS/MS) on dry seeds embryos and coats from a diversity panel of 204 pea genotypes, including wild and domesticated *Pisum* species and subspecies, as well as widely cultivated varieties. This allowed us to investigate the impact of evolution and domestication on the diversification and modification of SM in pea seeds.

Our results reveal a high metabolic diversity, with distinct SM compositions between seed embryos and coats, including many SM specific to each tissue. We also observed metabolic differences across species and subspecies, highlighting the influence of evolution and/or domestication on SM accumulation in seed defense (coat) and quality (embryo). In order to identify the genetic determinants involved in this accumulation, a metabolomic GWAS approach is currently being carried in order to identify loci/genes involved in the biosynthesis of selected agronomically relevant SM.
These findings highlight the diversity of SM in peas and pave the way for a better understanding of their role in the adaptation and domestication of this plant.

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Oral 13 - O13

MetaNetX: Enhancing metabolomics data integration through comprehensive reconciliation

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Résumé

Integrating metabolomics data with other omics data and computational models enables a more comprehensive understanding of biological systems and their mechanisms. The fusion of systems biology and metabolomics provides a powerful framework that links the study of biological systems with the analysis of small molecules in these systems. Chemical and compound databases are essential in this context, as they offer detailed information on the structure, properties, and interactions of molecules, supporting the identification and characterization of compounds, the study of metabolic pathways, and experimental design.

MetaNetX (https://www.metanetx.org) serves as a crucial resource for the reconciliation of metabolites and biochemical reactions, bridging major public databases such as ChEBI, HMDB, KEGG, Lipid Maps, MetaCyc, Reactome, SwissLipids and others. It creates a unified namespace for identifying, annotating, and cross-referencing metabolites and reactions, providing valuable insights for systems biology and metabolomics research.

Despite the automated nature of the reconciliation process, challenges such as incomplete stereoisomeric information and inconsistencies between databases persist. The reconciliation leverages molecular structures and cross-references to address these issues, with the reaction context as background, while occasional errors from external resources are manually reviewed and corrected. The MetaNetX reconciliation pipeline is continuously refined to enhance data accuracy and reliability. The resource is freely available as raw files, queryable through a SPARQL endpoint, and accessible via an ID mapper tool (https://www.metanetx.org/cgi-bin/mnxweb/id-mapper).

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Oral 14 - O14

Non-targeted metabolomics workflow for blood-microsampling devices

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Résumé

Blood and plasma are predilection matrices to monitor human health. Blood microsampling (BμS) has emerged as an alternative to these invasive sampling methods, especially for vulnerable groups such as infants or elderly. Several studies showed that BμS are suitable alternatives for analyzing endogenous metabolites and for metabolomics applications such as therapeutic drug monitoring, anti-doping, or toxicology analysis. Dried blood spots (DBS) have long been used for clinical applications, particularly for newborn screening. New quantitative BμS devices have emerged, such as volumetric absorptive microsampling (VAMS) or quantitative dried blood spots (qDBS).
Here we developed and optimized a non-targeted analytical workflow for BμS for polar metabolites with hydrophilic interaction chromatography (HILIC) and mid-polar metabolites with reversed-phase liquid chromatography (RPLC). Eight different extraction procedures were tested. The non-targeted HILIC-MS/MS method was developed with 63 standard molecules, including amino acids, co-enzyme A and derivatives, carnitines, organic acids, sugars, and biological metabolites. Analyses were performed on a Sciex ZenoTOF 7600. Data treatment and annotation were performed using mzmine 4.5 and in-house R scripts, with open and in-house libraries. Data were evaluated by the number of detected features, reproducibility and annotation rates. The workflow was tested on three commercially available BμS (DBS, VAMS, and qDBS).
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Oral 15 - O15

IsoDesign: software for optimizing the isotopic composition of labeled substrates in 13C-fluxomics experiments

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Résumé

Translator

The study of metabolic fluxes provides a detailed phenotypic and functional description of cellular metabolism, contributing to a better understanding of biological processes.

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Metabolic Flux Analysis (MFA) encompasses a set of approaches used to quantify biochemical reaction rates within metabolic networks, relying on mathematical modeling methods. 13C-MFA is an approach that leverages isotopic data from stable carbon-13 (13C) labeling experiments to calculate metabolic fluxes. The ability to compute fluxes of interest and their accuracy strongly depend on the experimental design, particularly on the choice of labeled substrates.
However, determining and selecting the optimal labeling configurations of substrates is a complex and time-consuming task, requiring the exploration of multiple labeling configurations within a vast solution space. This selection is even more challenging as it depends on optimizing flux accuracy, analytical capabilities, and the often high cost of labeled substrates, making the analysis of possible combinations even more complex.

To accelerate this process, we have developed IsoDesign, an open-source Python tool (https://github.com/MetaboHU
MetaToul-FluxoMet/IsoDesign) dedicated to optimizing the isotopic composition of labeled substrates for 13C-fluxomics experiments. With its intuitive graphical interface and the use of the influx_si software (https://github.com/sgsokol/influx) for flux calculation, IsoDesign facilitates the design of isotopic labeling experiments, improving accuracy and reducing the cost of 13C-MFA experiments.

Oral 16 - O16

Environmental drivers of metabolic and taxonomic temporal dynamics of freshwater biofilms and their role in the sensitivity to the chemical stress

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Résumé

Facing global changes, comprehending how environmental microbiomes respond to multiple stressors is essential to maintain ecosystem functions and services. In aquatic systems

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contaminated by chemicals, a critical challenge is understanding how shifts in microbial community composition and function modify their sensitivity to micropollutants. Here, we combined metabolomics and metagenomics to assess microbial activity, taxonomic/genic diversity, aiming to uncover the assembly mechanisms underlying these responses. This study pursued two main objectives: (i) investigating the synchronicity between the intrinsic dynamics of microbial metabolic activity (meta-metabolome) and structural biodiversity (species and gene diversity) over a one-year period, and (ii) evaluating how these natural fluctuations modulate the sensitivity of the meta-metabolome and photosynthetic processes to a representative herbicide, terbuthylazine (TBA). Monthly, biofilms were allowed to colonize glass slides in a pilot pond. Post-sampling, the biofilms were quenched, freeze-dried, and processed for comprehensive omics analyses. Simultaneously, sub-samples were exposed to graded concentrations of TBA for four hours to determine community sensitivity. Findings revealed an asynchronous and uncorrelated dynamics between the meta-metabolome and microbial composition, suggesting distinct assembly processes. Principal Component Analysis based on Aitchison distance and DIABLO analysis demonstrated that temperature significantly influences microbial taxonomy, while meta-metabolome structuring appears to be regulated by alternative factors such as nitrate availability. These variations likely alter TBA sensitivity, predominantly impacting metabolic functions. Ongoing investigations aim to clarify the roles of internal metabolism versus external water properties in driving these shifts. Overall, our study substantially deepens understanding of natural microbiome responses to stress.

Oral 17 - O17

Improving Model Interpretability in Metabolomics by Assessing Variable Importance Stability via Resampling

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Résumé

As a cornerstone of knowledge discovery in metabolomics, multivariate analysis helps to decipher and better understand biological processes. Matrix factorization methods are extensively employed to uncover trends and relevant groupings of observations, but also to highlight potentially related variables based on their contributions to model components. Metabolomic datasets are inherently characterized by high dimensionality, *i.e.* a large number of possibly noisy collinear variables (peaks, chemical shifts, ion features, etc.), while the number of samples is usually small, thus posing significant challenges for modeling. In particular, the stability of the model parameters useful for interpretation, such as variables contributions or weights, remains poorly evaluated in practice. To this end, a generic workflow based on bootstrap resampling and permutations is proposed to assess the stability of Variable Importance in Projection from (Orthogonal) Partial Least Squares regression models, a common criterion widely used in metabolomics to highlight interesting subsets of variables. Because it is computationally efficient and does not require assumptions about data distribution, this strategy, based on a stability index and diagnostic plots, is shown to be a relevant approach well suited to the needs of a wide range of applications. Results from different real case studies illustrate the potential of the proposed method for assessing the reliability and interpretability of meaningful variables and remove uninformative signals in metabolomics. The broad adoption of this type of methodology will undoubtedly help to achieve more consistent and reproducible results, ultimately advancing the understanding of metabolic signatures and their implications in biochemical events.

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Oral 18 - O18

Annotating 1D and fast 2D NMR spectra of complex mixtures in a metabolomics and lipidomics workflow

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Résumé

For metabolomics studies, 1D 1H NMR has been the most used experiment thanks to its short acquisition time and the possibility to achieve a global quantitative profiling with a simple sample preparation and to provide valuable structural information. However, annotation of metabolites in complex mixtures can be challenging because of crowded spectra and overlapping signals. By using 2D NMR, signals are spread along a second dimension, increasing NMR resolution. But this is at the expense of acquisition time, making classic 2D experiments incompatible with high-throughput batch acquisitions required for metabolomics studies. Recent developments have been made reducing the experiment time by using Ultra-Fast (UF) and Non-Uniform Sampling (NUS) 2D NMR making them usable in a metabolomics context. Furthermore, using the diversity of homo – and heteronuclear 2D experiments allows us to observe coupling between signals, providing further structural information about metabolites, thereby increasing confidence in compound annotation before identification. To study the contribution of these methods and their respective advantages, a detailed strategy was set up to proceed to the complete annotation of serum samples from pigs exposed or not to Bisphenol-A. This strategy was applied for both metabolomics and lipidomics datasets as 1D 1H, 2D UF COSY, NUS zTOCSY and NUS PS HSQC spectra were acquired on each phase following a Bligh and Dyer extraction. The metabolome and lipidome coverage and the complementarity of these techniques were successfully assessed and will be further compared to LC-HRMS RP and HILIC as well as GC-HRMS datasets acquired on the same samples.

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Metabolomic Analysis of Camellia: Revealing Phenotypic Traits and Industrial Potential

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Résumé

Artificial intelligence (AI) encompasses methods and algorithms that allow machines to perform tasks typically requiring human intelligence, such as image recognition, complex data analysis, and decision-making. Machine learning (ML), a central component of AI, relies on analysing large datasets to identify patterns and make predictions. Unlike traditional methods based on predefined rules, ML enables algorithms to learn from data and improve over time. In plant sciences, ML offers new opportunities for optimising varietal selection, predicting the biological activity of plants relevant to humans, and anticipating their applications in health, cosmetics, or agriculture.
The Camellia genus, known for its polyphenols and oils in cosmetics and its aesthetic value in horticulture, serves as a key example where ML can be impactful. The goal of its study is to identify promising varieties by optimising selection based on their compounds of interest and aesthetic traits. One major challenge is the long wait of 3 to 7 years for the camellia to flower, a critical phase for validating phenotypic attributes. ML applied to predictive metabolomics provides a solution by training models on leaf metabolomic profiles to predict floral characteristics before flowering. This method has achieved prediction accuracies of over 80% for flower shape and colour, revealing key families of metabolites involved.
Beyond accelerating varietal selection, this method could be used to predict biological activities of interest to humans, presenting new possibilities in health, cosmetics, and agriculture.

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Metabolomic signatures of two major respiratory pathogens in the reconstituted human airway epithelium model

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Résumé

Introduction

SARS-CoV-2 and influenza A viruses (IAV) are major pathogens for human respiratory infections. Several studies describe the impact of both viruses on host's metabolism (Ayres et al. 2020, Bahadoran et al. 2020, Rubayet Hasan et al. 2021). Nevertheless, none of those studies have exhaustively compared both viruses in a similar experimental set-up. Our study examines the impact of these viruses on the metabolism of human lung epithelium with an untargeted metabolomics approach.

Materials-and-methods

Reconstituted human airway epithelium (HAE) model was infected with SARS-CoV-2 (Wuhan strain) or IAV (H1N1 2009 pandemic strain) during 24 to 96 hours, and treated 48 hours after infections start or not with *Pseudomonas aeruginosa* flagellin. Infections were characterised by measuring TEER, inflammatory statement (Il-6), viral replication and transcriptomics profiles. Following cells collection, endometabolome was measured with an orbitrap LC-HRMS. Metabolomics data were processed with MZmine and MetaboAnalyst, then annotated with GNPS and Compound Discoverer in association with a homemade spectral

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database.

Results

Phenotype acquisition (TEER, Il-6, viral replication) and a K-means clusterisation method enabled us to define severity groups. LC-HRMS/MS analysis resulted on thousands of variables that were filtered (CV QC pool $< 30\%$, r2$>$ 0,7 for each variable). Non-supervised (PCA) or supervised (O-PLSDA) multivariate statistical analysis leaded to the identification of a VIP list associated to SARS-CoV-2 and/or IAV.

Discussion

We have identified metabolic disruptions distinctive for each virus and associate to a VIP list. Disrupted metabolic pathways may lead to the identification of new biomarkers specific to each infection types or severity levels.

Annotating the biotransformed metabolome based on untargeted metabolomics studies and in-source fragmentation in high-resolution mass spectrometry method development improves metabolite annotation

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Résumé

The gut microbiota significantly influences human pathophysiology through metabolic interactions. Several metabolic diseases, including steatotic liver disease, are associated with microbial metabolite biotransformations such as sulfation, glucuronidation and various conjugation reactions. Systematic annotation of these modified metabolites remains a major challenge in metabolomics, as they remain undescribed in databases and with limited reference standards availability.

In this study we have developed an ultra-high-performance liquid chromatography, high resolution mass spectrometry (UHPLC-HRMS) metabolomic strategy combining in-source fragmentation with targeted and untargeted profiling of mouse liver extracts to identify specific neutral losses (NL) of biotransformed metabolites. Metabolomic profiling of seven liver samples using C18 chromatography in positive and negative ESI modes detected 64,614 features, including 982 annotated metabolites, of which 542 metabolites passed linearity (r2$>$ 0.9) and reproducibility (CV%$<$ 30) filters. Among them, 181 were assigned to MSI level 1 and 360 to MSI level 2. An R script screened for 40 modification types, with 72.5% confirmed manually. Further targeted analysis revealed 333 and 223 NL signatures in positive and negative modes, with 146 and 82 annotated, respectively, identifying 29 and 24 modification types.

Our strategy enhances the detection of modified metabolites in liver tissue increasing the metabolic profile coverage up to 2.43%. Further work will focus on illuminating the dark metabolome, to better understand the microbial-host metabolic crosstalk and their impact on cardiometabolic disease. Leveraging analytical and chemoinformatics workflows to systematically annotate biotransformed metabolites will dramatically enhance annotation in biofluids and tissues to unravel novel markers associated with metabolic health and disease.

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Deciphering the biosynthesis of halogenated compounds in brown algal model using knock-out mutants, transcriptomics and metabolomics analysis

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Résumé

Some brown algae are known to concentrate halogens such as bromine or iodine and to produce halogenated metabolites maybe involved in signaling and/or defense during biotic interactions and physiological responses to environmental changes. These particular metabolites and their biosynthesis are still poorly described. In addition, the processes and function of halogenation remain uncertain in these marine organisms. We aim to explore the production of halogenated metabolites and their role in brown algae through functional genomics and omics approaches. One of the putative halogenating key enzymes, vanadium-dependent bromoperoxydases (vBPO), was inactivated using the CRISPR-Cas9 method in the model brown alga *Ectocarpus sp7*. The extinction of vBPO activity has been validated for 3 independent knock-out mutants. For metabolomic analysis, chemical extractions have

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been carried out using methanol and ethyl acetate and analysed on Liquid Chromatography-High-Resolution Mass Spectrometry. Data analysis was performed with analytical workflow including molecular networking for the comparison and description of (halo)metabolome in wild-type and KO strains. In addition, we will analyze gene regulation and metabolic patterns using transcriptomics and metabolomics on different strains upon oxidative stress in laboratory control conditions. All together, these data will provide new knowledge about the chemical diversity of halogenated metabolites and their biosynthetic pathways in brown algae.

Deciphering the Complex Interplay between Seed Exudates and Microorganisms during Seed Germination in Common Bean Genotypes

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Résumé

Seeds are crucial for plant reproduction, dispersal, and agriculture. Seed quality and vigour greatly impact crop production, referring to their ability to germinate rapidly and uniformly under varying environmental conditions, producing healthy seedlings that can withstand biotic and abiotic stress accentuated by global climate change. During germination, seeds release exudates, complex mixtures of organic and inorganic molecules, into the micro-environment surrounding them, known as the spermosphere. These exudates play a pivotal role in seedling development and overall plant fitness by influencing microbial

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selection, growth, and interactions in the spermosphere, ultimately shaping the plant's microbiome. Our research demonstrated that germinating seeds release diverse metabolites, peptides, proteins, and small RNAs in the exudates, some with functional properties like antimicrobial or antioxidant activities. This offers the possibility of utilizing these exudates as potential sources of new technologies for seed treatment applications, such as coating, pelleting, or priming, to improve seed quality. To understand the composition and functional properties of germinating seed exudates, we conducted our study using eight common bean seed genotypes produced under two contrasted locations in France. We investigated the diversity of specialized metabolites and microorganisms in the spermosphere and employed multi-omics data integration to identify correlations between spermosphere molecules and microorganisms. The approach used in our study identified potential molecules that could be used as candidates for developing strategies to enhance seed quality and improve crop productivity.

Oral Flash 6 - F6

Innovative methodological and analytical developments in the study of plant-soil fauna interactions: the contribution of metabolomics

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Résumé

Plant–soil interactions are modified by both biotic and abiotic factors, and understanding these dynamics is essential to predict ecosystem responses to climate change. Root exudates play a central role in rhizosphere dynamics through the release of primary and specialized metabolites, which are involved in defense mechanisms, competition, and symbiosis. However, the influence of soil fauna on root exudation remains poorly understood. To date, studying root exudation in soil matrices has been technically challenging, and the most commonly used systems hydroponic or hybrid fail to reproduce realistic interactions with soil fauna.

Presently, we investigate how soil fauna, specifically nematodes, modulate the quantity and composition of root exudates in *Linum usitatissimum*, and how these interactions are further influenced by increased temperature. Using the innovative ECOROOTS system, root exudates are collected *in situ* using nitrocellulose traps placed at the root apex, allowing for high-resolution metabolomic profiling via GC-HRMS (1-2). Additionally, molecular networks based on GC-HRMS data have been developed to support metabolite annotation.

Preliminary results indicate that the presence of nematodes significantly alters the exudation profile of *Linum usitatissimum*, an effect that is further amplified under a +4∘C. This methodology will be further integrated with HPLC-HRMS analyses, providing a robust multiblock approach for the investigation of root exudation in other plant species with allelopathic potential or agronomic relevance.

(1) V. Bohm *et al.*, mai 2025, doi: 10.1016/j.jcoa.2025.100205.

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(2) A. Levasseur *et al.*, juin 2025, doi: 10.1016/j.soilbio.2025.109771.

Elucidating the role of glucosinolate modifications regulated by high temperatures in Arabidopsis seeds

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Résumé

Glucosinolates (GSLs) constitute major antinutritional and defensive compounds massively accumulated in seeds of Brassicaceae species, including the model plant *Arabidopsis thaliana*. While most studies have focused on GSL role in biotic stress responses, the potential functions and regulation of GSLs in abiotic stress responses has been neglected. This is particularly true in seeds, for which few information are available. In this study, multi-omic analyses (untargeted metabolomics (LC-MS/MS) and transcriptomics (RNAseq)) allowed to identify a GSL network/pathway that is strongly modulated by high temperature (HT). Several new thioglucose acylated GSLs that were particularly induced by HT during Arabidopsis seed development were identified. Reverse genetics analyses showed that the SERINE CARBOXYPEPTIDASE-LIKE 17 (SCPL17) and BENZOYLOXYGLUCOSINOLATE 1 (BZO1) enzymes are involved in the sinapoylation and/or benzoylation of GSL thioglucose moieties. Acylation of GSL thioglucose moieties is suggested to have an important role in Arabidopsis seed responses to HT.

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Untargeted metabolomics combined with proteomics reveal the versatile effects of Mox-LDLs on endothelial cells

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Résumé

Myeloperoxidase-oxidized LDLs (Mox-LDLs) trigger endothelial cells and participate in atherosclerosis. However, the mechanisms behind Mox-LDLs stimulation are not fully understood. Therefore, we used an untargeted metabolomics approach combined with proteomics analysis to analyze human umbilical vein endothelial cells stimulated by Mox-LDLs. Cells (n=6) were exposed for 24 h to Mox-LDLs (0 or 100 μg/ml) with or without native LDLs (0 or 1 mg/m). Supernatant and cell lysate were then analyzed using liquid chromatography coupled to mass spectrometry. Using Workflow4Metabolomics work environment, MZmine, SIRIUS and MetGem, we selected and identified key metabolites influenced by Mox-LDLs treatment. For proteomics analysis, we used DIA-NN and the FragPipe-Analyst application to detect proteins differentially expressed after Mox-LDLs treatment. Metabolomics analysis revealed that sphingolipids, phospholipids and oxidized cholesterol-derived compounds increased in cells after Mox-LDLs exposition. The increase in sphingolipids was associated to an increase of 3-ketodihydrosphingosine reductase, an enzyme implicated in sphingolipid biosynthesis. We also observed an increased mitochondrial activity, probably resulting in intracellular reactive oxygen species (ROS). This can be correlated to the oxidation of the cholesterol-derived metabolites detected. Metabolomics analysis revealed an increase in di- and tripeptides, due to Mox-LDLs metabolism. Finally, a trihydroxy-unsaturated fatty acid was secreted by cells exposed to Mox-LDLs and could serve as a biomarker of Mox-LDLs exposure. Our study suggests that Mox-LDLs are internalized and degraded by HUVECs. They seem to induce mitochondrial activation and oxidative stress, likely ROS mediated. We suggest that HUVECs then activate cytoprotective antioxidant coping mechanisms (glutathione synthesis, heme oxygenase-1) to survive and adapt.

*Intervenant

Détermination de marqueurs hépatiques suite à une exposition aux PolyChloroBiphényles (PCB) chez le poulet par une approche métabolomique RMN 1D et 2D

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Résumé

Les polluants organiques persistants suscitent des préoccupations majeures en raison de leur toxicité, leur bioaccumulation et leur présence dans les aliments d'origine animale. Dans le cadre du projet ANR Sentinel (ANR-19-CE21-0011-02) visant à renforcer la surveillance de la sécurité alimentaire, les travaux portent sur une étude de cas dans l'objectif d'identifier des marqueurs endogènes suite à une exposition aux PCB chez le poulet par une approche non ciblée par RMN. Pour surmonter les limites de la RMN 1D, l'intérêt et les performances de la RMN 2D ont été évalués à l'aide de séquences 2D rapides.
Des extraits aqueux et lipidiques de foie de poulets exposés à de faibles doses de PCB (Aroclor 1260 : 4 ; 20 ; 200 μg/kg/jour) dans l'alimentation pendant 45 jours ont été analysés par RMN 1D et 2D (i.e. COSY ultrarapide, COSY rapide, TOCSY à échantillonnage non uniforme (NUS) et HSQC NUS) sur un spectromètre RMN 600 MHz. Des analyses statistiques multivariées ont été réalisées afin de différencier les animaux témoins des animaux exposés.
Les premiers résultats sur les extraits lipidiques montrent que la RMN 2D HSQC NUS permet de séparer les groupes témoins et contaminés, alors qu'aucune séparation n'est observée en RMN 1D, révélant ainsi des biomarqueurs. Ces résultats mettent en avant la valeur ajoutée de la RMN 2D pour améliorer l'identification des marqueurs. Des analyses multi-tableaux intégrant toutes les séquences 2D sont en cours. Notre méthodologie innovante ouvre la voie au développement de nouvelles solutions pour un suivi efficace, applicables à d'autres études nutritionnelles.

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Oral Flash 10 - F10

Exploring metabolic pathways and toxic effects of ten organic UV filters in Pocillopora damicornis via untargeted metabolomics

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Résumé

UV filters are the principal components of suncare products and are extensively released into coastal environments during the summer, affecting aquatic organisms, including reef-building corals. Due to their lipophilic properties, these compounds have a high potential for bioaccumulation, especially in corals, which are essentially composed of lipids. Research has shown that certain UV filters can be toxic to coral models, inducing mortality, disruptions in symbiotic relationships and microbial communities, as well as deformities in larvae. However, bioaccumulation studies on corals are scarce and mainly focus on the parent compounds, leaving the metabolic pathways of their transformation products largely unexplored. This study aimed to investigate the interactions between the tropical coral species *Pocillopora damicornis* and ten organic UV filters approved for use in the European market, with a focus on their metabolization processes and potential toxicity through an untargeted metabolomic approach. Corals were exposed to 1 mg/L of each UV filter for seven days, and their metabolomes were explored using an UHPLC-HRMS-ESI+ system. The majority of the annotated metabolites corresponded to UV filters biotransformation products. An in-depth analysis was conducted on octinoxate (ethylhexyl methoxycinnamate, EM), which showed the most significant effects on coral metabolome and phenotype during the exposure. A dose-response modeling approach (*DRomics*) was applied to the metabolomic data to further assess the dynamic relationship between EM concentration and metabolomic changes.

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Deciphering the molecular functions of defense signaling molecules in brown algae with a focus on the oxylipin pathway

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Résumé

The oxylipin pathway is known to be involved in defense signaling in plants and animals. In brown algae, an evolutionary independent eukaryotic lineage, oxylipins deriving from both C18- and C20- Polyunsaturated Fatty Acids are produced during stress defense responses. Their biosynthetic pathways and roles as signal molecules during biotic interactions are still incompletely known. Genomic approaches have identified several CYP5164 genes, which are homologous to the plant CYP74 gene family, that are involved in jasmonate biosynthesis. To decipher the biological functions of those genes in two brown algae from the Ectocarpales order, the free-living *Ectocarpus sp.7,* and the endophytic *Laminarionema elsbetiae*, targeted and un-targeted metabolomic analyses were performed to compare their global metabolomes in control and stressed conditions. LC-MS analysis was also used to mine for differences in the overall metabolic profiles and to investigate the occurrence or absence of specific oxylipins of CRISPR knock-out mutants for the CYP5164B1 and wild-type strains. In addition, recombinant CYP5164B1 proteins were produced to characterize *in vitro* biochemical activities by GC-MS and to identify brown algal-specific substrates. These approaches will

*Intervenant

indicate whether the profiles of mutant oxylipins are consistent with the previously determined catalytic activity of the recombinant enzyme. The combination of *in vivo* metabolomic approaches and targeted biochemical characterization will enable CYP5164 activity to be integrated into a more global metabolic context in a brown algal model and contribute to a better understanding of CYP-based defense and chemical signaling in brown algae during biotic interactions.

Glucose as a Versatile Glassing Agent for Hyperpolarizing Key Metabolites in Biological Studies

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Résumé

Dissolution-dynamic nuclear polarization (D-DNP) significantly enhances nuclear magnetic resonance (NMR) signals (1), paving the way for exploring cell metabolism. Among many targets, glutamine has drawn particular attention for its role in cancer and cardiovascular diseases, due to its high concentration in cancer cells, which proliferate more than healthy cells (2,3). In fact, as a key metabolite for cellular energy supply, glutamine's conversion into glutamate and alpha-ketoglutarate can be monitored over a biologically relevant timescale.
However, glassing agents used in standard DNP sample formulations can affect cellular media and kinetic analysis. While glycerol-d8, the most common glassing agent, is safe, it is not chemically inert and non-optimal to mimic physiological conditions. Here, we introduce a new formulation using glucose as a glassing agent, providing a biologically safe and cost-effective solution.

We first performed feasibility study on sodium acetate. Then, we optimized the formulation on glutamine achieving a maximum ^{1}H polarization of 69%. Glucose has demonstrated excellent biocompatibility, enabling the monitoring of the enzymatic conversion of glutamine to glutamate.

We have been able to monitor real-time glutamine metabolism with hyperpolarization in bacteria cells. These promising results suggest the potential for metabolic studies of glutamine and glucose, opening the way for applications in cancer cell research.

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(2) Dos Santos, K.; Bertho, G.; Caradeuc, C.; Baud, V.; Montagne, A.; Abergel, D.; Giraud, N.; Baudin, M. Chemphyschem 2023, 24 (12).
(3) Phan, L. M.; Yeung, S.-C. J.; Lee, M.-H. Cancer Biol Med 2014.

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Oral Flash 13 - F13

Exploration des cinétiques par RMN hyperpolarisée : du déséquilibre magnétique à l'analyse métabolique

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Résumé

Les études cinétiques sont essentielles pour comprendre les voies métaboliques. Déterminer les paramètres thermodynamiques et cinétiques d'une réaction enzymatique permet d'élucider les réseaux complexes de réactions biochimiques. La RMN (résonance magnétique nucléaire) s'est imposée comme une technique efficace pour suivre ces réactions en temps réel. Cependant, sa faible sensibilité limite son application aux réactions lentes ou impliquant des enzymes en faibles concentrations, bien en dessous des niveaux observables dans les compartiments cellulaires. Cette contrainte peut être contournée grâce aux techniques d'hyperpolarisation, comme la polarisation dynamique nucléaire (DNP). La dissolution-DNP (D-DNP) permet ainsi d'hyperpolariser un échantillon à 1.2 Kelvin avant sa dissolution, amplifiant le signal de 3 à 4 ordres de grandeur par rapport à la RMN conventionnelle (1).

La RMN distingue également les déplacements chimiques des molécules selon leur environnement. Pour le Glucose-6-Phosphate (G6P), le carbone C1 distingue deux anomères, α et β, en équilibre par mutarotation. Dans une étude précédente (2), nous avons déterminé à 27 ∘C une constante d'échange de kα = 0,042 ± 0,017 s^{1} et kβ = 0,028 ± 0,012 s^{1} via une réaction enzymatique impliquant la G6PDH.

Nous explorons ici la possibilité d'extraire ces constantes sans réaction enzymatique. Nous évaluons si un déséquilibre magnétique, induit par excitation sélective de certains signaux, peut fournir des informations cinétiques via la repolarisation par échange anomérique (3) et constituer une alternative robuste à l'approche enzymatique.

(1) Ardenkjær-Larsen et al., PNAS (2003), 100, 10158-63
(2) Soussi-Therond et al., JACS (2024), 146 (50), 34651-34660
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*Intervenant

Metabolomic Assessment of Trace Elements in Epilepsy: Focus on Zinc and Copper in Patients with Inherited Metabolic Disorders

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Résumé

Epilepsy is a major neurological disorder, ranking as the third leading cause of neurological conditions worldwide. It affects children and older adults, with these groups representing the fastest-growing segments of newly diagnosed patients. Childhood epilepsy, in particular, presents a wide variety of clinical manifestations and causes, including genetic predispositions, brain abnormalities, and metabolic disorders.
The role of trace elements, especially zinc and copper, in epilepsy has gained attention due to their importance in neuronal stability and function. Dysregulation in the homeostasis of these elements may contribute to seizures, and antiepileptic drugs (AEDs) can alter their serum levels, potentially influencing disease management.

This study aimed to evaluate the relationship between serum zinc and copper levels and epilepsy, alongside the impact of antiepileptic treatment. Serum levels of zinc and copper were measured using atomic absorption spectrometry in 25 epileptic patients, divided into three groups: untreated (n=6), monotherapy (n=13), and polytherapy (n=6). A control group of 100 healthy individuals was also included.

The results showed significant differences in zinc and copper levels between epilepsy patients and controls. Copper levels were higher in epileptic patients, especially those on polytherapy, while untreated patients had significantly lower zinc levels compared to controls. Patients on monotherapy had higher zinc levels than the control group.
These findings underline the critical role of zinc and copper homeostasis in epilepsy pathogenesis. Adjusting treatment strategies to restore balanced levels of these trace elements could improve epilepsy management in the future.

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A Personalized Metabolomics Approach to Prioritize Genomic Variants Identification in Rare Diseases

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Résumé

Nowadays, around 30% of patients suspected of having a rare genetic disease remain undiagnosed despite whole-genome or exome sequencing analysis. With the development and standardization of omics approaches, metabolomics could provide new insights and clues to help identify the genes responsible for these patients' diseases.
To test this, we analysed the metabolome in the plasma and blood cells of 46 patients clinically suspected of having genetic diseases, along with 30 healthy relatives and controls. Using the standardized Biocrates Quant MxP500 XL kit, which quantifies over 1,000 metabolites, we suggest a primary approach to highlight patient profiles that could guide genome reanalysis.

Briefly, after identifying outliers' profiles in both plasma and blood cells with significantly different metabolic profiles, we conducted a more in-depth investigation of these patients. Normal value ranges for each metabolite were established based on the control group, and patients' metabolites outside these ranges were highlighted. Aberrant metabolite concentrations and their associated metabolic pathways are currently used to prioritize genes for further genome or exome sequencing re-analysis.
This study is part of a wider multi-omics approach, called PRIOMICS, which we hope will soon contribute to establishing a pipeline to provide answers for undiagnosed patients.

*Intervenant

Poster 1 - P1

Metabolomic biomarkers of psychotic conversion in Ultra-High- Risk subjects: a pilot study

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Résumé

Introduction: Psychosis is a psychiatric condition that can become a chronic and severe psychiatric disorder affecting more than 1% of the population. The ultra-high risk (UHR)

patients have a transition rate to psychosis of 25% after three years. We aimed to identify circulating metabolomic biomarkers for psychotic conversion in UHR patients using nuclear magnetic resonance (NMR) spectroscopy.

Methods: We used samples from 35 UHR patients: 14 converters (UHR-C) and 21 non-converters (UHR-NC) at inclusion from the ICAAR cohort. Serum samples were analysed using the high-throughput screening IVDr NMR method. R and SIMCA were used for statistical analysis.

Results: Several lipoprotein parameters related to HDL and LDL metabolism were down-regulated in UHR-C compared to UHR-NC at inclusion. The 3 best lipoproteins to predict psychotic conversion at baseline were H4A1, H4FC, and L4FC (Area under the Curve (AUC)

*Intervenant

values were 0.81, 0.81, and 0.78, respectively). These lipoproteins were also negatively correlated to PANSS scores.
Discussion: Our study is the first to use NMR technology to identify biomarkers to predict the risk of psychotic transition in UHR subjects. This pilot study found lipoprotein parameters related to ApoA-1 and HDL-cholesterol (subclass 4) as potential biomarkers. These results need to be replicated on a larger sample. This study highlights the importance of the detailed analysis of circulant lipoproteins related to the brain using NMR technology in early psychosis to identify biomarkers of psychotic transitions and perhaps to better understand the physiopathology of psychosis.

Poster 2 - P2

On the classification of 1H NMR plants spectra (at an industrial scale) with a combined machine learning and metabolomic approach

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Résumé

This presentation will include the results obtained during an ongoing research project of my PhD thesis (in partnership with University of Lyon, France and an industrial partner, EVEAR extraction), on the modeling and intelligent classification of NMR spectroscopic data, using NMR spectra and AI models trained on 4 Nvidia L4 GPUs. The main goal is to develop a classification of plants extract from their NMR spectra database (4000 spectra), aiming to specifically identify the plants studied among a defined set.This methodology relies on detecting the unique characteristics present in each spectrum, allowing for accurate recognition of the molecules comprising the sample. First a similarity study I presented, from this, early results on a machine learning workflow based on a random forest (RF) algorithm have been obtained. On a side note, a comparison with several other machine learning models will be presented, mainly Support Vector Machines (SVM) and Decision Tree, where some algorithms enhance the results and provide further good predictions. This project promises to significantly improve the speed and accuracy of chemical compound identification on key industrial steps, offering potential applications in various scientific and industrial fields. It represents a notable advancement in the use of NMR spectroscopy, opening new perspectives for the exploration of spectroscopic data in research and beyond.

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Poster 3 - P3

Xtremomics ou comment la métabolomique permet d'explorer les confins de la résistance humaine

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Résumé

Les systèmes biologiques s'adaptent aux variations environnementales, mais les conditions extrêmes présentent des defis spécifiques pouvant induire des altérations physiologiques sévères. Notre objectif est d'identifier l'existence de systèmes métaboliques universels favorisant la résilience face à ces conditions.
Pour cela, nous avons réalisé l'analyse LC/MS du plasma et/ou urines de sujets placés en conditions d'hypoxie chronique liés à l'altitude (5300m), de plongeurs en séjour de plusieurs jours en capsule sous-marines sous atmosphère d'héliox (20%O2/70% He), de volontaires immobilisés pendant 2 mois en position couchée mimant les séjours spatiaux et de militaires en opération. Environ > 200 à 400 métabolites ont été annotés et permettent de stratifier le métabolome en unités fonctionnelles. Ces unités sont déclinables en amont en voies biochimiques et en aval en modules du système biologique. Ces trois niveaux d'organisation du vivant représentent les systèmes métaboliques de façon normalisée permettant un comparatif non biaisé des différentes situations.

*Intervenant

Cette modélisation permet de révéler des systèmes génériques déclinés à différents niveaux d'échelles (moléculaire/fonctionnel/module système) et communs à chacune des situations. Il s'agit du métabolisme du tryptophane en lien avec le microbiote et le métabolisme primaire de l'hôte, des systèmes de défense cellulaire associant le métabolisme du glutathion et de la sérine/threonine/glycine, et des adaptations du système cardiovasculaire et musculaire en lien avec les acides aminés branchés ou non et avec le transport des acides bilaires/amines/ions métal.
Il semble qu'un système métabolique de " rescue outcome pathway " générique semble s'activer en réponse aux conditions environnementales extrêmes diverses et assurer la survie.

Poster 4 - P4

Untargeted Metabolomic and Volatilomic Profiling of Fermented Products: Optimized Strategy for water and Milk Kefirs

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Résumé

Fermented foods are complex matrices whose chemical composition is influenced by microbial activity and fermentation conditions. Water and milk kefirs are attracting increasing interest due to their potential health benefits, yet their metabolomic and volatilomic profiles remain insufficiently characterized, particularly through untargeted approaches. This study aimed to establish a comprehensive analytical workflow combining UHPLC-HRMS (Orbitrap Exploris 240 ThermoFisher) for non-volatile metabolite profiling and GC-TQ-MS (GC 8890 - TQ 7010C Agilent) for volatile compound characterization.
A standardized sample preparation protocol was applied to both water and milk kefirs. Non-volatile metabolite analysis was performed in positive and negative ionization modes, using different chromatographic columns (HILIC Amide, C18, and HSS) to optimize metabolite separation and maximize molecular coverage. In parallel, Volatilomic analyses employed Dynamic Headspace Sampling (DHS) and Stir Bar Sorptive Extraction (SBSE), with or without salt addition, to enhance volatile compound extraction.

Data processing included peak detection, alignment, and normalization, followed by a comparative evaluation of detected and annotated ions across different chromatographic conditions. Statistical analyses were conducted to assess metabolic variations, while spectral libraries and computational tools refined metabolites and volatile compounds annotation.

This study establishes a systematic and reproducible workflow for untargeted metabolomic and volatilomic analyses, enabling comparisons of kefirs fermented under different conditions. The approach offers deeper insights into the biochemical transformations occurring during fermentation, contributing to a better understanding of kefirs' metabolic and volatilomic complexity and their potential functional properties.
Keywords: Untargeted Metabolomics, Volatilomics, Analytical Method Development, Fermented Products, Water Kefir, Milk Kefir.

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Poster 5 - P5

Apport de la métabolomique pour la mise en œuvre de solutions de lutte contre Heterodera carotae

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Résumé

Heterodera carotae est un nématode à kyste phytoparasite obligatoire. En absence de protection, son développement sur carotte est responsable de pertes de rendement allant jusqu'à 80 %. Depuis 2018, les moyens de luttes phytosanitaires (via la fumigation chimique des sols) sont interdits en France, impactant fortement la production de carotte à l'échelle de bassins de production entiers. Capable de parasiter uniquement le genre Daucus (1), H. carotae a développé des stratégies de reconnaissance de sa plante hôte via la perception des signaux chimiques qu'elle émet par ses racines (2). Cette spécificité a permis de développer une stratégie "d'éclosion suicide", qui correspond à leurrer le nématode sur la présence de sa plante hôte et dont l'efficacité a déjà été démontrée (3). Toutefois, son déploiement à l'échelle du bassin de production se heurte notamment à la difficulté de production d'exsudats racinaires en quantité suffisante. Identifier la(les) molécule(s) reconnue(s) par ce nématode pourrait contribuer à lever ce verrou. Pour ce faire, le projet ECLODERA repose sur la combinaison de deux approches complémentaires d'analyses métabolomiques (par LC-HRMS) d'exsudats racinaires. 1) L'analyse d'exsudats issus de 15 variétés d'apiacées différentes et 2) l'analyse d'exsudats prélevés à différents stades phénologiques. Les résultats obtenus ont permis d'identifier une molécule candidate. Pour confirmer cette structure, des approches de fractionnement bio guidés (via UHPLC) sont menées. (1) : Montarry et al., 2024 https://doi.org/10.1163/15685411-bja10361
(2) : Ngala et al., 2021 https://doi.org/10.3389/fpls.2020.602825
(3) : Ngala et al., 2024 https://doi.org/10.1016/j.apsoil.2024.105490

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Poster 6 - P6

Development of a Model for Assessing the Adsorptive Properties of Traditional Medicinal Formulations from Burkina Faso in Relation to Snake Venom Proteins

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Résumé

Abstract

Envenomation resulting from snake bites is commonly treated in Burkina Faso using traditional remedies derived from the calcination of plants. These treatments are administered either orally or topically, following incisions made at the envenomation site. This study aimed to develop a model for assessing the adsorption properties of these remedies concerning venom proteins, employing a metabolomics-based approach.
Traditional remedies were collected from three regions of the country. A preliminary physicochemical characterisation, including particle size analysis, was conducted, alongside an evaluation of the adsorption capacity of the remedies in comparison with activated charcoal (used as a positive control). Subsequently, the adsorption of toxic proteins from the venom was assessed by analysing intact proteins within the mixtures before and after adsorption using liquid chromatography–high-resolution mass spectrometry (LC-HRMS). The data generated were processed using the Workflow4Metabolomics (W4M) platform. Remedies exhibiting significant adsorption capacities were then subjected to proteomic analysis to identify the adsorbed proteins.

The findings revealed that the Kampti remedy and activated vegetable charcoal demonstrated significant adsorption capacities for venom proteins. Among the tested remedies, the Kampti preparation was particularly noteworthy for its ability to adsorb metalloproteinases, neurotoxins, and phospholipases.

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The metabolomic model developed in this study successfully demonstrated the adsorption capabilities of traditional remedies concerning venom proteins. Given the calcined nature of these preparations, ongoing metabolomic investigations aim to identify any residual secondary metabolites within the studied remedies.
Keywords: venom, proteomics, adsorption, traditional medicine, Burkina Faso

Poster 7 - P7

Connecting proteins to small molecules: transforming reaction curation in UniProtKB using Rhea

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Résumé

The expansion of multiomics data generation has highlighted a critical need for more comprehensive and accurate annotation of small molecules in commonly used databases, as they are essential for enabling meaningful biological interpretation. The UniProt KnowledgeBase (UniProtKB, at www.uniprot.org) is a reference resource of protein sequences and functional annotation that is commonly used for proteomics, transcriptomics, and genomics analyses. Here we describe how we extend the domain of applications of UniProtKB to include integrated analysis of metabolomics and other chemical data, through the curation of enzymes and transporters functions with Rhea, an expert curated knowledgebase of biochemical reactions (www.rhea-db.org) based on the ChEBI ontology of small molecular entities (www.ebi.ac.uk/chebi/). Building upon the current linkage of over 29 million proteins to 10,000 metabolites through Rhea, the effort to improve small molecule data integration in UniProtKB will leverage advanced machine learning to capture enzyme and transporter chemistry knowledge at scale. This development improves interoperability with other data and knowledge resources dealing with metabolites, such as the metabolomics data repository MetaboLights, the protein structure repository PDB, the pathway knowledgebase Reactome, and the Gene Ontology. It provides enhanced support for metabolic modeling, multiomics data integration and analysis, and the use of advanced machine learning approaches to predict enzyme function and biosynthetic and bioremediation pathways.

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Poster 8 - P8

Fast isomer distinction and quantification in complex mixtures using direct introduction - high-resolution ion mobility - mass spectrometry (DI-IM-MS)

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Résumé

Simultaneous detection and quantification of multiple isomers in complex biological matrices remains challenging due to matrix interferences and, in particular, their very high structural similarity, resulting in overlapping detection signals. Although ion mobility-mass spectrometry (IM-MS) enables efficient isomer separation without the need for an additional separation system like liquid chromatography (LC), achieving high-resolution ion mobility conditions capable of differentiating multiple structurally related species remains difficult.
In this study, an untargeted approach was developed on a trapped ion mobility spectrometry time-of-flight (TIMS-ToF) to achieve high-throughput and high-resolution ion mobility conditions. This approach enables comprehensive compound characterization in a complex matrix, improving detection coverage over a wide ion mobility range while maintaining high analytical sensitivity and high-throughput analysis. This strategy effectively distinguishes isomers with close mobility values and allows their rapid detection.

Beyond its capability for advanced isomer characterization, this approach can be used for the rapid and simultaneous quantification of isomers in complex mixtures. Under optimal conditions, the standard addition method can be employed to accurately measure target compounds in biological samples. To demonstrate its applicability, this method was applied to breast milk analysis, enabling the identification of various human milk phenotypes and the quantification of key isomers of human milk oligosaccharides. This method is also suitable for the chiral metabolite analysis and the determination of their enantiomeric excess, such as amino acid enantiomer analysis.
This study highlights the potential of our proposed approach for isomer distinction and quantification, offering promising applications in metabolomics, biomarker discovery, and complex mixture analysis.

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Poster 9 - P9

1H-NMR profiling of eggplant and pepper fruits during their development

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Résumé

For *Solanaceae*, the primary metabolite contents of ripening and ripe fruits have been widely studied in tomato. However, less data are available for other species such as pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.) during fruit development to study their metabolism. We used 1H NMR profiling to produce quantitative metabolite data of pepper and eggplant fruit along development.

Polar compounds were extracted from lyophilized fruit powder with an ethanol-water series. NMR analyses were performed on pH-adjusted extracts containing EDTA. Metabolite absolute quantification was achieved using a 500-MHz-NMR-spectrometer and external calibration-range solutions. 1D-NMR spectra were processed with NMRProcFlow web tool(1). Annotation of the 1D-1H-spectra was performed as previously described(2,3,4), and using additional common 2D-NMR and less common 1D-selective-gradient COSY and TOCSY experiments.

Twenty-four metabolites were determined in pepper (including two compounds partially identified: a trans-4-hydroxyproline-like-compound, and a hydroxycinnamic-acid containing-compound) and 27 in eggplant. Nineteen common metabolites were quantified in both fruit species including three soluble sugars and one sugar-alcohol, five organic acids and nine free amino acids. Spectra and data are available at recherche.data.gouv repository and ODAM dataexplorer and described in Roch et al. (2024). The metabolite quantification data allowed seeing common changes during development for the two species and their main compositional differences, and also comparison with tomato data.

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Poster 10 - P10

Steroidomic Characterization of Adrenocortical Tumors: A Non-Targeted LC-MS/MS Approach on Tissue Extracts

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Résumé

Adrenal cortex produces various steroid molecules, including mineralocorticoids, glucocorticoids and androgens, thanks to an enzymatic cascade reaction taking cholesterol as precursor. This pathway is altered in adrenocortical tumors, leading to a steroid excess that manifests with varying degrees of symptomatology. These alterations are not fully elucidated by targeted serum profiling using routine LC-MS/MS. The aim of this study was thus to perform untargeted steroid profiling directly on adrenal tumors tissue extracts to establish a steroid fingerprint able to discriminate different tumor types. Samples include normal adrenals, unilateral (Adrenal carcinoma, cortisol-producing and non-functioning adenomas), and bilateral tumors (Micronodular and macronodular adrenal pathologies), which were extracted in methanol after a grinding step. Five samples of each tumor type were analysed by an untargeted HPLC-MS/MS method with an Agilent 1260 Infinity II system, equipped with a CORTECS T3 C18 120Å column (2.7 μm; 2.1 mm x 150 mm), with a water/acetonitrile gradient, coupled to an ESI-tims-TOF (Bruker) in the positive ion mode. The LC method was validated by the efficient separation of a standard mix of 14 steroids and allowed the further detection of unknown molecules in some specific tumor types, whose annotation is still under progress. For the first time, steroidogenesis alterations in adrenocortical tumors are being explored by untargeted steroid profiling directly in adrenal tissue. This study paves the way for the discovery of new biomarkers for earlier tumor diagnosis.

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Poster 11 - P11

Metabolic Impacts of Genetic Modifications to Spermidine Biosynthesis in Escherichia coli

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Résumé

Intestinal bacteria play a crucial role in metabolism, influencing overall health by producing various metabolites. Signals from these microbial metabolites have important and diverse effects on host physiology. One example is spermidine, a polyamine which is gaining attention for its potential immunomodulatory and anti-inflammatory properties.
In the context of the holobiont, the probiotic strain Escherichia coli Nissle has been genetically engineered to overproduce and secrete spermidine. Previous studies have shown that enhancing the production of enzymes involved in spermidine biosynthesis and export in this strain significantly increases extracellular spermidine levels. However, in vivo experiments in mice revealed viability issues with the modified strains, indicating that these genetic modifications affect other essential cellular processes.
To investigate these effects, we perform 13C-labeling experiments under microaerobic cultivation conditions. This setup aimed to more accurately mimic the oxygenation conditions of the intestinal environment, providing physiologically relevant insights into cells metabolism. Using a high throughput fluxomic workflow composed of automated robotic platforms for cell cultivation and sample preparation, coupled with mass spectrometry and NMR analysis, we performed an exhaustive exploration of intracellular fluxes in E. coli mutants.
This study illustrates how high-throughput fluxomic experiments have enhanced our understanding of the metabolic impacts of various genetic modifications in E. coli. By performing labelling experiment, and analyzing extensive isotopic datasets, we can generate highly detailed maps of carbon fluxes in the central carbon metabolism. This approach enables the identification of bottlenecks that may adversely affect strain viability and facilitates the proposal of modifications to optimize spermidine production.

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Poster 12 - P12

Beyond the Label: Unveiling the Metabolomic Fingerprint of Turmeric and Hawthorn Supplements

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Résumé

Herbal food supplements (HFS) are gaining in popularity, but their chemical composition and quality can vary considerably. A non-targeted metabolomics approach using LC-HRMS was used to analyse the composition of commercial products based on turmeric and hawthorn. These 2 plants were chosen for their different consumption patterns. In one case, the bioactive metabolites are fairly well known and in the other not, but in both cases, clinical evidence exists. By increasing our knowledge of the phytochemical composition of complex matrices, consumption is safer and our understanding of their physiological/therapeutic effects is more rational. In the case of turmeric, the results showed considerable phytochemical variability between the 19 products tested, particularly in terms of curcuminoids and tumerones. The main discriminating factor was formulation, with manufacturers being very inventive in improving curcumin absorption. We correlated content with the commercial price of HFS, with surprising results. For hawthorn, the manufacturing extraction method was the main discriminating factor, although different species were authorised among the 17 products tested. Herbal teas generally showed the highest levels of compounds, while extracts showed the lowest levels, despite being more expensive. This study highlights the phytochemical variability of products and therefore the potential for inequivalent biological effects without the consumer being informed. This suggests the need for stricter regulations and more extensive phytovigilance.

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Poster 13 - P13

Technological developments and opportunities with Workflow4Metabolomics 4.0

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Résumé

Background

Metabolomics data analysis is a complex and multistep process, which is constantly evolving

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with the development of new analytical technologies, mathematical methods, bioinformatics tools and databases. By a common effort from two French national infrastructures as MetaboHUB and the IFB (European ELIXIR French node), and supported by the RFMF, Workflow4Metabolomics (W4M) endeavours to break through the barriers that are obstructing data analysis practices in this field.

Combining technological innovation, expertise in data analysis and support to the community

Workflow4Metabolomics 4.0 is built around three pillars. First, it provides free open-source Galaxy-based workflow possibilities for MS- and NMR-based data analysis. By the adjustable nature of Galaxy, W4M tool suit evolves with methodological innovations, when in the meantime it enables traceability, reproducibility and favours interoperability with other complementary resources. Second, W4M is keen to enable adequate training to people willing to improve their data analysis understanding and skills. Thus, it provides various learning contents: free online tutorials, yearly "Bring Your Own Data" on-site trainings, and lately a dedicated protocol (**https://doi.org/10.1002/cpz1.70095**). Third, W4M is held by experts in Metabolomics, computer science and data analysis, agreeing to give some of their time to provide guidance to the community (e.g. by providing support through the IFB's open forum.

Results and impact

By gathering a large variety of tools with standardised input/outputs, W4M web portal increases LC/GC-MS(MS) and NMR workflows' efficiency. We highlight how current advances, along with community training contribute to comprehensive open data analysis practices worldwide.

Keywords:
Data analysis workflows, Metabolomics, Galaxy, Training

Poster 14 - P14

Bacterial microcompartments as platforms for synthetic methylotrophy in Escherichia coli

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Résumé

Methanol assimilation presents a metabolic challenge due to the toxicity of formaldehyde and hydrogen peroxide. An imbalance between assimilation and oxidation can lead to formaldehyde accumulation, compromising cell viability. Natural methylotrophs overcome this issue by finely regulating enzyme expression (bacteria) or compartmentalizing metabolism within peroxisomes (yeasts).
To implement a synthetic methylotrophic pathway in *Escherichia coli*, enzyme compartmentalization offers a promising alternative. Bacterial microcompartments (BMCs), protein shells that encapsulate enzymes, could help limit the toxicity of metabolic intermediates. An empty BMC (eBMC) can be produced in *E. coli* by expressing shell proteins from the Pdu BMC of *Citrobacter freundii*, enabling targeted enzyme encapsulation via specific peptides.

The METASYS team has designed a methylotrophic pathway in *E. coli*, combining methanol dehydrogenase (Mdh) and dihydroxyacetone synthase (Das), further optimized by overexpressing glycerol dehydrogenase (GldA). While this pathway allows methanol assimilation, it does not support growth on methanol as the sole carbon source.
In this study, we aimed to evaluate whether enzyme compartmentalization within an eBMC could improve the efficiency of this pathway. We assessed the impact of enzyme encapsulation using ^{13}C isotopic labeling, tracking methanol incorporation into central metabolites. Our results indicate that encapsulation does not significantly enhance metabolic flux but highlights key bottlenecks limiting its efficiency. Further work is underway to optimize this approach and develop a functional methylotrophic microcompartment.

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Poster 15 - P15

Améliorer la contextualisation des données du produit du métabolisme végétal par l'annotation systématique de la littérature

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Résumé

L'annotation de la littérature scientifique sur les matrices végétales constitue un défi majeur pour la contextualisation des données en métabolomique. En l'absence d'un système d'indexation standardisé comparable à celui du domaine médical, des initiatives comme Planteome, développant des ontologies spécialisées pour les espèces végétales, demeurent largement sous-exploitées.
Le projet "FORVM Plants" propose d'utiliser des modèles de langage (LLM) pour établir des associations entre composés chimiques et concepts ontologiques. Cette approche vise à améliorer l'interprétation des données expérimentales issues de matrices végétales en métabolomique. Pour surmonter le manque d'annotation, nous proposons une approche d'annotation automatique de la littérature scientifique par des termes ontologiques basée sur les modèles de

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langage (LLM). Cette méthode exploite la "similarité sémantique" des LLM, leur capacité à capturer le sens des mots et des phrases au-delà de leur forme lexicale. Combinée à la méthodologie établie dans le projet FORVM desease, qui établit des associations statistiquement significatives entre composés chimiques et concepts ontologiques de l'ontologie MeSH (Medical Subject Headings), cette stratégie ouvre de nouvelles perspectives pour la contextualisation des données expérimentales étudiées au sein des plateformes de métabolomique. Les résultats préliminaires obtenus via la plateforme Big Data Metabolomics Semantic Data Lake ont permis de générer des annotations et des associations pertinentes pour l'identification de biomarqueurs. Actuellement, nous validons l'utilisation de ces connaissances dans trois domaines : la réponse des Brassicacées au stress hydrique et osmotique, la caractérisation des variétés de cannabis riches en THC et en CBD, et l'étude du métabolisme redox des tomates.

Poster 16 - P16

Exploring Metabolic Mysteries: Urine GC/MS Analysis in Inherited Disorders Among Moroccan Patients

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Résumé

The analysis of organic acids in urine is a crucial element in the assessment of the patient suspected to have an inborn error of metabolism (IEM). Urine comprises a diverse array of organic acids, numbering in the hundreds, originating from various sources, encompassing both normal and aberrant metabolic processes. Additionally, these organic acids may stem from the metabolism of drugs. The aim of this study is to highlight the contribution of GC-MS in the assessment of organic acids, which allows accurate rapid urinary chemical analysis after derivatization.

This method allowed us to diagnose 8 patients with tyrosinemia type I , 3 patients with vitamin B-responsive disorders (B1, B8, B12), 2 patients with vitamin B2 deficiency, 2 patients with isovaleric aciduria, others with glutaric aciduria, 3-hydroxyisobutyric aciduria, pyrimidine disorders, and fatty acid oxidation disorders... Urine GC/MS analysis also enables the identification of biomarkers associated with the intestinal microbiota, as observed in three patients, providing an opportunity to connect complex metabolic pathways to the etiology of various diseases.

This diversity of diagnoses demonstrates the effectiveness of the method in detecting a wide range of metabolic disorders, thereby reinforcing its clinical utility for targeted and individualized patient management. Urine GC-MS analysis is a powerful tool for the diagnosis of inherited metabolic diseases. Using this method that provides a rapid diagnosis, enabling affordable and accessible care, thereby preventing the development of irreversible complications and/or morbid decompensations.

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Poster 17 - P17

Understanding the chemical and genetic basis of white mustard resistance to the pollen beetle

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Résumé

Selection of a suitable host plant is essential for phytophagous insects to complete their life cycle. Among plant traits that influence this process, specialized metabolites are widely recognized as key drivers of host plant selection. The pollen beetle (*Brassicogethes aeneus*) is a major pest of oilseed rape (*Brassica napus*), in which no source of resistance has been identified so far. In previous studies, we found that a related species, the white mustard (*Sinapis alba*), shows high levels of resistance to this insect. However, the mechanisms underlying this rejection were still unknown. Since pollen beetles reject *S. alba* after a short walk on flower buds, the involvement of perianth chemistry in the resistance of this plant was explored. Using bio-guided fractionation, several sub-fractions of perianth chemical compounds associated with a deterrent effect on pollen beetle feeding were identified. UPLC-MS analyses of these subfractions revealed several candidate metabolites involved in resistance, for which annotation is in progress. In parallel, two bi-parental segregating were developed for QTL analyses and two genomic regions on chromosomes S06 and S09 were identified . In particular, co-localizing QTLs for pollen beetle resistance were found on chromosomes S06 in each population.Overall, our results provide a better understanding of the mechanisms underlying the rejection of *S. alba* by the pollen beetle. Validation of QTL effects and candidate compounds will give oilseed rape breeders access to strong elements for rapid transfer of this resistance from *S. alba* to oilseed rape.

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Poster 18 - P18

Etude des effets multigénérationnels d'une exposition intra-utérine à faibles doses et faibles débits de doses de Césium 137 par analyses métabolomiques et lipidomiques

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Résumé

L'évaluation des risques sanitaires associés aux expositions *in utero* à faibles doses de rayonnements ionisants (RI) sur la santé des générations futures est cruciale en radioprotection. Ces expositions peuvent influencer le développement fœtal et la santé à long terme. Notre hypothèse scientifique, ancrée dans le concept de la DOHaD, postule qu'une exposition intra-utérine à faible dose de RI, augmentant le stress oxydant, pourrait perturber la programmation épigénétique et fonctionnelle des cellules somatiques et germinales. Ces perturbations pourraient engendrer des modifications métaboliques, accentuant la sensibilité aux stress environnementaux ultérieurs.
Pour explorer cette hypothèse, des souris gravides (C57BL/6J) ont été exposées à 100 mGy de Césium 137 (3.6 MBq.L-1) par ingestion durant la gestation. Après sevrage, leurs descendants ont été nourris avec un régime standard ou riche en lipides, considéré comme une autre source de stress environnemental. Des analyses métabolomiques par LC-MS (OrbitrapTM Q ExactiveTM Plus) en mode positif et négatif, avec une colonne C18 et HILIC, sur le plasma de la progéniture (mâles et femelles, âgées de 10 semaines, N=15) montrent qu'environ 10 % du métabolome plasmatique est impacté par l'exposition intra-utérine aux RI, avec un effet notable du sexe et du régime alimentaire.
L'enrichissement via les bases de données KEGG et SMPDB a révélé que les métabolites discriminants participent à des processus biologiques tels que le stress oxydant, l'inflammation et le métabolisme énergétique. Ces signatures métabolomiques plasmatiques pourraient prédire le risque de maladies multifactorielles. Des analyses approfondies et des études supplémentaires sont en cours d'investigation pour consolider ces résultats.

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Poster 19 - P19

"Antioxidantomics": A Novel Strategy for Identifying Antioxidant Biomarkers in Complex Natural Extracts - Case Study of olive (Olea europaea) Leaf Extract

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Résumé

Natural extracts are rich sources of bioactive compounds with potential applications in cosmetics. However, the inherent complexity of these natural extracts makes identifying specific bioactive constituents challenging. This study presents an innovative "antioxidantomic" approach using an on-line analytical system for selective identification and characterization of antioxidant biomarkers (1) in one olive (*Olea europaea*) leaf extract. This system (on-line hyphenation of high-performance liquid chromatography - diode array detector - electrospray ionization high resolution-mass spectrometry - bio-chemical detector (HPLC-DAD-ESI HRMS-BCD)) allows simultaneous acquisition of the HPLC fingerprint, mass fragmentation data, and real-time antioxidant activity profiles against peroxyl radicals (ROO∘, ORAC assay) within a single chromatographic run.
Bio-chemical detection revealed over twenty antioxidant biomarkers in the olive leaf extract. Leveraging chromatographic behavior, UV spectra, and HRMS/MS data, coupled with literature searches and database comparisons, ten of these biomarkers were identified as phenolic compounds, several confirmed with analytical standards. Further work will focus on characterizing the remaining unidentified biomarkers and developing automated data processing workflows to streamline the correlation of BCD activity peaks with HRMS data for rapid biomarker identification. This on-line "antioxidantomic" strategy offers a powerful tool for efficiently pinpointing bioactive components in complex natural extracts, accelerating the discovery and development of novel cosmetic ingredients.
(1) Development and Validation of an On-Line HPLC-DAD-Antioxidant Assay (ORAC)/ESI-HRMS System to Identify Antioxidant Compounds in Complex Mixtures, L. Paillat and al., J. of Chrom. Sci. Vol. 61 (6), pp. 530-538, 2023

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Poster 20 - P20

Utilisation de la métabolomique non ciblée pour caractériser la transition de stade de Leishmania infantum.

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Résumé

La leishmaniose représente la deuxième maladie parasitaire la plus mortelle dans le monde causant environ 70 000 morts chaque année à travers 97 pays (OMS, 2020). A ce jour, les traitements disponibles sont peu efficaces et induisent un grands nombre d'effets secondaires (Feng, 2022). L'une des formes les plus mortelles de la maladie (leishmaniose viscérale) est causée par *Leishmania infantum*, un parasite qui se développe chez le phlébotome et atteint sa forme infectieuse dans les glandes salivaires. Il est alors transmis sous forme promastigote à l'Homme lors d'un repas sanguin et est phagocyté par les macrophages dans lequel il se transforme en amastigote (Burza, 2018). Le stress oxydant généré par le macrophage est impliqué dans cette transition de stade mais la nature exacte des espèces réactives de l'oxygène en cause reste inconnue. Dans ce contexte, comprendre les mécanismes responsables de la transition de stade parait être un enjeu majeur qui permettrait le développement de nouvelles stratégies thérapeutiques. Des études préliminaires menées au laboratoire ont mis en évidence le rôle potentiel du monoxyde d'azote (NO). Afin de confirmer ces résultats une étude métabolomique non ciblée a été menée sur *L. infantum*. L'objectif était de caractériser le métabolome du parasite sous ses formes promastigote et amastigote et d'évaluer l'effet du radical NO. Nous avons ainsi pu mettre en évidence des biomarqueurs spécifiques de chacun des stades et montrer que le traitement des promastigotes avec un donneur de NO entraine un changement du métabolome du parasite, rapprochant leur profil de celui des amastigotes.

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Poster 21 - P21

Multi-Omics Biomarker Profiling and Network Analysis for Early Diagnosis of Localized Radiation Injury in Mice

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Résumé

Exposure to high-dose irradiation either from an accident, of malicious acts or medical treatment, may lead to skin radiation burnings and underlying tissues damages, potentially leading to Localized Radiation Injury (LRI). LRI severity depends on absorbed dose, exposure duration and irradiated tissue volume and potentially leading to deep ulceration and necrosis through unpredictable inflammatory waves. Therefore, identification of novel non-invasive molecular biomarkers for early diagnostic of LRI would be of great benefit for the patient's medical management.
Biomarker profiling was performed in mice plasma samples (N = 15/group) collected after hindlimb irradiation at different doses (control, 20, 40 and 80 Gy), before symptom apparition, using multi-block sparse Partial Least Square Discriminant Analysis on several multi-omics data blocks to simultaneously discriminate dose groups and estimate the multi-scale correlations between the different data blocks: metabolomics, lipidomics, clinical and physiological parameters.

In addition, to understand the biological role of the identified biomarkers in the aetiology of LRI upon different doses, network analysis of correlations between manual-curated functional groups to which biomarkers can be assigned was performed.
Good predictive power to separate each dose groups from the others was shown for metabolomic or lipidomic data (AUC: 0.77-0.95), while the other blocks were much less performant (AUC:0.50-0.96). Network analysis showed significant decrease in node degree and closeness while node betweenness was higher for each dose groups compared to the control group.
In conclusion: metabolomic and lipidomic data can be used for biomarker prediction and each dose group has a different structured network compared to control.

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Poster 22 - P22

Characterization of two RNA modification enzymes involved in human pathologies

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Résumé

RNA modification enzymes have become of interest due to their roles in maintaining the efficiency, fidelity and regulation of protein synthesis by chemically modifying key factors (eg. tRNA, mRNA, rRNA). In my thesis, we use biochemistry and mass spectrometry (MS)-based approaches to characterize two RNA modification proteins, ALKBH8 and TRMT9B. ALKBH8 is a tRNA modification enzyme associated with intellectual disorder that modifies the wobble uridine. The first aim of my thesis is to study the effect of pathogenic ALKBH8 mutations on enzyme function and stability. TRMT9B is a putative tumor suppressor protein with a strong structural similarity to ALKBH8. Thus, it is proposed to also be an RNA modification enzyme, however, its substrate and therefore function in disease, especially cancer, is still unknown. The second aim is hence to identify the substrate of TRMT9B and the modification it catalyses.
A multi-pronged approach utilizing *in vitro*, *in vivo,* and structural studies is in place to achieve these goals. Biology-wise, techniques used include the development of a methylation activity assay, phenotypic studies on knockout cell lines, and cryo-EM.
In the interest of studying modified RNA for characterization of pathogenic ALKBH8 mutants and potentially the TRMT9B substrate, we are also implementing relevant MS-based approaches including: LC-MS and MS/MS for identification and quantification of modified nucleosides, modification mapping on partially digested RNA samples using MALDI FT-ICR, chemical derivatization to increase sensitivity of uridine detection, and separation and analysis of oligonucleotides by using ion-pairing reagent-free methods.

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Poster 23 - P23

The genetic architecture of the metabolome across several organs illuminates responses to nutritional and pharmacological challenges

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Résumé

Genetic variants disrupting metabolic homeostasis play an important role in the pathogenesis of complex disorders. However, how genetic variants may alter metabolic traits in a tissue-specific manner remain elusive. Here, we deploy a systems genetics strategy to untargeted metabolomics to dissect the tissue-specific genetic architecture of metabolomic traits. Using a rat recombinant inbred panel as a model of human metabolic syndrome, we profiled the metabolome in liver, peritoneal fat, heart, aorta and brain by untargeted ultra-performance liquid chromatography coupled to mass spectrometry generating > 16,764 metabolomic features per tissue, among which 2,072 were replicated across all 5 tissues. Tissue metabolites are highly heritable (H2> 27%), mostly polygenic. We identified 4,131 metabolomic features under genetic control (mQTLs), with > 48% of these being tissue-specific and > 200 mapping to the *Abcb4* transporter locus acting a master regulator of liver and serum phospholipids. Metabolite-phenotype correlation networks predicted the response to high-fat diet and anti-hypertensive drugs, opening avenues in understanding the genetic basis of metabolic flexibility and their application in precision medicine.

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Poster 24 - P24

Profilage métabolique : une approche rapide et innovante pour décrypter le mode d'action des biofongicides

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Résumé

Face aux préoccupations croissantes concernant l'impact des pesticides sur l'environnement et la santé, l'Inulagreen, extrait d'inule visqueuse développé par AkiNaO, émerge comme une alternative prometteuse. Cependant, son mode d'action (MOA) demeure mal compris, un défi commun à de nombreuses biosolutions. La compréhension du MOA est cruciale pour évaluer les risques d'impact sur les organismes non cibles et le développement potentiel de résistances, tout en optimisant son application.
En l'absence de données génomiques sur le champignon phytopathogène, une approche métabolomique multiomique et multiplateforme (MSXM, MetaToul) a été mise en œuvre. Des analyses ciblées ont été réalisées en lipidomique (GC-MS, LC-MS, GC-FID) ainsi que sur les métabolites du cycle énergétique (IC-MS). Parallèlement, une approche non ciblée par UHPLC-HRMS en phase inverse a permis d'examiner le métabolisme central du pathogène. L'objectif est de comparer les profils métaboliques des champignons traités par l'Inulagreen à ceux exposés à des fongicides de synthèse, dont les modes d'action sont référencés, en supposant qu'un même MOA affecte le champignon de manière similaire. Au total, 12 traitements ont été évalués, incluant l'Inulagreen, avec au moins deux fongicides par mode d'action et cinq réplicats biologiques par condition.
Les données ont été analysées par clustering hiérarchique, révélant des similarités entre l'Inulagreen et certains fongicides. Les résultats préliminaires suggèrent que l'Inulagreen perturbe la biosynthèse de l'ergostérol, un composant clé des membranes cellulaires fongiques, et pourrait altérer leur perméabilité. En parallèle, des similarités ont été observées avec un fongicide ciblant la voie de la β-tubuline, suggérant ainsi un mode d'action multisite.

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Poster 25 - P25

Characterization of candelilla resin (Euphorbia cerifera) using a multi-technique approach

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Résumé

Makeup consumers prioritize long-lasting wear without sacrificing comfort and increasingly search for natural ingredients. Currently, only solutions formulated with petrochemical ingredients offer this level of performance.
Candelilla resin (*Euphorbia cerifera*) is one of the natural ingredients identified as a potential replacement for petrochemicals. While effective, its composition remains poorly understood (1,2,3), necessitating updated toxicological assessments.

First, chemical diversity was demonstrated using high-performance thin-layer chromatography (HPTLC). Targeted volatile and semi-volatile organic compounds were studied by gas chromatography coupled with a flame ionization detector and mass spectrometry (GC/FID/MS) to determine the residual presence of solvents, polycyclic aromatic hydrocarbons, and allergens. Inorganic elements as heavy metals were also detected at trace-level concentrations using X-ray fluorescence (XRF).

Two dereplication strategies were used to characterize less volatile organic compounds. The first one using liquid chromatography (LC) and supercritical fluid chromatography (SFC), hyphenated to high-resolution mass spectrometry (HRMS). Annotation of terpenoid compounds were used to propose semi-quantification with charged aerosols detection (CAD). However, precise structural annotation was compromised by in-source fragmentations and absence of referenced spectra in databases. To overcome this issue, an offline coupling of centrifugal partition chromatography (CPC) and nuclear magnetic resonance (NMR) spectroscopy was performed to decipher structures of major triterpene isomers.
This combined approach identified 70-80% of the resin's composition, showcasing the strengths and limitations of each technique and establishing a robust methodology for characterizing natural resins.

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Poster 26 - P26

Expanding the clinical use of DBS: through a metabolomic and a lipidomic evaluation

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Résumé

DBS (Dried Blood Spot) are used for the screening of neonatal diseases and to monitor some adults' diseases in clinical context. DBS is a self-sampling device which can be sent by mail allowing everyone to get access to biological analysis. Their use in new contexts has been widespread: carrying out anti-doping tests or detection of cancer.
In this work, we propose to compare quantitative data and untargeted analysis data between DSB and serum, the blood sample of reference, to assess the potential of DBS.For quantitative data, we quantified 6 short-chain fatty acids, 20 bile acids, 20 tryptophan intermediates and 8 organic acids from TCA cycle by LC-QqQ-MS and around 500 lipids by SFC-QTOF-MS. Untargeted metabolomics and lipidomics have been carried out by a LC-Q-Orbitrap-MS.

Concerning metabolomics, among the quantified compounds (MassLynx) 34 compounds are

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present in both samples, each one allowing to detect 5 unique metabolites. From the untargeted point-of-view, 100 level 1 compounds are presents in both samples and around 50 are specific to one sample (W4M, Xcalibur). For lipidomics, more compounds are quantifiable (MZmine) and detected (W4M, LipidSearch) in serum than in DBS (124 vs.330 and 434 vs. 608 respectively).
DBS seems suitable for the widespread use of metabolomics in a clinical context whereas more development is required in lipidomics. Still, in both, several biomarkers are presents and can be used for detection and monitoring of diseases. These results make it possible to envisage the use of DBS in both quantitative and exploratory metabolomics analyses.

Poster 27 - P27

Unravelling the phytochemical profile of pubescent oak (Quercus pubescens Willd.) for wines and spirits aging.

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Résumé

Most of wood solutions used for aging wines and spirits are made from oak (barrels, staves, chips). The two species traditionally used are sessile oak (*Quercus petraea* Liebl.) for wines and pedunculate oak (*Quercus robur* L.) for spirits aging. The oak forests from Centre-Val de Loire region are renowned for the quality of their trees and staves. However, with climate change, these forests are becoming vulnerable, and other oak species, especially the pubescent oak (*Quercus pubescens* Willd.) reputed to be more resistant to drought, could be subject to assisted migration to conserve the specific biodiversity of the oak grove. We also know that properties of pubescent oak wood for staves are improving upon some populations, and we can now envisage the use of these trees in cooperage, since it is widely recognized that the chemical composition of the wood influences the organoleptic character of wines.

Accessing the phytochemistry of pubescent oak therefore represents a major challenge for predicting changes in the taste and olfactory qualities of wines and spirits as they age. A non-targeted metabolomics approach using UHPLC-Q-TOF-HRMS, combined with multivariate statistical analysis and a HRMS/MS molecular network study, has been carried out to evaluate the chemical composition of pubescent oak, comparing it to pedunculate and sessile oaks.

As preliminary results, multivariate analysis shows that pedunculate and pubescent oak first separate themselves from sessile oak in the same component, then separating themselves in an other component, suggesting there is a more common phytochemistry between *Q.robur* and *Q.pubescens* than with *Q.petraea*.

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Poster 28 - P28

Exploration of Specialized Metabolites in Mucor spp. Using LC-MS/MS Molecular Networking

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Résumé

The genus *Mucor*, part of the order Mucorales, represents a major group of zygosporous fungi, formerly placed in the obsolete phylum Zygomycota, rejected due to its polyphyletic character. As early-diverging fungi, *Mucor* species occupy a basal position in the fungal tree, unlike "higher" fungi (Ascomycota, Basidiomycota). They are among the most primitive terrestrial fungi, with fossils dating back to the Precambrian (800million to 1.4billion years). *Mucor* species show remarkable metabolic and ecological diversity (1,2); some are used in food fermentation, others are opportunistic pathogens (1). Yet, their specialized metabolism remains poorly understood, (few identified metabolites, mainly volatile organic compounds) (3,4). They are the only fungi known to possess St1-type carbohydrate sulfotransferases (personal communication).This study investigates the metabolomic diversity of four *Mucor* strains from plant and insect holobionts. Untargeted LC-MS/MS data were processed using MZmine (v3.6.0) and MetGem (v1.4.1) to build molecular networks and identify structurally related compound clusters (5). This approach supports the annotation of known metabolites, discovery of analogues, and novel structures (6), while revealing strain-specific chemical signatures. New compounds will undergo fragmentation analysis and isolation for structural elucidation. Genomic data will support identification of biosynthetic pathways.This work contributes to the chemodiversity landscape of early-diverging fungi and highlights the utility of molecular networking in natural product discovery.

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Poster 29 - P29

NMRProcFlow: A graphical and interactive tool dedicated to batch processing of 1D spectra for NMR-based metabolomics and qNMR analysis.

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Résumé

NMRProcFlow**1** (nmrprocflow.org) is a web-based tool for processing batches of 1D 1H, 13C and 31P NMR spectra. It was initiated for 1H-NMR-based metabolomics and developed at Bordeaux-Metabolome-Facility in interaction with NMR specialists and the French metabolomics community, and finalized as part of the French MetaboHUB infrastructure. NMRProcFlow covers all the steps involved in processing spectra and preparing quantification data for export without the need for programming skills, in a graphical and interactive way. NMRProcFlow allows you to:

- save and export processing files for replay on a set of similar NMR spectra (processing reproducibility).

- visualise the levels of experimental factors in the set of NMR spectra using a spectral-viewer

- facilitate links between experimental design and subsequent statistical analysis

- facilitate interaction between biologists, chemists and NMR spectroscopists, which is crucial for metabolomics or authenticity studies.

For qNMR, exemplary use-cases concern the analysis of ascorbic acid in acerola-based food supplements**2**, of a range of metabolites in urine**3**, and of pyroglutamic acid in wine**4**.

Since 2017, NMRProcFlow has been widely used by the international NMR-based metabolomics community and beyond with an average of over 4,000 sessions/year on the nmrprocflow.org

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online instance, and 148 citations in the Web-of-Science. It can be used online or downloaded**5**.

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Funding: MetaboHUB_2.0 (ANR-11-INBS-0010), ANR_WAP-NMR (ANR-21-CE21-0014)

Poster 30 - P30

Molecular networking: setting up to address new analytical challenges

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Résumé

With new challenges arising from the novel needs of our stakeholders, L'Oréal R&I is compelled to explore alternative and cutting-edge methods to conduct analyses more efficiently and quickly.
In the context of the big data era, landmark advances in bioinformatics tools have recently enhanced the field of natural products research, putting today's natural product chemists in the enviable position of being able to perform the efficient targeting/discovery of previously undescribed molecules by expediting the prioritization of the isolation workflow. Among these advances, MS/MS molecular networking has appeared as a promising approach to dereplicate complex mixtures, leading to a real revolution in the "art of exploring the unknown from the already known". This new approach was tested internally to investigate a sample of PHMB, a plant-based raw material derived from the mixture of multiple biomasses.

This study aimed to expand the current understanding of known bioactive molecules and identify novel active compounds within a complex matrix. This investigation was conducted in collaboration with Ometalabs, a start-up specialized in the analysis of untargeted tandem mass spectrometry data, enabling the measurement, connection, visualization, and interpretation of complex chemical information.

This poster details the methodology used to construct a molecular network from a PHMB sample. The workflow encompasses several software applications, which are individually assessed. It also details the conception of an internal database used for dereplication purposes. The generated network is visualized, highlighting matches with both internal standards and an external database. Structural similarities observed within the identified molecules are also presented.

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Poster 31 - P31

Cellular Heterogeneity Analysis: An Innovative Approach to Single-Cell Metabolomics

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Résumé

Cellular heterogeneity, arising from variations in gene expression, physiological environment, and cell age, significantly influences the phenotype of cultured cells. A better understanding of this heterogeneity is essential in all fields, from biotechnology to healthcare. Single-cell analysis provides a wealth of information for studying cellular physiology and pathology.Among single-cell "omics" approaches, metabolomics stands out by offering rapid and dynamic insights into cellular functions due to the metabolome's immediate response to environmental changes (seconds or less).
However, single-cell metabolomics faces substantial challenges, including low metabolite levels, their endogenous and exogenous presence, and the inability to amplify metabolites.

Recent advancements in mass spectrometry approaches now enable single cell analysis mainly by miniaturizing cell handling, incubation, and enhancing chip-coupling concepts for analyte ionization through the interfacing of microfluidic chips and mass spectrometers.

Here we propose an innovative miniaturized system that combines microfluidics with nanoESI-MS for high-throughput on-line detection of cellular metabolites. On a specific homemade chip, Suspended cells are isolated from a fermentor by trapping, rinsed on-line to remove exogenous metabolites, lysed during gas-assisted electrospray, and monitored in real-time via MS analysis.

This system is currently being optimized and validated using synthetic cells with a specific lipid composition of PC, PG, and PE. Once validated, it will be applied to more complex cell cultures to study cellular heterogeneity.
We anticipate that this technology will serve as a foundation for more sophisticated designs, with the medium-term goal of coupling metabolomic and genomic analyses of single cells to gain a comprehensive understanding of cellular heterogeneity.

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Poster 32 - P32

Biochemical and Metabolomic Characterization of the Network Controlling Glucosinolate Modifications in Seed Responses to High Temperature

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Résumé

In the context of climate change, understanding how plants cope with abiotic stresses, and more specifically heat stress, is crucial. Specialized metabolites (SMs) play essential roles in response to stress. In particular, glucosinolates (GSLs) are found in Brassicaceae species, including the model species *Arabidopsis thaliana*. While the role of GSLs in responses to herbivores has been widely studied, their potential functions in coping with abiotic stresses have been neglected, particularly in seeds.
SM modifications (or decorations) largely modified the activity and localisation of these compounds. Thioglucose acylated and/or R-chain acylated GSLs were heavily accumulated in developing Arabidopsis seeds subjected to heat stress. Untargeted metabolomic and reverse genetic analyses showed that the acyltransferase SERINE CARBOXYPEPTIDASE-LIKE 17 (SCPL17) and the 2-OXOGLUTARATE-DEPENDENT DIOXYGENASE (AOP3) are involved in the network controlling those modifications. To confirm and further investigate the role(s) and function(s) of SCPL17 and AOP3 in GSL acylation, these enzymes are being produced through heterologous expression in *Escherichia coli* and insect cells.

The production of these proteins will provide more insights into the network controlling GSL modifications in response to heat stress in Arabidopsis seeds. Moreover, it will enable the production of the thioglucose acylated GSLs induced by heat stress, providing the opportunity to investigate their potential role in helping plants cope with such conditions.
Uncovering the function of these novel metabolites could lead to innovative strategies in crop heat stress tolerance management.

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Poster 33 - P33

Multi-omics Integrative approach of the combined effects of biotic and abiotic contaminants on fish

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Résumé

In their natural environments, aquatic animals are exposed to a variety of environmental stressors that are commonly divided into biotic and abiotic ones and it is difficult to establish the relative importance of one or the other on their physiology.
The main objective of the INTERACTION project is to assess the impact of separate or combined biotic and abiotic stresses on the European eel (*Anguilla Anguilla*), a critically endangered species exposed to various types of contaminants in the environments it crosses on migration. The targeted biotic contaminant is the parasitic nematode *Anguillicola crassus*, which is known to significantly compromise migration1. The abiotic contaminant is Bisphenol S, an emerging pollutant whose effects on aquatic organisms are of growing concern2. Using a holistic untargeted multi-omics approach (transcriptomics, proteomics, metabolomics, microbiomics, histo-peptidomics MALDI imaging) we will test the hypothesis of a negative synergistic effect of these two types of stressors. For that, we will i) highlight the main biological pathways involved in response to these 2 contaminants (individually or in combination) and ii) develop biomarkers in skin mucus of the eel to assess its health status using noninvasive methods. Global experimental design and analytical workflow, Bisphenol S quantification by LC-MS and eel mucus metabolomics optimizations will be presented here.

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Poster 34 - P34

Analyse métabolomique de la cicatrisation cutanée comparée entre des modèles in vitro et in vivo

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Résumé

Dans le cadre de l'étude des processus de cicatrisation cutanée, une approche métabolomique LC-HRMS a été mise en œuvre afin de comparer les profils métaboliques issus de deux modèles expérimentaux : un modèle *in vitro* basé sur des explants de peaux humaines lésées et cultivées dans des conditions contrôlées, et un modèle *in vivo* reposant sur des prélèvements cutanés humains réalisés à différents temps physiologiques de la cicatrisation.
Les écouvillons issus des prélèvements de surface des échantillons et les explants ont été extraits, puis les phases aqueuses ont été analysées par UHPLC (I-Class, Waters) couplée à un spectromètre SYNAPT G2-Si (Waters) par electrospray. Le traitement des données a été effectué avec XCMS (Workflow4Metabolomics), avec alignement, filtrage des blancs et des QC. Après analyses statistiques multivariées, les variables discriminantes présentant un VIP> 1 et une p-value < 0.05 ont été annotées grâce à CAMERA et notre base de données interne de temps de rétention. Les métabolites annotés ont été identifiés sur un spectromètre LTQ-Orbitrap XL (Thermo Scientific) à l'aide de notre base de données interne MS/MS et des bases externes.
Suite aux analyses métabolomiques, environ 3500 variables ont été obtenues en modes positif et négatif après nettoyage des données. Les analyses statistiques ont permis l'obtention de modèles statistiques valides et robustes. L'annotation des variables discriminantes a conduit à l'identification de 56 métabolites à partir du modèle *in vitro*, et 35 à partir des prélèvements cutanés *in vivo*.

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Poster 35 - P35

Dereplication of chloronorlichexanthones in lichen extracts : ASAP-MS/MS and Multivariate Analysis

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Résumé

Chloronorlichexanthones were obtained by synthesis, using orsellinic acid and phloroglucinol as major building blocks. Four monochloronorlichexanthones were analyzed by ASAP MS and MS/MS. Small R-script based algorithms were used to process the MS files. Their homogeneity as replicates and the relevancy of their discrimination power as single samples were evaluated with MetaboAnalyst 6.0 online platform. After few steps, the discrimination method was powerful enough to start with real sample application. The final optimized protocol was applied on two cases, extracted and raw material, from respectively *L. alboflavida* and *M. antiqua* lichen. The discrimination process allowed the recognition of the isomers of monochloroxanthones by their MS/MS spectrum in negative ionization mode.

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Poster 36 - P36

The Metafollow project: One-year longitudinal follow-up to assess intra-individual metabolites variations in healthy subjects

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Résumé

In healthcare, most metabolomics studies focus on pathologies by studying inter-individual and/or inter-group variations in metabolites concentrations. However, patient care paradigm is currently changing towards a more personalized approach. Thus, to integrate metabolomics into personalized medicine, understanding normal intra-individual metabolite variations is crucial to identify deviations that may indicate early pathological changes. Currently, our knowledge of these "normal" variations remains limited. To bridge this gap, it's essential to first study the metabolome of healthy individuals over a defined period.

For this purpose, we conducted a one-year longitudinal study involving 30 healthy volunteers. Blood, urine, and saliva samples were collected weekly for the first ten weeks, followed by monthly collections for ten months, according to the recommendations of the EuBIVAS (EFLM Working Group on Biological Variation). The analysis was first focused on blood samples and as metabolites quantification is absolutely required here, NMR appeared to be the most useful analytical tool. Over the ten-week period, metabolites were classified based on their variability, ranging from the least to the most fluctuating. A similar classification pattern emerged from the monthly samples, confirming the consistency of metabolite variability over time. Additionally, we applied an innovative approach, Metabolomic Informative Content (MIC), to stratify individuals based on the variation in their metabolome.

Our preliminary results enable the stratification of blood metabolites according to their short- and long-term variability. Ongoing urine and saliva analyses will further refine our understanding of normal metabolome variations, providing a more comprehensive foundation for the future application of metabolomics in personalized medicine.

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Poster 37 - P37

Explorer la chimio-diversité des Streptomyces : une voie vers la découverte de nouveaux antibiotiques

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Résumé

Les actinomycètes marins présentent une grande diversité chimique et constituent une source importante d'antibiotiques, comme la tétracycline. Une mini-revue de 2025 rapporte la découverte de 45 nouveaux composés antibactériens issus d'actinomycètes marins en 2024. Ces molécules, appartenant à différentes classes (polykétides, alcaloïdes, macrolactames et peptides), ont été étudiées pour leurs structures et leurs activités biologiques.
Dans cette étude, nous avons analysé quatre souches d'actinomycètes isolées de lichens marins, dont les séquences d'ARNr 16S étaient identiques à *Streptomyces fulvorubeus*. Les extraits bruts de ces souches ont montré une forte activité antibiotique contre SARM. Les formules moléculaires des composés majeurs ont été déterminées par LC-MS, puis annotées grâce à des bases de données telles que LOTUS, Chemnetbase, MoNa et SIRIUS.

Malgré leur proximité génétique, ces souches présentent des profils métaboliques très variés. Une analyse par réseau moléculaire (FBMN sur la plateforme GNPS) suivi de l'étude des fragmentations des composés majoritaires des fractions actives a permis d'annoter et de propager les annotations des 20 métabolites, répartis en trois familles chimiques : depsipeptides, bipyridines et macrolactames à acide tétramique polycyclique (PTMs). Bien que deux tiers des composés aient déjà été décrits, certains analogues pourraient être inédits.
Ce travail montre que le genre Streptomyces est le plus grand producteur bactérien de métabolites secondaires aux propriétés bioactives et souligne l'intérêt de l'analyse par réseau moléculaire pour l'exploration de la chimio-diversité.

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Poster 38 - P38

Uncovering the hidden ecological roles of specialized metabolites in rapeseed seed exudates

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Résumé

Biocontrol strategies are being developed to identify biobased alternatives to synthetic pesticides for seed treatment. The spermosphere is the microenvironment surrounding germinating seeds and plays a crucial role in this context. It consists of primary and specialized metabolites, seed-exuded molecules, and the microbial communities they attract.
In this study, we characterized the seed spermosphere of ten rapeseed genotypes grown under two different crop management systems using LC-MS/MS untargeted metabolomics. Glucosinolates (GSLs) were among the most abundant metabolites detected in seed spermosphere and showed significant variation among genotypes. While the role of GSLs in plant herbivore interactions is well established, their ecological function in seeds and seed exudates remains poorly understood.

We mapped all identified GSLs and their degradation products (isothiocyanates) to their biosynthetic pathways, and quantified their concentrations in both seeds and exudates. Subsequent bioassays with exudates from rapeseed accessions with contrasting GSLs contents on several seed pathogens (bacteria and fungi) revealed that the high-GSL-concentration genotype exhibited the strongest inhibitory effect against some seed pathogen. Several GSL and isothiocyanate compounds are being tested for their specific inhibitory effects on pathogen growth. Furthemore, multi-omic analyses are underway to study genes and enzymes involved in GSL biosynthesis, transport, degradation and decoration during seed exudation.
These data uncovered a large metabolic diversity in rapeseed spermosphere and highlighted a potential ecological role for GSLs in seed-pathogen interactions during germination.

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Poster 39 - P39

Semi-targeted metabolomics on metabolites of P. aeruginosa to evaluate the mechanism of action of new anti-virulence agents

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Résumé

The spread of multidrug-resistant bacteria, especially *Pseudomonas aeruginosa*, is a major public health issue. Its ability to produce biofilms grants it protection against the host immune response and antibiotics, leading to chronic infections. Some virulence pathways of this pathogen are regulated by the quorum sensing (QS), a bacterial communication system, which appears as pharmaceutical targets of choice. The aim is to target the QS with molecules that improve antibiotics efficiency without generating additional selection pressure. Following encouraging initial results with an indazole-quinolole hybrid as anti-virulence agents (AVAs) (Hanot and al., 2024), new hybrids targeting QS, have been synthetized and their mechanism of action have been studied by semi-targeted metabolomics.
Colonies of *P. aeruginosa* were cultivated on 96-well plates with five AVAs. After ultracentrifugation, the amount of biofilm was estimated by optical density and the supernatants were analyzed by LC-MS in ESI+ and ESI- modes on an UPLC-QtoF after a simple protein precipitation. Data preprocessing was performed using MassHunter – Quantitative Analysis. Univariate analysis were conducted to compare the different AVAs.
Confirmed by chemical standards, nine metabolites, signaling molecules and secondary metabolites linked to *pqs, las* or *rhl* systems or virulence factors, were identified. Two AVAs have shown interesting inhibitory proprieties on the secretion of signaling molecules of *pqs, las* and *rhl* systems (PQS, BHL and odDHL). Moreover, they led to a strong inhibition of pyoverdine production. Thus, the bacteria could be weakened, making it more vulnerable to antibiotics.

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Poster 40 - P40

Dual Workflow Integration: RPLC-HRMS/MS and SFC-HRMS/MS for comparative metabolomic profiling of Trichoderma reesei

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Résumé

Trichoderma reesei is a filamentous fungus extensively studied for its cellulase enzymes production. Nonetheless, its specialized metabolism remains underexplored and could also be industrially exploited. Specialized metabolites are produced in response to environmental stresses and offer valuable bioactive potentials. Untargeted metabolomics traditionally relies on reversed-phase liquid chromatography coupled to high-resolution tandem mass spectrometry (RPLC-HRMS/MS), which provides broad coverage and sensitivity, yet has limitations in separating highly polar metabolites using water/acetonitrile as an eluent.

To address this gap, supercritical fluid chromatography (SFC) coupled with HRMS/MS can be considered as a complementary approach with orthogonal selectivity, efficient separation of nonpolar compounds, and reduced solvent consumption.

The ethyl acetate/butanol extracts from the cellulase hyperproducer strain RUT-C30 were analyzed through both workflows (RPLC and SFC), and their datasets exhaustively compared via molecular networking using MetGem (1). Common *Trichoderma* metabolite families, including sorbicillinoids, peptaibols, coumarins, and previously unreported structures, were successfully detected by both approaches. However, significant differences emerged, notably with SFC-HRMS/MS revealing unique clusters composed of highly nonpolar metabolites such as triglycerides, not detectable by RPLC. Conversely, some polar compounds showed weaker separation in SFC compared to RPLC, highlighting distinct selectivity.

The comparison highlights the significant complementarity between RPLC and SFC. Their distinct selectivities make it possible to cover a wider chemical space, allowing a more exhaustive exploration of the metabolome from this complex matrix. Combining both approaches could be a key for more comprehensive metabolic profiling.
(1) Olivon F *et al.*, Analytical Chemistry, 2018, 90, 23, 13900-13908.

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Poster 41 - P41

Advancing diagnostics in inherited metabolic diseases through enhanced metabolic modeling of lipid metabolism

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Résumé

Accurate modeling of lipid metabolism is essential for understanding cellular function and predicting metabolic phenotypes, particularly in the context of inherited metabolic diseases (IMDs). As part of the Horizon Europe Recon4IMD project, we focus on expanding and refining the representation of lipid metabolism to support predictive metabolic modeling using frameworks like COBRA and genome-scale models such as Recon3.
We systematically enumerated over 2.2 million lipid-specific reactions by leveraging the SwissLipids and Rhea databases. These reactions were annotated with RInChIKeys and structured for integration into MetaNetX, ensuring compatibility with established computational modeling pipelines. This comprehensive dataset enables the enrichment of existing metabolic networks with detailed lipid biochemistry, supporting simulations of lipid fluxes under physiological and pathological conditions.

To facilitate access and reuse, we developed pyrheadb, a Python package that translates Rhea's biochemical knowledge into structured reaction networks and metabolic models, now openly available at GitHub. Using this tool, we applied reaction templates to fill 62 previously disconnected gaps in lipid metabolism, generating over 1,300 candidate reactions for curation and integration.

Further, we extended this approach to stereospecific reactions, generating over 2,000 stereochemically detailed variants using atom mapping. For complex glycosphingolipids, we processed glycan-based structures from the SphinGOMAP resource, creating 2,000 stoichiometrically balanced reactions from stepwise sugar elongation.

By improving chemical specificity and expanding reaction coverage, our work enables more robust, lipid-inclusive simulations within COBRA-based frameworks, advancing metabolic model precision and predictive capacity for lipid-related disorders.

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Poster 42 - P42

An overview of metabolites identified in Leucojum aestivum (Amaryllidaceae) bulbs and in vitro bulblets using LC-MS and GC-MS

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Résumé

Leucojum aestivum, a bulbous medicinal plant belonging to the Amaryllidaceae family, produced numerous alkaloids known to exhibit a wide range of biological activities such as Galanthamine, an acetylcholinesterase inhibitor used for the treatment of Alzheimer's disease and lycorine at the clinical trial stage for its anticancer properties. Our research works explored the possibilities of producing these alkaloids using biotechnological methods, including plant and endophytes *in vitro* cultures. For that purpose, HPLC coupled to a mass spectrometer (MS) was used for the analysis of galanthamine and lycorine in bulb extracts of *L. aestivum* and in their *in vitro* cultures. GCMS was used to investigate underivatized alkaloid mixtures from these extracts. Endophytic bacteria belonging to the Bacillus genus and a strain of *Paenibacillus lautus* were isolated from *in vitro* bulblets of this plant. Their ability to produce Amaryllidaceae alkaloids was studied and the phytochemical analysis using LC-MS and GC-MS revealed the presence of five and ten Amaryllidaceae alkaloids, respectively. In addition, Proton Nuclear Magnetic Resonance (1H NMR)-based metabolomics combined with multivariate data analysis was chosen to compare the metabolism of *L. aestivum in vivo* bulbs, and *in vitro* bulblets with those of the endophytic bacteria community. Primary metabolites were quantified by quantitative 1H NMR (qNMR) method. The results showed that tyrosine, one precursor of the Amaryllidaceae alkaloid biosynthesis pathway, was higher in endophytic extract compared to plant extract.

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Poster 43 - P43

Unraveling the impact of microbiota xenobiotic interactions on the mosquito metabolome through clustering-based analyses

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Résumé

Urbanization is reshaping ecosystems exposing organisms to a wide range of anthropogenic chemicals. These xenobiotics can affect physiology, behavior, and ecological interactions. While some species adapt genetically over time, others may also benefit from functions carried by their microbiota *(i.e*, host-associated microorganism). The Asian tiger mosquito,

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Aedes albopictus, particularly during its aquatic life stage, is directly exposed to xenobiotics in urban environments. Previous studies suggest that such exposure can induce developmental costs. Here, we investigated how glyphosate, melamine or metformin, at both high and environmental doses, affect mosquito metabolism, and how the microbiota modulates this response across larval development. To achieve a comprehensive metabolic profiling, we employed a combination of complementary metabolomics techniques, including GC-MS, LC-FLUO and LC-MS-QTOF. Our results show that mosquito metabolic profiles are mainly shaped by the presence of a microbiota as well as the developmental stage, whereas xenobiotic exposure alone induces marginal shifts. However, integrative modeling and clustering analyses revealed distinct metabolic clusters and biomarkers in response to microbiota-xenobiotics interactions *in insecta*. Metabolite annotation and pathway mapping further provided insight into key metabolic pathways involved. These findings highlight the central role of microbiota in modulating mosquito metabolism, notably under chemical stress and suggest that metabolic plasticity may contribute to the adaptive success of *Ae. albopictus* in anthropized environments. Developing new analytical strategies enhances our understanding of complex interactions and paves the way for novel experimental designs to address emerging biological questions.

Poster 44 - P44

Effets d'un stress métallique au cuivre sur une plante modèle, l'inule visqueuse

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Résumé

Pour lutter contre le mildiou de la vigne, les viticulteurs utilisent de la bouillie bordelaise où le principe actif est le sulfate de cuivre. Cela a causé une contamination en cuivre des sols de vignes ce qui peut causer des perturbations chez la plante. Une présence massive d'inule visqueuse (*Dittrichia Viscosa*) a été observée dans des friches agricoles dont le sol renferme des concentrations élevées en cuivre. L'objectif de mon projet est de caractériser le stress causé par le cuivre sur cette plante, l'inule visqueuse, par une approche métabolomique non-ciblée. Sur la base de profils métabolomiques, une optimisation de l'extraction des métabolites des feuilles de l'inule a été réalisée grâce à un plan d'expérience Box-Behnken. Ensuite, 15 plants d'inule visqueuse ont été cultivés dans un phytotron avec des sols non dopés (5 plants), dopés avec 50 mg/kg de cuivre biodisponible (5 plants) et dopés avec 500 mg/kg de cuivre biodisponible (5 plants). Après 2 mois dans le phytotron, des feuilles ont été prélevées et des analyses en UHPLC-HRMS ont été réalisées sur ces échantillons. Des analyses multivariées ont été appliquées pour mettre en évidence une séparation des échantillons suivant la dose de cuivre. Des variables discriminantes ont été recensées et des propositions d'identification de molécules appartenant aux familles des flavonoïdes, des sesquiterpènes ou des dérivés d'acides quiniques de ces variables ont été faites. Notre hypothèse est que ces familles de molécules possèdent une activité antioxydante qui permettrait de réduire le stress oxydatif causé par le cuivre.

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Poster 45 - P45

Combining Mass Spectrometry and Nuclear Magnetic Resonance for the Annotation of Metabolites in Pea Root Exudates

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Résumé

Plants release large amounts of exudates containing a wide diversity of metabolites into the soil. These compounds vary greatly both in quantity and quality depending on plant development (species and growth stage) and environmental conditions (biotic and abiotic stresses). In addition, they are involved in a wide range of interactions at the plant-soil interface, particularly between the plant and its rhizospheric environment.
They serve as essential carbon sources for soil microorganisms, promote the recruitment of beneficial microbes, and play key roles in root defense against pathogens and environmental stresses (e.g., drought) by modulating microbial communities and influencing physicochemical processes (such as pH).
Given their crucial role in shaping rhizosphere properties, it is essential to characterize the precise composition and function of root exudates. Our previous studies have independently used nuclear magnetic resonance (NMR) and mass spectrometry (MS) coupled with liquid chromatography (LC) to identify metabolites in pea root exudates. However, challenges such as isomeric complexity or low ionization efficiency in MS, and the low abundance of some compounds in NMR, have limited compound annotation. In this context, cross-comparing MS and NMR datasets can enhance compound identification and improve annotation confidence.
To address this, we have developed a protocol for sample preparation compatible with both analytical techniques. Preliminary results demonstrate that the combination of MS and NMR enables easier compound identification at confidence level 2. Furthermore, each technique allowed the detection of distinct sets of metabolites, underlining their complementarity and reinforcing the value of this combined analytical approach.

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Poster 46 - P46

Optimisation des effets santé des bioactifs du colza : de la graine à l'assiette

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Résumé

Le tourteau de colza, coproduit riche en protéines issu de la trituration des graines, contient également des composés bioactifs d'intérêt tels que les polyphénols et les glucosinolates. Ce projet vise à valoriser ces molécules dans une approche intégrée " du champ à l'assiette ", en optimisant leur présence et leur impact santé.
Deux variétés de colza (Mambo et Bonanza), aux profils antioxydants contrastés, ont été transformées en tourteaux selon deux procédés : l'un classique, avec cuisson à haute température (méthode conventionnelle), et l'autre plus respectueux des composés thermosensibles, permettant de mieux préserver la qualité des bioactifs. Ces tourteaux ont été incorporés à la ration de vaches laitières. Le lait produit a ensuite été administré à leurs veaux et à des souris ob/ob, modèle de stress oxydatif. Un autre groupe de souris a reçu directement des extraits de tourteaux.

Pour comparer les effets antioxydants des bioactifs natifs (extraits) versus ceux biotransformés (dans le lait), les plasmas des vaches, veaux et souris ont été analysés par métabolomique ciblée (UHPLC-MS/MS). Des analyses statistiques multivariées ont permis d'identifier les métabolites discriminants entre les groupes. Le lait a également été analysé pour évaluer sa qualité nutritionnelle et antioxydante selon l'origine des tourteaux.
Les premiers résultats suggèrent que le procédé de fabrication influence fortement la disponibilité et l'activité des bioactifs dans le lait. La biotransformation via l'animal pourrait renforcer certains effets bénéfiques, soulignant l'intérêt d'optimiser l'ensemble de la chaîne de production pour valoriser le tourteau de colza comme vecteur de santé.

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Poster 47 - P47

Real-time online monitoring of metabolites in biological processes using NMR

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Résumé

Metabolomics is widely used to provide a detailed picture of the cell growth process through metabolic profile analysis of microorganisms. However, reliable real-time monitoring of metabolites is still a challenge during industrial production, and new methods are urgently needed to accelerate the development of bioproducts and to avoid batch losses.
In this study, we evaluate the feasibility of quantitative NMR real-time monitoring of metabolite kinetics from a small-scale lactic fermentation of *Lactobacillus lactis* (Dynamic approach). Sequential aliquots were also collected at different time points of the fermentation and analyzed using end-point NMR (Pseudo-dynamic approach). NMR experiments were run on an Ascend 600 MHz spectrometer equipped with a 5 mm QCI cryoprobe using the insightMR flow tube unit (Bruker Biospin). Acquisition parameters and flow rate were optimized to comply with quantitative experiments.

Over 30 metabolites from 13 chemical classes were quantified within the pseudo-dynamic approach, with a concentration range between 0.1 and 60 mM. Metabolite quantification from NMR spectra recorded during the dynamic approach required further optimization to overcome resolution issues due to the nature of the medium and chemical shift deviations. A total of 11 metabolites were quantified with a good R2 (> 0.7). Efforts to improve metabolite recovery between the two approaches are ongoing.
This method provides a standardized and sensitive methodology for studying metabolites kinetics during lactic fermentation. NMR real-time monitoring using insightMR flow tube unit offers new insights into metabolite dynamics to better understand both microbial physiology and bioprocesses paving the way to 'precision fermentation' for industrial purposes.

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Poster 48 - P48

FragHub: A Mass Spectral Library Data Integration Workflow

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Résumé

https://pubs.acs.org/doi/full/10.1021/acs.analchem.4c02219 Open mass spectral libraries (OMSLs) are critical for metabolite annotation and machine learning, especially given the rising volume of untargeted metabolomic studies and the development of annotation pipelines. Despite their importance, the practical application of OMSLs is hampered by the lack of standardized file formats, metadata fields, and supporting ontology. Current libraries, often restricted to specific topics or matrices, such as natural products, lipids, or the human metabolome, may limit the discovery potential of untargeted studies. The goal of FragHub is to provide users with the capability to integrate various OMSLs into a single unified format, thereby enhancing the annotation accuracy and reliability. FragHub addresses these challenges by integrating multiple OMSLs into a single comprehensive database, supporting various data formats, and harmonizing metadata. It also proposes some generic filters for the mass spectrum using a graphical user interface. Additionally, a workflow to generate in-house libraries compatible with FragHub is proposed. FragHub dynamically segregates libraries based on ionization modes and chromatography techniques. It also automatically fills in missing chemical compound ontologies and intelligently completes instrument information using a decision tree, thereby enhancing data utility in metabolomic research. The FragHub Python code is publicly available under a CC-BY-NC 4.0 license, at the following repository: https://github.com/eMetaboHUB/FragHub. FragHub offers ease of use with fully integrated Python executables for Windows, Linux, and macOS. Generated data can be accessed at 0.5281/zenodo.14761050.

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Poster 49 - P49

High-Throughput Targeted Metabolomics Library Generation on a Novel Mass Spectrometer Applied to Microbiome Analysis

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Résumé

Purpose: The main objective of this poster is to showcase the capabilities of the Thermo Scientific™ Stellar™ mass spectrometer in developing and implementing a comprehensive LC-MS library for high-throughput, sensitive, and selective quantitation of fecal metabolites.
Methods: Three LC columns were used to separate compounds before analysis on the Thermo Scientific Stellar instrument with the Thermo Scientific™ Vanquish™ Horizon UHPLC system: Hypersil GOLD™ C18 reversed-phase, Accucore™-150-Amide-HILIC, and Acclaim™ Trinity™ P2.
Results: This study demonstrated the successful development and implementation of a fecal metabolites LC-MS library using the Stellar mass spectrometer, highlighting the instrument's capability for high-throughput quantitation analysis without compromising data quality.

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Poster 50 - P50

Age-related metabolic reprogramming in the African turquoise killifish (Nothobranchius furzeri)

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Résumé

While human lifespan is increasing, the extended longevity is accompanied by a growing incidence of age-associated diseases, often driven by metabolic dysregulation. Yet, the organ-specific evolution of metabolism during ageing remains poorly characterized. The African turquoise killifish (*Nothobranchius furzeri,* referenced thereafter as ATK), a short-lived vertebrate model, offers a unique opportunity to study systemic ageing processes. Here, we investigate age-related metabolic reprogramming in this model using in vivo stable isotope tracing with uniformly labeled 13C-glucose and targeted quantitative metabolomics.
Experiments were conducted in young and old adult male ATK, with glucose administered via water infusion and intraperitoneal injection. Mass spectrometry-based analysis of 13C incorporation enabled mapping of glucose fluxes in plasma and various organs (visceral adipose tissue, liver, muscle, brain). Metabolite profiling revealed distinct age-related shifts in both content and utilization across tissues. Despite age, a consistently low reliance on glucose metabolism was observed, suggesting alternative substrates such as amino acids and fatty acids may play a dominant role.
This work establishes a methodological framework for metabolic studies in ATK and provides insights into age- and tissue-specific metabolic remodeling. These findings contribute to the understanding of age-related evolution of metabolism in this emerging model of vertebrate ageing.

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Poster 51 - P51

Rôle de la médiation chimique entre Euphydryas desfontainii et Cephalaria leucantha

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Résumé

Le Damier de Godart, *Euphydryas desfontainii* (Godart, 1819), est un papillon de jour protégé et en régression. Cela a conduit à son classement dans la catégorie " En Danger Critique " lors de son évaluation dans la récente liste rouge des Lépidoptères Rhopalocères et Zygènes d'Occitanie. L'espèce est extrêmement localisée en France avec seulement 3 stations connues à ce jour, toutes situées dans les Pyrénées-Orientales et l'Aude. *E. desfontainii* utilise en France la Céphalaire blanche *Cephalaria leucantha* (L.) Schrad. ex Roem. & Schult., 1818 comme unique plante-hôte lors de son cycle larvaire, tandis qu'à l'état imaginal c'est le Thym *Thymus vulgaris* L., 1753, qui fait office de plante nourricière principale. La présence et la survie de l'espèce sur un site sont donc étroitement conditionnées par la présence et l'abondance de ces 2 plantes.
Par une approche métabolomique et transcriptomique, nous allons tenter de répondre à deux questions qui pourraient, *in fine*, orienter des actions de conservation de la dernière population de Damier de Godart en France.

Quel est le rôle de la médiation chimique dans la relation entre *E. desfontainii*, sa plante hôte (*C. leucantha*) et sa plante nourricière (*T. vulgaris)* ?
Le stress lié au pâturage impacte-t-il cette communication en stimulant la production de composés chimiques susceptibles de favoriser la ponte du Damier de Godart sur sa plante hôte ?

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Poster 52 - P52

Unlocking Workflow for Untargeted Fluxomics

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Résumé

Stable isotope tracer studies are increasingly applied to explore metabolism by analyzing tracer incorporation into metabolites. Recent advances in untargeted LC/MS approaches for isotopic studies have emerged and offer potent methods for expanding the dimension and complexity of the metabolic networks that can be investigated. However, the full potential of untargeted approaches in isotopic studies remains underexploited due to the intricate nature of isotopic data and the lack of comprehensive, robust workflows and tools for effective data processing and interpretation. To address these limitations, we present the development of an integrated and transversal pipeline for untargeted fluxomics, covering all stages from sample preparation to data contextualization. This pipeline is designed to ensure automation, interoperability, and optimization of isotopic profiling approaches using mass spectrometry (MS) and nuclear magnetic resonance (NMR). A central component is the development of a software (IsoGroup), designed as a modular tool to improve isotopic clustering in MS data. By combining automated processes with advanced computational tools, our workflow enhances the reproducibility, efficiency, and interpretability in untargeted fluxomics, facilitating the extraction of meaningful insights from complex isotopic datasets. This evolving, integrative workflow aims to lower technical barriers and promote the broader adoption of untargeted isotopic studies in systems biology.

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Poster 53 - P53

STUDY OF THE BIOACCUMULATION IN THE AMPHIPOD HYALELLA AZTECA BY LC-MS/MS AND MASS SPECTROMETRY IMAGING.

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Résumé

Studies have shown that the presence of pollutants released into the environment by human activity creates an imbalance in the ecosystem and poses a real risk to human health. Thus, regulatory institutes such as REACH are increasingly challenging manufacturers to assess the impact of their products through environmental studies, such as bioconcentration assessment.
Bioconcentration is the absorption of a contaminant and its accumulation in the tissues of organisms following direct contact with the surrounding environment. Since 2012, the OECD guideline 305 was released to guide companies in their aquatic environmental approach, but the test is described to be carried out on fishes, considered as laboratory animals which hampered its use for cosmetic industry.

The Fraunhofer Institute has developed an alternative test based on non laboratoy animal model, i.e. (Hyalella azteca), an amphipod crustacean (bioindicator). Since 2024, this protocol has been recognized by the OECD under guideline 321.

This study presents the analytical approach from development to validation of studying fungicides, as a reference compounds. A specific LC-MS/MS method was developed to monitor the concentration of the substances in the organism and in the aquatic environment to evaluate the bioconcentration factor (BCF).
Currently, research is underway to refine these environmental assessments using a mass spectrometry imaging (MSI) approach. The objective is to determine the distribution of parent compounds and their biotransformation products within the organism. MALDI-FTICR MSI method has been developed to locate the reference substance in Hyalella azteca organisms and also to locate lipid tissues where the substance is bioaccumulated.

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Poster 54 - P54

Developing a novel MS imaging technique to decipher redox metabolism in tomato flower.

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Résumé

Redox metabolites play a vital role in regulating signalling pathways essential for both developmental and environmental responses in plants. These responses encompass critical processes such as the development of reproductive organs, including pollen formation in flowers and early fruit growth. Preliminary transcriptomic and metabolomic findings suggest that a precise balance between reactive oxygen species and antioxidants is necessary for tomato flower fertility. For a better understanding of redox mechanisms, it is essential to map the spatial distribution of these compounds within the flower.

We developed mass spectrometry (MS) imaging methods using MALDI and DESI techniques, which are typically suited for large molecules like proteins and lipids. However, analysing small polar compounds such as redox metabolites presented challenges. We optimised the MS imaging workflow, from sample preparation, including matrix crystallisation, to MS analyses. Key challenges included the ionisation and detection of small analytes and their diffusion due to polarity. We thus compared different matrices (9AA, DHB, CHCA), laser frequencies and intensities and optimised MALDI source parameters to address ionisation issues. To minimise metabolite diffusion, we tested various embedding media (CMC, gelatin), thaw-mounting, and double-sided tape for section adhesion.

These methodological advancements enabled the differential spatial mapping of central and specialised antioxidants, such as ascorbic and chlorogenic acids, in heat-stressed tomato flowers. Future work will compare DESI with MALDI MS imaging. Overall, this method development will facilitate redox studies in plants, particularly under environmental stresses like temperature waves, which significantly impact fruit production, in tomato and fruit trees particularly.

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Poster 55 - P55

A multimodal study using mass spectrometry imaging to explore age-related metabolic changes in a fish model

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Résumé

Zebrafish is a valuable model in aging research due to its metabolic and genetic similarity to humans. This study aims to investigate the metabolic profile of zebrafish at different ages through an integrated metabolomics approach using HPLC-HRMS/MS and Mass Spectrometry Imaging (MSI) in order to understand the metabolic alterations associated with age and age-related diseases. For this purpose, Zebrafish samples were collected at different stages of development and analyzed using HILIC-HRMS and DESI-MSI systems. Metabolic analyses were associated with histological and microscopic analyses to provide an in-depth exploration of zebrafish metabolic profiles. Our multimodal approach revealed distinct metabolic profiles in the different tissues and developmental stages of zebrafish. HPLC-HRMS/MS experiments allowed the annotation of different classes and molecular species in zebrafish. Comparative analyses between young and old individuals highlighted significant changes in key metabolic pathways, especially those related to energy metabolism. Taurine was found to be more abundant in old zebrafish, while lactate, glucose, glycerol-3-phosphate and some amino acids were more abundant in the young animals. The metabolic profiles of the embryos were also studied and showed differences in abundance compared to adult zebrafish. DESI-MSI revealed specific spatial distributions of amino acids, organic acids, lipids, and other small molecules in the different tissues. Histological and microscopic studies allowed to correlate the spatial distribution of metabolites with tissue structure. The multimodal approach enabled the identification of age-related changes in metabolic pathways, particularly in energy metabolism, providing potential biomarkers for further investigation.

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Poster 56 - P56

Real-time metabolomic on E.coli cell cultures

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Résumé

Fermentation monitoring is a powerful tool for bioprocess development, optimisation and control. Several parameters can be tracked including temperature, dissolved oxygen, pH, metabolites, etc... As metabolites represent the final step of biological regulation, they are key indicators. Metabolic changes can occur very rapidly, and traditional metabolomic analyses are time-consuming. Therefore, online high-resolution time course analysis is required. To achieve this, we adapt a real-time metabolomic workflow (Cortada-Garcia, et al., 2022). As a proof of concept, we monitored metabolites from an *E.coli* culture and the incorporation of 13C after a spike of 13C glucose. A set of modules, including culture pump, solvent pumps, 6 and 10 ports valves and filtration valve, links the bioreactor to the mass spectrometer. A workflow manager, developed in Python, was used to pilot fluidic system and launch analysis sequences. The *E. coli* culture was analysed every minute using flow injection mass spectrometry at resolution of 90 000 for m/z = 200 (Exploris 240, Thermo Scientific). An integrated solution for real-time data processing, enabling direct monitoring of the bioreactor is currently in progress. The system will be capable to measure, identify and quantify metabolites, visualize the results and modulate the fermentation process if needed.
The next steps of this development involve improving metabolome coverage by MS, implementing metabolite quantification with internal standard or by benchtop NMR in series. This approach should be transposable to other types of culture, such as adherent cells.

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Poster 57 - P57

New LC-HRMS Methods for Quantification and Isotopic Profiling of Polyamines

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Résumé

Polyamines are a family of aliphatic compounds with one or more amine functions, playing a crucial role in proper cellular functioning. They are involved in various cellular processes such as growth, proliferation, apoptosis, autophagy, and microbiota-host interaction. Spermidine, in particular, is involved in anti-inflammatory functions and intestinal homeostasis. For the functional study of these metabolites, we needed a method to quantify them and perform isotopic profiling analyses. We thus developed a 45-minute liquid chromatography method coupled with high-resolution mass spectrometry (LC-HRMS) with separation on a PFPP column (Supelco), allowing not only quantification but also isotopic profiling. This method was validated to enable precise and absolute quantification (RSD $<$ 20%) of all intermediates with high sensitivity over a wide linear range (from 0.125 to 50 pmol). The isotopic profiling of these molecules was also validated for analyses of culture extracts and supernatants of Escherichia coli. In addition to the intermediates of the polyamine pathway, this method allows the detection of metabolites from the methionine cycle as well as amino acids. This method was applied to track over time the production and excretion of different polyamines by genetically modified Escherichia coli mutants designed to overproduce and secrete spermidine. The results highlighted differences in the production of these molecules by the different strains, as well as differences in the incorporation of carbon-13. These results provide a better understanding of the evolution of intracellular metabolism in response to the genetic modifications made.

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Poster 58 - P58

Préparation d'échantillons pour l'utilisation combinée de RMN et LC-HRMS en métabolomique

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Résumé

En métabolomique non ciblée, la chromatographie liquide couplée à la spectrométrie de masse haute résolution (LC-HRMS) et la résonance magnétique nucléaire (RMN) sont couramment utilisées. Leur utilisation combinée reste relativement rare bien qu'elles soient complémentaires permettant d'augmenter la couverture du métabolome et fiabiliser l'annotation. Préparer un seul échantillon à analyser avec les deux techniques malgré leurs contraintes spécifiques permettrait d'assurer l'homogénéité des analyses et pallier un éventuel manque de quantité. Le tampon phosphate 0,2 M est habituellement utilisé pour les analyses RMN, évitant ainsi les décalages trop importants de déplacements chimiques. Cependant, les phosphates sont déconseillés pour l'analyse LC-HRMS en electrospray en raison de leur cristallisation dans la source, entrainant une perte du signal et une altération de l'appareil.

L'un des objectifs de ce projet est de développer un protocole de préparation unique des échantillons compatibles avec les deux techniques. Dans un premier temps, le tampon est réduit à 0,05 M en phosphates afin de tester sa capacité à tamponner l'urine, une matrice à large gamme de pH. Les écarts de pH entre les échantillons réduits à des valeurs acceptables limitent les décalages de déplacements chimiques en RMN, partiellement corrigés grâce à des algorithmes de réalignement (CluPa, par exemple). Les échantillons ont ensuite été analysés en LC-HRMS, correspondant à une centaine d'injections. La stabilité du signal mesuré des métabolites et le maintien du voltage du détecteur n'ont pas été altérés.
Une réduction de la concentration en phosphates serait un bon compromis pour l'analyse d'un échantillon unique en LC-HRMS et RMN.

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Poster 59 - P59

Functional analysis of metabolic networks and host-microbiota interactions using multimodal Metabolomics

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Résumé

A Methodological Approach Using Mass Spectrometry, NMR, DESI Imaging, and Modeling

The intestinal microbiota plays a key role in determining animal growth trajectories. However, the mechanisms underlying this mutualistic relationship remain poorly understood. Metabolomics offers a powerful approach to explore these interactions and provides valuable insights into how metabolic networks influence health and disease.

The complexity of metabolic networks and their interactions with the host microbiota presents a major challenge in systems biology. Our goal is to develop analytical methods that allow investigation of simplified models, from *in vitro* systems to *in vivo* studies, in order to better understand the interactions between host and microbial partners. To address this challenge, we have developed a multimodal strategy combining Mass Spectrometry (MS), Nuclear Magnetic Resonance (NMR), DESI Imaging, and Computational Modeling to study the metabolic dynamics of host–microbiota interactions. MS and NMR allow detailed profiling of metabolites, while DESI imaging enables high-resolution spatial mapping of these molecules directly within tissue sections. By integrating these datasets through computational modeling, we

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aim to reconstruct metabolic networks and predict system-level responses under various conditions. This comprehensive approach enhances our understanding of microbial contributions to host metabolism and has potential applications in personalized medicine, biomarker discovery, and therapeutic development.

Poster 60 - P60

Investigating How Sugar Compartmentalization in Tonoplastic SWEET Mutants Impacts Photosynthesis Under Ambient and Elevated CO Conditions

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Résumé

According to the National Oceanic and Atmospheric Administration, atmospheric CO concentration increased by 100 ppm between 1960 and 2020 due to human activities. At this continuous rate of increase, concentrations are expected to reach around 1000 ppm by 2100. Elevated CO typically stimulates photosynthesis and vegetative growth; nonetheless, it often results in unpredictable effects on yield. Since sugars regulate photosynthesis through negative feedback, a key factor underlying this complexity is sugar compartmentalization. Tonoplastic transporters such as SWEET2, SWEET16, and SWEET17 mediate passive bidirectional sugar fluxes across the vacuolar membrane (Tingshan *et al.*, 2022) and may play a pivotal role in buffering cytosolic sugar concentrations, particularly under elevated CO conditions where energy-dependent processes are impaired.
This work aims to understand the contribution of these transporters to subcellular sugar partitioning under ambient (400 ppm) and elevated CO (1000 ppm) conditions, using *Arabidopsis thaliana* wild-type and *sweet* mutant lines. To access the subcellular metabolome, non-aqueous fractionation was used, a technique that separates intracellular compartments based on density while preventing metabolite diffusion or redistribution, thereby preserving the *in vivo* subcellular distribution. Fractions were analysed using both targeted enzymatic assays and GC-MS profiling, allowing the quantification and distribution of soluble sugars across vacuolar, cytosolic, and plastidial compartments.

By integrating non-aqueous fractionation with metabolic profiling under contrasting CO conditions, this work aims to determine whether deficiencies in tonoplastic passive sugar

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transporters alter subcellular sugar distribution, offering new insights into how intracellular sugar homeostasis is regulated and its potential impact on plant productivity under future climate scenarios.

Poster 61 - P61

Impact of pesticides, their mixture and transformation products on freshwater periphyton metabolism.

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Résumé

Pesticides are widely used in agriculture and often reach aquatic ecosystems where they can impact exposed organisms. During their transfer from fields to rivers, pesticides undergo abiotic degradation, forming transformation products (TPs), whose identities and toxicities are mostly unknown. Aquatic periphytons as a complex communities of microorganisms (e.g. microalgae, bacteria, etc.) playing a key role in ecosystem functioning. Thus, they are relevant for assessing impact of micropollutant on ecosystem functions. Despite increasing insights into chemical stressors, limited information exists about the combined effects of pesticides and their TPs on such microbial communities. To tackle this knowledge gap, our study investigates the toxicity of the herbicide terbuthylazine (TBA) and its TPs on periphyton using a multi-descriptors and metabolomics approach. Periphyton was exposed for four weeks to TBA, its TPs TBA-desethyl, and weathered TBA (wTBA), at two concentrations (5 and 150 μg/L). To reflect environmental conditions, TBA-desethyl was also tested in mixture with two ubiquitous contaminants glyphosate and AMPA (GA) at environmental levels. Control and GA-only treatments allowed for proper comparison. Results showed that most descriptors (e.g., photosynthetic yield, biomass, heterotrophic enzymatic activities) were strongly impacted by TBA but not or slightly by its TPs or mixtures with glyphosate. However, metabolomics revealed distinct profiles for each treatment, with TBA at 150 μg/L being the most disruptive. Interestingly, GA-containing mixtures showed no additional metabolic impact. These findings highlight metabolomics as a sensitive tool for detecting subtle effects of TPs and chemical mixtures, emphasizing its value in ecological risk assessments of aquatic microbial communities.

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Poster 62 - P62

Monitoring the impact of cork stoppers on red wine aging using 1H-NMR metabolomics

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Résumé

In this study, we used 1H-NMR metabolomics to monitor the influence of micro-agglomerated cork stoppers on the chemical evolution of red wines during bottle aging. Syrah wines from six wineries, matured in stainless steel tanks or oak barrels, were sealed with corks of four different oxygen transfer rates (OTRs), then aged for 24 months. The wines were analysed using a combination of unsupervised multivariate analysis such as PCA and HCA, and supervised multivariate approaches, namely OPLS-DA. We identified distinct chemical signatures based on maturation method, timepoint (initial *vs.* 24-months), and based on cork OTR. At the initial stage, univariate analysis revealed that wines matured in tanks exhibited higher levels of methanol and lower levels of ethyl lactate, acetic acid, myo-inositol, and isobutanol compared to barrel-aged wines, reflecting differences in oxygen exposure. During bottle aging, we observed a decrease in pentosides and monomeric flavan-3-ols, alongside increased levels of gallic acid, consistent with known breakdown of hydrolysable tannins over time. Notably, acetoin concentration correlated positively with cork OTR, suggesting its potential as a marker of oxygenation during aging. These results demonstrate that 1H-NMR metabolomics provides a robust, high-throughput platform for metabolomic profiling in enology and highlight the strategic role of cork permeability in modulating wine aging. Leleu, G., Garcia, L., Homobono Brito de Moura, P. H. B., Da Costa, G., Saucier, C., Richard, T. (2025). Cork impact on red wine aging monitoring through 1H NMR metabolomics: A comprehensive approach. Food Research International, 203. doi:10.1016/j.foodres.2025.115772

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Poster 63 - P63

La biogéographie influe la capacité naturelle du sol à résister à la pourriture noire des racines du tabac

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Résumé

La résistance d'un sol aux maladies est une propriété essentielle pour la santé des plantes, attribuée à l'action de phytoprotection du microbiote, mais les facteurs biogéographiques qui déterminent cette résistance ne sont pas encore compris, notamment en ce qui concerne le rôle des réponses physiologiques du végétal.
Pour tester l'importance de la géographie et de la géologie dans la résistance, des sols ont été prélevés à Morens (Suisse) et en Savoie (France) et utilisés pour cultiver des plants de tabac, inoculés ou non avec l'agent pathogène de la pourriture noire des racines *Thielavipsis basicola*. Les niveaux de sévérité de la maladie ont été mesurés pour confirmer le statut du sol, résistant ou sensible. Les communautés microbiennes ont été caractérisées par métabarcoding et métagénomique pour prendre en compte leurs particularités taxonomiques et fonctionnelles, et les profils métaboliques des plantes ont été déterminés pour prendre en compte les réponses des plantes en relation avec les conditions de résistance et la pression de maladie.

L'hypothèse selon laquelle la résistance du sol à la maladie est une propriété déterminée à la fois par la géographie et la géologie a été validée par les approches de métabarcoding et de métagénomique. Ces résultats mettent également en évidence des mécanismes de résistance distincts dans les deux régions.

Le traitement des données métabolomiques obtenues sur les feuilles des plants de tabac montre des familles de métabolites spécialisés, impliqués dans la résistance, différentes entre les deux zones géographiques considérées, en accord avec les autres approches.

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Poster 64 - P64

Determination of correction factors to quantify different compounds with a standardised method to analyze wine in NMR metabolomic

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Résumé

Wine is a high value agri-food product, which makes it a target of fraud. It is therefore necessary to continue developing tools that allow better traceability of wines to prove or disprove their authenticity. Several methods already exist, from a simple organoleptic test to more advanced technologies including isotopic techniques or residual radioactivity measurements. However, with counterfeiting becoming increasingly sophisticated, more complex methodologies are needed for its detection. In this context, metabolomics tools can offer an innovative approach to study wine authenticity (1).
The present work is part of the development and validation of an open metabolomic approach based on proton nuclear magnetic resonance (1H-NMR) to guarantee wine authenticity, using standardised, flexible, and accessible protocols. More precisely, this work focuses on calibration methods with determination of correction factors by spiking the sample with known concentrations for each metabolite. To obtain these correction factors, semi-automatic and fully automated treatments of 1H-NMR spectra are compared. Validation tests were conducted using other metabolite concentrations, and the results were consistent and promising.

The overall results represent a significant advancement towards establishing a standardised and open methodology for certifying wine authenticity using 1H-NMR metabolomics.

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Poster 65 - P65

Untargeted metabolomic analysis of saliva from patients suffering from pollen-food allergy syndrome : towards a better understanding of the mechanisms underlying the allergic reaction and identification of predictive biomarkers

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Résumé

Pollen-food allergy syndrome is highly prevalent, with 30 to 60% of food allergies associated with a pollen allergy. Symptoms extend from oral syndrome to respiratory or gastrointestinal, or even anaphylactic shock in presence of cofactors such as exercise. Skin pricks tests and IgE measurement are commonly used for its diagnosis, but oral food challenge (OFC) are required notably to initiate an oral immunotherapy. Our study aims to analyse metabolic mediators in saliva samples collected during an OFC from allergic patients with oral syndrome.
Patients suffering of birch pollen allergy in association with oral allergy syndrome to apple were submitted to an OFC. Increasing doses of apple were ingested by patients and saliva were sampled at different timepoints before, during and after ingestion. Non-targeted metabolomic analysis was carried out using an analytical workflow based on liquid chromatography coupled to high-resolution mass spectrometry. Metabolite annotation was performed using an in-house spectral database containing 1200 standards. Statistical analyses were performed using omics-dedicated R packages.

In samples collected at the onset of the allergic reaction, 87/210 metabolites annotated were significantly different between patients and non-allergic controls. Pathway enrichment analysis evidenced that arachidonic acid, tryptophan and polyamines metabolisms were particularly involved. Interestingly, analysis of the corresponding metabolites in samples collected before the OFC allowed establishment of a good PLS-DA predictive model.

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Our study highlights the potential of non-targeted metabolomic analysis of saliva in the context of an OFC to study metabolic pathways activated during an oral syndrome, but also to predict allergic state of patients.

Poster 66 - P66

CABiosE : Comparative analyses of biostimulant effects on tomato crop and their associated microbiota, under water stress conditions.

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Résumé

The aim of the project is to study the response mechanisms induced by biostimulants on tomato plants under semi-controlled conditions. In particular, the objective is to determine the direct contribution of BS on plants and their indirect contribution, through the variations in rhizosphere and phyllosphere microbiota.
We planned a three-stage approach: Plant – Product – Soil. The product approach aims at screening the most effective products for biostimulation in tomato under water stress. BS of various natures are tested: algae extract, vegetal extract, mineral. The soil approach is designed to assess whether BS influences the microbiota of various types of soil.

The first experiment in 2024 under water stress conditions allowed selecting two BS among seven, under semi-controlled conditions, in a greenhouse. Another experiment in 2025 under water stress conditions and integrating two different soil conditions is dedicated to study the effects of these two BS on plants and plant microbiota. Phenotypic observations and measurements are planned to generate a growth curve. Samples will be taken from leaves and fruit at different stages of growth for metabolomic analysis and from soil and plant tissue for analysis of the rhizosphere and phyllosphere microbiota. The aim of combining metabolomics and metagenomics analyses is to assess the influence of BS on the phyllosphere and rhizosphere microbiota communities and on their functions.
Thanks to this project we hope to gain insights into the mechanisms by which plants develop resilience to abiotic stress in response to BS applications directly and on the indirect contribution *via* the microbiota.

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Poster 67 - P67

Unlocking metabolic insights: photo-CIDNP NMR applied to the discrimination of tea

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Résumé

The versatility of NMR for analyzing complex mixtures has been described in health, food or plant sciences.These advantages have been highlighted in metabolomics, where NMR has contributed to the comprehension of metabolic profiles, identifying and quantifying components in the mM range.1
However, minor compounds suffer from intrinsic sensitivity limitations, palliated with the development of several hyperpolarization methods.2 Among them, the photo-Chemically Induced DNP (photo-CIDNP)3 by light irradiation is cost-effective and easy to implement on any NMR instrument. A proof-of-concept for the potential of photo-CIDNP in metabolomics studies is shown,4 but the optimization of the method for the sequential analysis of a large number of samples is still a challenge.

Using a 1-scan 1D sequence, we have already obtained a sensitivity enhancement of over 20-fold of flavonoid derivatives at the micromolar range for 1H NMR. Here we present the optimization of: sample preparation, light irradiation and NMR acquisitions over a range of concentrations adaptable for metabolomics. The method is supported by LC-MS to determine the ratio of sample degradation, and the identification of the byproducts. The optimal conditions are applied to the detection of flavonoids in different varieties of tea offering a complementary tool for the discrimination of such samples in a fast and efficient manner, paving the way for a unique metabolomics protocol dedicated to highly conjugated compounds.

1) Nagana Gowda, G. A. et al Anal. Chem. 2017

2) Eills, J. et al Chem. Rev. 2023

3) Okuno, Y. eMagRes 2017
4) Kuhn, L. T. et al Anal. Chem, 2023

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Poster 68 - P68

Discrimination of South Tyrolean wines by their cultivation practices: A detailed mass spectrometric approach

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Résumé

Climate change profoundly impacts viticulture, causing, among others, increased water stress and earlier harvests that affect wine quality and character. It also alters the concentration of phenolic compounds, key to wine structure and colour. Furthermore, climate-related diseases like powdery mildew, downy mildew, and grey rot threaten yields, prompting heavy pesticide use with serious environmental and health consequences. To address these challenges, viticulture is shifting toward sustainable practices such as biological control, preventive strategies, and disease-resistant grape varieties. These new varieties can reduce pesticide use while producing wines with phenolic, anthocyanin, and volatile profiles similar to traditional Vitis vinifera, although with some distinctive features.

In this context, a joint project by the University of Bordeaux and the University of Bolzano compares French (Bordeaux & Pays d'Oc) and Italian (South Tyrol) wines using metabolomics. It focuses on different cultivars (local, international, and resistant), terroirs, and cultural practices (organic vs. conventional), employing NMR and MS, combined with HPLC and GC. An initial profiling of 61 monovarietal wines from Pays d'Oc using UHPLC-HRMS/MS revealed clear distinctions based on grape variety and cultural practices. The targeted analysis included over 45 polyphenols; the untargeted approach used Full Scan HRMS with data-dependent fragmentation.

This methodology is now applied to 59 South Tyrol wines, with HS-SPME-GCxGC-ToF/MS for volatile profiles and HPLC-QQQ-MS for anthocyanins and polyphenols. These advanced spectrometric tools aim to develop robust, transferable methods to better differentiate wines by their cultural practices.

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Poster 69 - P69

Development of a dual-extraction method to profile hydrophilic and lipophilic molecules in processed fruit products

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Résumé

Fruits and vegetables are complex matrices characterized by high variability and are subject to diverse conventional and innovative processing methods. Systematically studying their hydrophilic and lipophilic metabolite pools presents a considerable challenge. The UMR-SQPOV addresses multidisciplinary research questions, aiming to valorize plant biodiversity for healthy, sustainable, and diversified food systems. The expertise already includes evaluating the impact of processing and gastrointestinal digestion, using targeted approaches, on polyphenols, carotenoids, and vitamins to assess plant food nutritional quality. However, the broader impact of the production–processing–consumption–digestion continuum on plant composition remains largely unexplored, although a diversity of their phytochemicals display interesting biological activities (antinflammatory, antitumoral,...). This study aims to develop and implement a dual-extraction protocol to simultaneously access and profile hydrophilic and lipophilic metabolites in fruit and vegetable products and to give a deep insight through untargeted metabolomics approaches. Preliminary extraction tests, using four solvent combinations, were conducted on strawberry purée and nectar, as well as thermally processed tomato products. Untargeted metabolomics will be performed using UHPLC-HRMS (Orbitrap Exploris 240, Thermo Scientific), with both ESI and APCI ionization sources in positive and negative modes. Data processing and interpretation will involve tools such as MSDIAL, MZmine, SIRIUS, MetaboAnalyst, and GNPS, aligned with mQAQC and MSI guidelines. Combining methods and analytical platforms is essential to maximize metabolite coverage, considering matrix complexity, molecular heterogeneity, and interactions among micro- and macroconstituents. This system-based approach aims to capture the dynamic evolution of metabolite pools throughout processing and gastrointestinal digestion, providing new insights into food transformation and nutrient bioaccessibility.

*Intervenant

Poster 70 - P70

Tolerance of leaf-associated microbial communities to the biofungicide Kasugamycin: new insight from meta-metabolomic approach.

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Résumé

Biopesticides are increasingly used as eco-friendly alternatives to synthetic pesticides in agriculture, but little is known about the tolerance and resilience of microbial communities to these molecules in aquatic receiving ecosystems. To address this gap, meta-metabolomics offers an innovative ecotoxicological approach by providing a snapshot of the biochemical response of microbial communities to exposure to (bio)pesticides. In this context, this study investigates the tolerance mechanisms of leaf-associated microbial communities exposed to the biofungicide kasugamycin. Leaves colonized by natural microbial communities were exposed under controlled conditions to 10 and 100 mg/L of kasugamycin for 4 weeks. At days 1, 7, 14, and 28, non-targeted meta-metabolomics, structural descriptors (fungal and bacterial biomass), and functional descriptors (respiration and enzymatic activities) were assessed. Additionally, Pollution-Induced Community Tolerance (PICT) was evaluated via laccase activity assay on days 7 and 28. Results showed a decrease in bacterial density and community respiration, but an increase in fungal biomass, under kasugamycin exposure. ANOVA-simultaneous component analysis (ASCA) analysis of metabolomic profiles revealed significant effects of both time and kasugamycin concentration. The classes of metabolites most influenced by the relative abundance of kasugamycin are peptides and amino acids. These changes suggest that kasugamycin disrupts protein synthesis, consistent with its known mode of action. Tolerance to kasugamycin in pre-exposed communities appeared after 7 days, but disappeared by day 28. Further analysis is ongoing to improve metabolite annotation and better understand the biochemical changes involved and identify biomarkers of microbial community tolerance.

*Intervenant

Poster 71 - P71

Élucidation et Découverte Intégrées par Approches OMICS de la variabilité pigmentaire florale de la Pédiculaire

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Résumé

Les Pédiculaires (genre *Pedicularis*) sont des plantes herbacées de la famille des *Scrophulariaceae*, répandues dans les milieux montagnards frais des Pyrénées orientales. Leur diversité florale, oscillant du jaune au rose, témoigne de variations pigmentaires dont les déterminants moléculaires restent mal caractérisés.
Dans ce travail, nous avons comparé les empreintes chimiques d'extraits de fleurs jaunes et roses de *Pedicularis*, obtenues par LC-HRMS Q-Exactive Plus en mode ESI+ et couplées à une trace UV, afin d'explorer le métabolome via les réseaux de similarités spectrales. Parallèlement, une étude transcriptomique a été menée pour identifier les voies de signalisation impliquées dans la biosynthèse pigmentaire.

L'analyse métabolomique non ciblée a révélé plusieurs clusters d'anthocyanes spécifiquement enrichis dans les fleurs roses. Parmi eux, l'ion majoritaire, annoté comme une Cyandin-coumaroyl-rhamnoside, est totalement absent chez les spécimens jaunes. La trace UV a confirmé le profil d'absorption caractéristique des pigments anthocyaniques.

Les données transcriptomiques ont mis en évidence la surexpression significative de gènes-clés de la biosynthèse des anthocyanes dans les fleurs roses, suggérant une activation coordonnée des voies flavonoïdes. L'intégration multiomique a permis d'établir des corrélations robustes entre l'abondance relative de l'ion majoritaire et l'expression de ces enzymes.
Ce poster et ces résultats illustrent la complémentarité des approches métabolomique et transcriptomique pour élucider les déterminants moléculaires de la variabilité florale chez *Pedicularis*, offrant de nouvelles perspectives pour l'étude de la coloration végétale et de ses adaptations à son environnement.

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Poster 72 - P72

Fusion de données inter laboratoires en métabolomique non-ciblée : importance de la standardisation des workflows de traitement des données.

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Résumé

La métabolomique non ciblée par chromatographie liquide couplée à la spectrométrie de masse haute résolution (LC-HRMS) permet une large couverture du métabolome d'échantillons biologiques. Cependant, l'hétérogénéité des données complique la comparaison inter-laboratoires, limitant son application à large-échelle. Dans ce contexte, cette étude vise à évaluer l'apport de la standardisation des workflows de traitement des données pour la fusion de données inter-instruments.
Un essai inter-laboratoire a été mené par deux plateformes de l'INBS MetaboHUB, utilisant des instruments différents (QToF et Orbitrap), en harmonisant les autres étapes : préparation de 86 échantillons de plasma et de contrôles qualité (pools et dilutions), séquence analytique, méthode chromatographique. Pour le retraitement des signaux, les pratiques des deux laboratoires ont été harmonisées afin d'obtenir un workflow unique sous Workflow4Metabolomics. Les seuils des critères qualité ont été évalués pour optimiser le filtrage des ions et permettre la comparaison des deux appareils.

Les résultats mettent en évidence l'impact de paramètres tels que la détection des pics et le regroupement sur la qualité de l'alignement des signaux. L'apport des filtres, notamment sur les blancs et les corrélations des contrôles qualité, est démontré comme favorisant la comparabilité entre deux instruments de résolutions différentes. L'analyse s'appuie à la fois sur une évaluation globale des ions extraits et sur une approche ciblée portant sur 50 métabolites d'intérêt détectés par les deux laboratoires.
Ce travail souligne l'importance d'utiliser des outils de diagnostic pour évaluer la qualité du prétraitement des données, et de workflows standardisés pour garantir l'interopérabilité des données de métabolomique non ciblée.

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Poster 73 - P73

OLIVE (Off Line Instrument for Volatile Extraction) - Prototype automatisé d'extraction de l'espace de tête d'aliment pour l'analyse des COV (composés organiques volatils)

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Résumé

Plus de 7000 COV ont été isolé des aliments. Ces molécules présentent toutes une pression de vapeur caractéristique de leur volatilité, ont une masse $<$ 400 Da et possèdent une très large gamme de polarité. Cette grande diversité de propriétés physico-chimiques rend difficile de représenter le " volatolome " total d'un aliment par une extraction liquide-liquide ou bien par distillation sous vide. Le prototype que l'on vous présente est une méthode qui permet de piéger et détecter les COV par mesure dynamique d'un espace de tête. Il offre la capacité de piéger les molécules très volatiles comme l'acétaldéhyde et peu volatils comme la β-ionone .

Le prototype comprend quatre unités de mesure qui travaille en simultané et constituées d'un flacon laveur, dans lequel l'échantillon est introduit puis placé dans un flux d'azote contrôlé. Les temps d'incubation, d'extraction vers des pièges Tenax ® sont gérés par un programme développé en C++ implanté sur un Raspberry pi.
Nous vous présentons les résultats des COV détectés dans 10 variétés d'abricots frais et transformés en confiture. Nous identifions une soixantaine de COV communs à toutes les variétés d'abricots avec des différences qualitatives et quantitatives entre les matrices fraiches et transformées . Même si ces COV ne représentent pas les 216 composés du volatolome des abricots (VCF database), ce prototype nous permet de produire une signature aromatique montrant un pouvoir discriminant des variétés et des conditions de transformation.

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Poster 74 - P74

Exploring the effect of a prolonged space storage on grapevine cane metabolism

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Résumé

Developing more resilient agriculture requires a deeper understanding of how plants respond to extreme environments. Space travel offers a unique opportunity to expose plants to conditions that cannot be replicated on Earth, such as microgravity and cosmic ionizing radiation. In this study, we investigated the effects of a 10-month exposure to space conditions on grapevine (*Vitis vinifera*) canes stored aboard the International Space Station (ISS). Upon their return to Earth, we conducted both untargeted and targeted analyses of polyphenols in wood tissues coupled to an analysis of the xylem tissues integrity by microscopy. Our results revealed that cane storage in space conditions did not affect xylem integrity nor induce tissues necrosis. Our study shows that space conditions induce a strong effect on the metabolic profile of the canes. The untargeted analysis reveals distinct overall metabolic profiles between canes stored in ISS and their respective controls stored on Earth. Moreover, the analysis of the compound class annotation revealed that space conditions promoted the accumulation of specialized metabolites such as polyphenols, at the expense of compounds associated with primary metabolism. Specific analysis of polyphenol highlighted an accumulation of compounds such as gallic acid, flavan-3-ols, and stilbenoids compared to ground-stored controls. As polyphenols are key contributors to plant defense and stress resilience, this finding indicates that exposure to the space environment may trigger biosynthetic pathways associated with protective responses without compromising tissue integrity.

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Poster 75 - P75

Towards new antiobiotics through metabolomics: the power of marine fungi revealed by new eco-inspired cultures;

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Résumé

Antimicrobial resistance constitutes a major public health issue, requiring the development of new therapeutic molecules. Marine fungi and their specialized metabolites represent an underexploited source of potential antibiotics. However, under conventional laboratory conditions the number of observed metabolites remains limited relative to the biosynthetic gene clusters (BGCs) present in genomes. To induce the expression of some of these BGCs remaining cryptic, some strategies have been successfully developped, such as the OSMAC approach using different media including host-derived media for strains from holobionts and co-cultures of microbial partners. However, co-culture methods are mainly performed using pairs of microorganisms, which does not reflect what naturally occurs within marine microbiomes.
This project aspires to develop eco-inspired multicultures of different fungal strains sampled from the seaweed *Palmaria palmata*, aiming to highlight metabolic inductions in microbial consortia when cultured on a reconstituted seaweed-based medium.

First experiments have been conducted on four fungal strains which were cultivated on 12 different culture media including a *P. palmata*-derived media. Extracts were tested on environmental or pathogenic bacterial strains, and analyzed by UHPLC-HRMS/MS to construct bioactive molecular networks. This allowed us to find the best host-derived medium for antibacterial and original compounds production.
Co-cultures involving 2 to 4 of the strains have also been engaged, and MS-based chemometrics analyses led to highlight the induction of specific signals in some of the microbial consortia. Promising microbial consortia will be up-scaled in order to isolate the most promising compounds issued from these eco-inspired host-derived media.

*Intervenant

Poster 76 - P76

Etude multi-omique longitudinale de la composition métabolique et taxonomique de l'écosystème kéfir de fruit

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Résumé

Le kéfir de fruit est une boisson issue de la fermentation en plusieurs étapes d'une solution de saccharose avec des fruits, classiquement figue sèche et citron. L'inoculum utilisé est composé de granules macroscopiques – les grains de kéfir - contenant des levures, des bactéries lactiques, des bactéries acétiques, incorporées dans une matrice d'exo polysaccharides. Le kéfir constitue donc un écosystème complexe dans lequel une vingtaine d'espèces de microorganismes coopèrent sur la base d'échanges métaboliques dans l'espace et dans le temps.

L'étude repose sur la caractérisation de cet écosystème symbiotique par métabolomique et génomique. Les données métabolomiques obtenues par LC-MS(/MS) ont été collectées sur le surnageant de boisson de kéfir à différentes étapes de la fermentation (après 0, 24, 48 et 72 h), après lyophilisation et re suspension de manière à concentrer 25 fois. L'analyse multivariée PLS-DA des données LC-MS a révélé une bonne séparation des temps de fermentation. La librairie R DRomics a été utilisée de manière à regrouper les métabolites par profil temporel. L'annotation des métabolites a été réalisée à partir des données LC-MS/MS, construction de réseaux moléculaires et interrogation de bases de données. Parallèlement, les génomes des souches constitutives du consortium (15 bactéries et 4 levures) ont été examinées pour leur potentiel de production de métabolites spécialisés en utilisant l'outil Antismash, permettant d'identifier des systèmes de biosynthèse de peptides, polycétides et saccharides.

L'intégration de ces données devrait permettre de caractériser la composition chimique du kéfir de fruit et sa dynamique, en lien avec sa composition microbienne.

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Poster 77 - P77

REFRAMED : nouvelles approches en spectrométrie RMN pour la compréhension des facteurs de risque associés aux maladies cardiovasculaires

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Résumé

Les maladies cardiovasculaires (MCV) sont une cause majeure de mortalité à l'échelle mondiale et un taux plasmatique élevé de cholestérol lié aux lipoprotéines de basse densité (LDL-C) constitue un facteur de risque majeur de ces dernières. L'hypercholestérolémie familiale (HF), maladie génétique héréditaire se caractérisant par une forte réduction de la clairance hépatique des LDL et une augmentation de leur taux plasmatique, favorise le risque de MCV. Cependant, une très faible proportion de patients HF ($<$ 4%) ne développe pas de MCV ; la découverte de nouveaux biomarqueurs associés à cette cardio-protection pourrait ouvrir la voie à de nouvelles cibles thérapeutiques.

Le microbiote intestinal, source de métabolites bioactifs tels que le TMAO impliqué dans le risque cardiovasculaire, constitue une matrice d'intérêt émergente pour l'identification de biomarqueurs associés au risque cardiovasculaire. Le projet REFRAMED a pour ambition de trouver de tels biomarqueurs dans le microbiote intestinal en analysant les selles de patients HF par une approche multi-plateforme combinant la métabolomique et la lipidomique afin d'explorer le plus largement possible les métabolites microbiens impliqués dans l'inflammation et l'athérogenèse. La complémentarité des méthodes de MS (LC- et GC-MS) et de RMN (paillasse, 1D, 2D rapides et hyperpolarisée) qui seront appliquées favorisera une couverture élargie du métabolome.

La première étape du projet consiste à tester divers protocoles d'extractions biphasées-Folch, Bligh&Dyer, Matyash- afin de trouver la meilleure méthode de préparation commune aux

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différentes méthodes analytiques appliquées. Les résultats préliminaires comparant les profils métaboliques et lipidiques avec chacune des méthodes et chacun des protocoles d'extractions seront présentés.

Poster 78 - P78

Maximizing metabolome coverage and metabolism in Microcystis aeruginosa : A comparative evaluation of monophasic and biphasic extraction-based workflow

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Résumé

Cyanobacterial blooms are a phenomenon of growing international concern, due to their potential ecological, human and economic impacts. Recently, some studies have highlighted the potential role of metabolic interactions between heterotrophic bacteria and cyanobacteria in the phycosphere in promoting cyanobacterial blooms. In this context, there is a growing need to comprehensively depict metabolome and metabolism of cyanobacteria and heterotrophic bacteria consortium. Thus, this study aims to explore various metabolomic workflows in order to provide a comprehensive picture of the chemical landscape and so the metabolism for twelve strains of M. aeruginosa. Thus, our objective was to determine the complementarity and the specificity of these workflows in terms number of annotated metabolites and classes. To such an end, biphasic (MTBE/MeOH/HO leading to a hydrophilic and lipophilic extracts) and monophasic extraction (MeOH/HO leading to a single extract) coupled to UPLC-HRMS/MS analyses were compared. Biphasic extracts were separated according an hydrophilic and a lipophilic chromatographic gradient while monophasic extract

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was only separated on hydrophilic gradient prior data acquisition. Data were processed by combining MS-DIAL, MS-CleanR and SIRIUS. Monophasic extraction based-workflow highlighted marked differences in the chemical landscapes of the twelve cyanobacteria. In particular, some specialized metabolites (microcystins LA, LF) were specific to some strains. Data from biphasic workflow is under investigation. Overall, this work will support a better understanding of the role of metabolism and metabolic interaction in M. aeruginosa bloom formation.

Poster 79 - P79

Exploring the complementarity of fast multi-pulse and multi-dimensional NMR methods for metabolomics: a chemical ecology case study

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Résumé

The overwhelming majority of NMR metabolomics studies rely on the acquisition of 1D 1H fingerprints. 1D 1H spectroscopy has the advantage of being high-throughput and relatively simple to process. However, 1D proton spectra are hampered by severe peak overlaps arising from the diversity of analytes present in samples, associated with a limited frequency range and to the spreading of 1H multiplets. This issue is further compounded by the peak shifting that occurs within sample groups due to pH, concentration or ionic strength variations, making peak integration a complex task. The NMR toolbox includes several promising alternatives for the analysis of complex mixtures, but their application to metabolomics remains scarce. In this study, we evaluate the potential and complementarity of several high-throughput multi-pulse and multi-dimensional NMR methods for metabolomics. Through a chemical ecology case study, three methods are investigated, offering a continuum of methods with complementary features in terms of resolution, sensitivity and experiment time. Ultrafast 2D COSY, adiabatic INEPT and SYMAPS HSQC are shown to provide a very good classification ability, comparable to the reference 1D 1H NMR method. A detailed analysis of discriminant buckets upon statistical analysis shows that all methods are highly complementary, since they are able to highlight discriminant signals that could not be detected by 1D 1H NMR, in particular in highly crowded regions of the 1H spectrum. Overall, the combination of these recent methods within a single NMR metabolomics workflow allows to maximize the accessible metabolic information and raises exciting challenges in NMR metabolomics.

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Poster 80 - P80

Exploring new stilbenoid-producing plant seeds as potential sources of bioactive compounds

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Résumé

Phenolic compounds are specialized metabolites produced by plants including phenolic acids, flavonoids, tannins and stilbenoids such as resveratrol and ϵ-viniferine. These molecules are of significant interest, particularly for their roles in both human and plant health. Stilbenoids can be produced either consitutively or in response to environmental stimuli. They have been isolated from various plant organs including roots, leaves, bulb, flowers or seeds. To date, they have been identified in around 150 species of Monocotyledons and Dicotyledons. However, considering that Angiosperms comprise nearly 250 000 species, there remains a vast diversity of plants yet to be explored. In this study, we selected more than 200 species from local and international botanical gardens to investigate their stilbenoid content. Seeds will be the focus of the intial analysis, as very little information is currently available for this organ. After grinding, polyphenols will be extracted and analyzed using non-targeted metabolomics via UHPLC-HRMS. Particular attention will be paid to the stilbenoid profile.

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Poster 81 - P81

Enhancing Metabolite Identification in Complex Natural Product Mixtures with an Integrated 13C NMR and LC-HRMS2 Approach

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Résumé

Dereplication studies using UPLC-HRMS^2 have become the gold standard for analyzing complex mixtures of natural products. Molecular networks derived from HRMS^2 fragmentation patterns, combined with database associations, enable metabolite annotation with varying levels of confidence. Our approach integrates NMR data into the dereplication study (MixoNat) using structural databases to enhance the certainty of node annotations.

Our strategy involved analyzing the same fractions by both NMR and LC-HRMS2, leveraging high-resolution capabilities to generate detailed fragmentation data. By creating molecular networks and annotating nodes obtained from HRMS2, we compared these nodes with structural hypotheses observed by NMR to improve the confidence of annotations. This integration enabled more robust metabolite identification, particularly in complex natural product mixtures. The MixoNat software facilitated the comparison of NMR and MS data, significantly enhancing the accuracy of metabolite annotations.

This integrated approach was applied to the study of *Inula montana L.*, a plant known for its bioactive compounds. The combination of UPLC-HRMS^2 and 13C NMR provided comprehensive insights into the plant's metabolome, revealing novel metabolites and confirming known structures with high confidence. This methodology not only advances the field of natural product research but also demonstrates the potential for broader application in metabolomics studies

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Poster 82 - P82

Development of a semi-quantitative targeted DESI-QqQ approach to visualize analyte distribution in biological samples

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Résumé

Mass spectrometry imaging (MSI) is an analytical technique that provides spatial molecule distribution information of a sample. The combination of a DESI (Desorption ElectroSpray Ionization) source with a triple-quadrupole mass spectrometer (QqQ) is a recent improvement, enabling targeted metabolite imaging using Multiple Reaction Monitoring (MRM) transitions to reach higher sensitivity. However, the lack of TIC disables signal normalization and so, the comparison between section analysis as well as the availability to follow a dysregulation over time.

Our objective is to develop a semi-quantitative DESI-QqQ imaging method to visualize the distribution of a set of molecules. In order to compare the sections, we added a solution of internal standards at known concentrations on the tissue section by spraying it before ionization (DESI). This approach allows the calculation of intensity ratios between the targeted metabolites and their corresponding internal standards, enabling comparative analysis across different tissue sections or experimental conditions.

The proof-of-concept application of this approach to rat brain sections is a first step toward robust relative quantification of targeted metabolites by DESI-MSI, with promising perspectives for comparative and longitudinal studies.

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Poster 83 - P83

NMR as a new tool in fluxomic studies: measuring the efficiency of betaglucosidase in natural extracts of Vanilla pods

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Résumé

Vanilla has a dominant position in the global flavor market and ranks as the second most expensive spice worldwide after Saffron. Botanically, vanilla is a tropical perennial plant of the Orchidaceae family, and there are three main cultivated species: *Vanilla planifolia*, *Vanilla pompona*, and *Vanilla tahitensis*. The aromatic complexity of vanillas is of scientific interest and involves making hybrids of these main cultivars and the quantity of vanillin seems to be the primary asset in the quality of the flavor of the pods. The aim of the study was to evaluate the efficiency of the enzymatic reaction of β-D-glucosidase as vanillin producer. For this purpose, we adapt the use of a coupled reaction (spectrophotometric method) to 1H-NMR. Indeed, para-nitrophenyl-β-D-glucopyranoside reacts specifically with the vanilla β-D-glucosidase to produce 4-nitrophenol. These molecules are easily monitored by 1H-NMR in the aromatic region and with automation a good resolution is achieved with one measure every 90 seconds during 30 minutes. Using this method, it was found that two hybrids from the society Eurovanille produced 7.85 and 1.5 μg/min/mg DW of 4-nitrophenol. Therefore, differences in enzymatic efficiency may therefore be due to genetic variation. Keywords: NMR, Vanilla, β-D glucosidase

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Poster 84 - P84

Isotope-assisted assessment of matrix effects in different extracellular media and its impact on the reliability of relative metabolite quantification

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Résumé

Introduction: Analysis of the extracellular media's metabolome of different cell lines can make a major contribution to understanding the mechanisms of cell proliferation. In this context, non-targeted LC-HRMS profiling offers the advantage of covering a wide range of metabolites. However, significant cell line-dependent variations in terms of nutrient uptake and metabolite secretion can lead to drastically and chemically different culture supernatants, thereby resulting in matrix effects and potential quantification biases. In this study, we sought to assess the impact of these variations on the MS-based relative quantification of 20 relevant metabolites also measured by NMR.

Materials and Methods: Extra-cellular media of seven diffuse large B-cell lymphoma (DLBCL) cell lines were supplemented with 20 stable isotope-labeled compounds. The spiked concentrations were adjusted based on quantitative 1H-NMR data obtained from the original culture media. The resulting samples were profiled by LC-HRMS. For all 20 metabolites, peak areas were collected using a non-targeted processing workflow (W4M) and a targeted reprocessing method (Skyline software).

Results and perspectives: The relative quantification results were compared with each other and with 1H NMR data. Notable discrepancies were noted between NMR and LC-MS measurements. By using isotopically-labeled standards, the proportion of signals whose measurement bias was imputable to matrix effects could be determined. Thus, a more robust LC-HRMS method could be developed. Despite the apparent similarity of cell supernatant samples, the use of isotopically-labeled standards should be considered for semi-quantitative studies, given the sometimes-dramatic variations in culture media composition between cell lines.

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Poster 85 - P85

Analitycal study of life style exposure and impact of biologiocal function through hair analysis.

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Résumé

The worldwide use of chemicals and human exposure to them has drawn attention to the potential consequences for human health. Information on the exposure of the general population remains very limited in most countries. In this study, hair analysis was conducted as a first step to investigate the exposure of 204 urban women living in two different cities to 110 molecules and 30 metabolites representing lifestyle exposures. In a second step, the relationship between detected molecules and hormone ratios was evaluated by investigating the association between exposure biomarkers and hormone levels. Results show that approximately 90 targeted molecules and metabolites were found in the hair samples, with concentrations ranging up to 1070 pg/mg. The concentrations of 38 chemicals (e.g., p-nitrophenol, diethyldithiophosphate, λ-cyhalothrin, permethrin, carbendazim, and tebuconazole) were significantly different between women in the two cities, indicating regional differences in exposure. Using elastic net regression, we demonstrated a correlation between some chemical markers and thyroid hormones (T4, T3, rT3, T2); corticoid hormones (cortisol, tetrahydrocortisol, cortisone, and tetrahydrocortisone); and steroid hormones (pregnenolone (P5), progesterone (P4), 17α-hydroxyprogesterone (17OHP4), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate DHEAS, androstenedione AD, testosterone T, estrone E1, and 17β-estradiol E2. These results can provide baseline information on the exposure of the adult female population in an urban setting to lifestyle-representative chemicals and the impact of this exposure on biological function through hormone homeostasis. This study demonstrates that hair is a robust means of measuring the impact of urbanization on a person's lifestyle and can reflect the body's biological function

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Poster 86 - P86

Impact des variations des métabolites énergétiques sanguins sur l'oxygénation du cerveau chez des rats sevrés précocement

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Résumé

Le projet européen Prometeus vise à améliorer la prise en charge des prématurés dans les services hospitaliers en individualisant la nutrition afin de la rendre optimale au développement de l'enfant. L'objectif est de modéliser la corrélation entre les taux sanguins de glucose, lactate et bêta-hydroxybutyrate et l'oxygénation cérébrale.
Dans ce contexte, notre étude évalue le métabolisme sanguin de ces trois métabolites lors de challenges hypo- et hyperglycémiques à l'aide de la méthode des traceurs marqués au 2H et 13C, dans un modèle de rat sevré précocement. Nous établirons ensuite un lien entre le métabolisme et l'oxygénation cérébrale par mesure IRM dans ces mêmes conditions.
Nous avons tout d'abord développé le modèle animal en séparant les ratons de la mère 13 jours après la naissance. Lors de la mise en place du challenge hypoglycémique, nous avons constaté que l'anesthésique utilisé, l'isoflurane, induisait une augmentation de la glycémie de 50%. Cependant, une mise à jeun de 2h permet de limiter cette hyperglycémie. A l'inverse, un jeune d'une nuit, comme fait dans beaucoup de publications, stabilise la glycémie mais induit une augmentation des corps cétoniques de 300%. Ces résultats soulignent l'importance des conditions expérimentales, notamment dans les études métaboliques. Les prochaines étapes de ce projet comprennent i) le suivi de l'utilisation des substrats énergétiques, basé sur l'infusion de traceurs, l'échantillonnage sanguin et la spectrométrie de masse et ii) la cartographie du débit sanguin cérébral et du taux métabolique cérébral de l'oxygène à l'aide de l'IRM quantitative.

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Poster 87 - P87

A Systematic Analysis of Untargeted LC-MS Methods for Human Metabolomics Studies Using the GNPS Public Spectral Library

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Résumé

Untargeted metabolomics using LC-HRMS is a powerful tool in various research domains, including large-scale clinical studies. Current validation criteria for untargeted methods typically rely on a limited set of representative analytes, mainly due to their high cost and limited availability. This study proposes a data-driven strategy for evaluating untargeted LC-HRMS methods leveraging the GNPS public spectral database as an accessible alternative to chemical standards to enhance method evaluation. Commercial human serum samples were first assessed under a total of 23 LC-HRMS methodologies proven for high-throughput analyses from 6 metabolomic platforms using an Orbitrap Exploris™ 240 Mass Spectrometer. The metabolites annotated with the GNPS library were used for the evaluation of the chemical coverage, reproducibility and linearity of the methods. The reliance of GNPS for non-targeted annotation was validated with chemical standards in the methods presenting the best performance. As a proof of concept, these methods were applied to characterize the metabolome of human serum clinical samples. A total of 1482 unique metabolites were annotated using all the methods. Iterative DDA allowed us to increase the number of unique compounds with high-quality fragmentation spectra up to 50%. GNPS annotations were validated using chemical standards, achieving over 80% spectral similarity with a false-positive rate of less than 5%. This robust evaluation strategy allowed us to identify the most informative complementary methods (RP and HILIC) while maintaining the confidence and reliability of the results in a cost-effective scenario and broadening their applicability and feasibility of the proposed strategy across diverse research contexts.

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Poster 88 - P88

Processing, annotation and integration of 1D and fast 2D (UF-COSY, NUS-TOCSY, NUS-HSQC) NMR lipidomic datasets

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Résumé

This work forms part of a national project within the MetaboHub infrastructure (*Fil rouge* santé), which aims to study the redundancy and the complementarity of metabolomics/lipidomics analytical methods. These methods include mass spectrometry and nuclear magnetic resonance, using both targeted and untargeted approaches. This project is applied to the study of the tumor suppressor p53. Here, the objectives are to process lipidomic NMR datasets, annotate the spectra in the most extensive manner and integrate the four datasets through a multiblock statistical analysis.

*Intervenant

Lyophilized livers of 112 mice (fed or fasted) of 8 different genotypes were extracted using the Bligh and Dryer protocol. Four different NMR acquisitions were performed on the lipidic extracts: 1D and fast 2D NMR (UF-COSY, NUS-TOCSY and NUS-HSQC). Several software programs were used to integrate the spectra and to perform an exhaustive annotation of the peaks according to their lipidic classes.
The spectra show different concentrations depending on whether the mouse was fed or fasted, and to some extent depending on their genotypes. Furthermore, the lipidic classes present in the liver were successfully annotated, by taking advantage of the complementarity between the four datasets. The multiblock integration currently ongoing will allow to give information about which of the four datasets discriminate best the samples. Future perspective might be to better comprehend whether some lipids are responsible for the concentration differences within genotypes.

Poster 89 - P89

The catabolism of branched-chain amino acids and tyrosine has a low contribution to the mitochondrial metabolism during senescence in Brassica napus L.

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Abstract

Winter oilseed rape (WOSR) is characterized by a low nitrogen remobilisation efficiency during developmental leaf senescence (DLS) compared to cereal crops. The catabolism of branched-chain amino acids (BCAA) and tyrosine plays a significant role in the recycling of protein-bound nitrogen and the fuelling of mitochondrial respiration during stress-induced senescence. In this study, we elucidated the role of BCAA and tyrosine catabolism during DLS in WOSR. To this end, we employed a multi-faceted approach, integrating transcriptional fingerprints, mitochondrial respiration measurements and 13C-labelling experiments during both DLS and dark-induced senescence (DIS). In general, the transcriptional regulation of BCAA and tyrosine catabolism was weakly correlated with apparent catabolic fluxes during DLS and DIS in WOSR. The absolute quantification and 13C-analysis of organic and amino acids by GC-MS showed that the catabolism of BCAA and tyrosine had a comparable contribution to the functioning of the tricarboxylic acid (TCA) cycle during DLS and DIS in WOSR. Isotopologue analysis of TCA cycle intermediates provided evidence that BCAA and tyrosine catabolism contribute minimally to the functioning of the TCA cycle during senescence. The role of BCAA and tyrosine catabolism could be essentially devoted to the recycling of nitrogen during senescence.

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Poster 90 - P90

L'ingénierie du microbiote des graines modifie la physiologie de la plantule et son métabolome lors du développement précoce

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Résumé

Le microbiote des graines, présent dès les premières étapes du cycle de vie de la plante, influence la germination, la levée et le développement des plantules. Sa modulation par inoculation ouvre des perspectives prometteuses en agriculture. Pourtant, les interactions précoces plante-microbiote et leurs effets sur le métabolisme des plantes restent peu comprises. Nous avons ici combiné essais de germination/levée, métabolomique non ciblée (GC-MS) et métabarcoding (gyrB, ITS1) pour caractériser les liens entre vigueur des graines et génotype, métabolome des graines matures et microbiote associés pour huit variétés de haricot commun (Phaseolus vulgaris L.). Plus de 300 métabolites ont été identifiés, dont certains, impliqués dans les métabolismes énergétique, lipidique, phénylpropanoïdes et nucléotides, sont associés à une meilleure vigueur. Des taxons microbiens indicateurs, tels que Cladosporium, Alternaria et Sphingomonas, ont également été corrélés à cette vigueur. Pour explorer les effets fonctionnels du microbiote des graines, nous avons ensuite inoculé des graines de haricot avec quatre communautés microbiennes synthétiques différentes. Le métabolome et le microbiote des plantules cultivées en terreau non stérile ont été suivis à différents stades précoces (de la graine à la plantule 7 jours après semis) et dans plusieurs compartiments. Les communautés inoculées modifient précocement le métabolome, notamment dans l'axe hypocotyle-radicule (stade imbibition) puis dans les cotylédons et feuilles (J+4). Elles se transmettent préférentiellement aux parties aériennes (> 60 % d'abondance relative), en cohérence avec les profils métaboliques. Ces résultats soulignent l'importance des interactions précoces plante–microbiote et ouvrent la voie à une ingénierie microbienne ciblée grâce aux approches métabolomiques.

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Poster 91 - P91

Quantification relative des disruptions métaboliques consécutives à différentes disruptions endocriniennes : une modélisation " homologue " à la mécanique du point utilisée en physique

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Résumé

La recherche de **biomarqueurs métaboliques** combine les méthodes statistiques multivariées de modélisation du métabolome et la connaissance *a priori* de **métadonnées** pour en réaliser une analyse supervisée. Mais les métadonnées sont rarement " questionnées ". Nous évaluons la qualité des métadonnées à partir des données clustérisées du métabolome, ceci en s'appuyant sur les méthodes d'**enchâssement** (embedding) développées par Coifman et Lafon (2005) sur des cartes de diffusion. Aux variables métabolomiques traitées par l'**algorithme PHATE** (Potential of Heat-diffusion Affinity-based Transition Embedding, Moon et al, 2019) nous joignons des **indicatrices** synthétisant les métadonnées. Leurs coefficients sont ajustés par **programmation non-linéaire** (Nelder-Mead) *via* la minimisation de la p-value d'une **MANOVA** à une voie décrivant la distribution des individus en clusters, à partir des coordonnées des individus calculées par la fonction **Phate()** sur trois dimensions.

Ce clustering est appliqué aux données métabolomiques produites par **RMN 1D** sur des **sérums** collectés sur **cyclistes** professionnels ou Elite amateur dont les **métadonnées endocriniennes** permettent de les classer *a priori* selon leurs valeurs normales ou non en

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cortisol, en testostérone ou en IGF1 (Paris et al, 2021).

Les scores transformés en coordonnées sphériques permettent d'établir les distances mesurées entre barycentres des différents clusters. i) Des faux-positifs sont projetés dans le cluster des témoins ; des faux-négatifs sont projetés dans des clusters de cas anormaux. ii) Les trajectoires métaboliques différenciant les témoins des cas en anomalie endocrinienne positive sont orthogonales à celles différenciant les témoins des cas en anomalie négative.

Cette procédure permet ainsi de fiabiliser les métadonnées.

Poster 92 - P92

Organic cultivation of garlic - expansion of the range of varieties through selection of plant genetic resources.

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Résumé

The increasing demand for organically produced garlic (Allium sativum) is challenged by the limited availability of high-quality site-adapted varieties. Although the genetic diversity of garlic is extensive, selection of suitable accessions for organic farming remains underdeveloped. The metabolic composition of garlic, particularly its organosulfur compounds, and bioactive molecules, plays a crucial role in its agronomic performance, sensory properties, and health benefits. Additionally, factors such as soil composition, climate, and fertilization significantly influence its metabolic profile, affecting both yield and flavor. However, despite the existence of genebank collections, large-scale metabolic fingerprinting efforts have not yet been applied to unlock the full potential of garlic genetic resources for organic farming. This study aims to integrate metabolomics, a high-throughput analytical approach that provides a detailed biochemical snapshot of plant metabolism, allowing the identification of key metabolites linked to desirable traits such as stress resilience, yield optimization, and enhanced flavor. Using both targeted and untargeted metabolomic techniques, including liquid and gas chromatography-mass spectrometry, this study analyzes the metabolic diversity of different garlic accessions from the IPK genebank planted under different organic farming conditions. The effects of genotype-environment interactions, including soil composition and sulfur fertilization, will be assessed to understand their impact on the metabolic expression of garlic. The integration of metabolomics into garlic breeding presents an innovative approach to overcome the limitations of traditional selection methods, which rely primarily on phenotypic traits. By leveraging metabolomics, this approach enables the development of virus resilient site-adapted garlic cultivars with improved agronomic performance and sensory attributes.

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Poster 93 - P93

WHEN ANAYSIS MEET STATISTICS - A STUDY CASE ON BIOACTIVES EXPLORATION IN HAWTHORN EXTRACTS

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Résumé

The cosmetics and beauty industry thrives on consumer preferences, with a growing emphasis on natural ingredients that are safe for consumers and beneficial for the environment, being non-ecotoxic and sustainably sourced.
In this context, a study was conducted to identify the 100 plants or ingredients of interest in cosmetics. Hawthorn (*Crataegus monogyna*) was ranked among the top 10 plants of interest as it is very common in Europe and is responsible for a wide range of human effects, including antioxidant, anti-inflammatory, anti-aging and anti-depressant(1).

Efficacy evaluation of Hawthorn extracts have confirmed the anti-aging properties of this plant; however, the active ingredients are not elucidated due to its chemical complexity. In the present study, a statistical correlation study on composition-activity relationship using untargeted UPLC-HRMS and RT-qPCR evaluation was applied to explore the bioactive components of Hawthorn.

The results achieved using two prediction models (supervised OPLS and Pearson's coefficient) indicated that 16 compounds (from the 405 followed) could contribute to gene modulation and thus be considered as possible anti-aging biomarkers. Some compounds were successfully characterized based on HRMS and MS/MS data but most of them remain unknown.

Perspective of this work will include the confirmation of characterized structure via co-injection of standards, the characterization of unknown compounds, and the evaluation of characterized structures to confirm their anti-aging properties.

(1) Nazhand A. et al. Hawthorn (Crataegus spp.): An Updated Overview on Its Beneficial Properties. Forests 11 (2020) 564-585.

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Poster 94 - P94

Phase I and II biotransformation investigations to characterize differences in zearalenone metabolism between human and rat primary hepatocytes

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Résumé

Zearalenone (ZEA) mycotoxin is a widespread contaminant that is found in human food and grains fed to livestock(1). In the European Union (EU), the presence of ZEA in food commodities is regulated(2). Zearalenone has a high estrogenic activity in vitro and in vivo. The present work is part of the ADME4NGRA (EFSA) project, which aims to develop *in silico* and *in vitro* assays in order to setting up PBK models to estimate the bioavailable concentration of compounds after oral uptake in the model organisms' rat and human. Interspecies differences of ZEA metabolism are studied in pooled primary human hepatocytes (PHH) and pooled primary rat hepatocytes (PRH). PHH and PRH in suspension were exposed to three non-toxic ZEA incubation concentration during four hours. Kinetic assays were performed in two biological replicates with three technical replicates. At different incubation timepoints, metabolism was stopped and ZEA and its potential metabolites (phase I and II) formed were extracted to be analyzed in UHPLC-HRMS system. A UHPLC-HRMS method was developed for the simultaneous quantification of parent compound ZEA and five of its phase I metabolites (α-ZEL, β-ZEL, α-ZAL, β-ZAL and ZAN) in cells culture medium. Early timepoints (15 min) allow quantification of β-ZEL (phase I metabolite) in PHH and PRH. Additionally, phase II metabolites (such as glucuronides or sulfate conjugates) were found in the full scan acquisition and then using a semi-quantitative method.

(1) Mauro et al., " 10.1016/j.fct.2018.04.027 ".

(2) Mendez-Catala, Wang, et Rietjens, "10.1002/mnfr.202100443".

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Poster 95 - P95

Développement d'une approche métabolomique pour évaluer l'impact de l'Éco-Exposome Portuaire sur les juvéniles de poissons

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Résumé

Compte tenu des activités anthropiques, la qualité des eaux portuaires est perturbée par des polluants tels que des éléments traces métalliques, des contaminants organiques et par des communautés microbiennes provenant d'effluents. Tous ces apports chimiques et biologiques, qui font partie de l'éco-exposome portuaire, constituent un risque pour les écosystèmes des ports et les espèces qui se développent dans les environnements côtiers. Chez les poissons, le risque écologique induit par les accumulations aiguës de polluants est connu, en revanche, leurs effets sur l'état physiologique des poissons in vivo et dans le cas d'une pollution chronique faible sont peu documentés. Il n'existe aujourd'hui aucun bio-indicateur du bon état physiologique des juvéniles de poissons in vivo. C'est dans ce contexte que nous proposons de développer une approche basée sur la métabolomique non ciblée afin d'évaluer l'impact de l'éco-exposome portuaire sur des juvéniles de poissons. L'espèce modèle choisie dans notre étude est le sar à tête noire (*Diplodus vulgaris*), poisson côtier d'intérêt halieutique. Les zones d'étude, contrastées d'un point de vue écologique, se situent dans le port de Port-Vendres (Pyrénées-Orientales) et dans une nurserie naturelle à 2 km au Nord du port (zone témoin). Les captures ont été effectuées par plongée à 3 stades de développement sur 2 années. Après extraction et analyse par UHPLC-HRMS, les résultats montrent une discrimination des poissons des ports et de la zone naturelle au deuxième stade de développement ; il semblerait que le métabolisme des acides aminés (tryptophane, méthionine, phénylalanine, alanine) soit affecté par l'environnement.

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Poster 96 - P96

Skin's lipidomic Analysis

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Résumé

Skin, our protective barrier, is covered by a hydrolipidic film composed of sweat and sebum. Within each skin layer, from the stratum corneum to the epidermis, various lipids play crucial roles in skin health, although some mechanisms are not well understood.
To better understand these lipids and their roles in skin health, combining lipidomic workflows with the development of specific non-invasive sampling methods allows for the targeting of lipids within these different layers. To be effective, a study based on criteria like skin types, gender, ages (40 volunteers per group), 2 sampling times (T0, Tx), generally involves around 500 samples, and about 100 lipids are measured.

Fresh superficial lipids (most likely related to sebaceous lipids) show significant variations depending on skin type. The difference between dry, normal, and oily skin is manifested not only by the quantity of lipids but also by their composition, particularly in the ratio of unsaturated to saturated fraction. Once on the skin, lipids undergo compositional changes due to the presence of microflora and other processes such as oxidation.
Further research is needed to fully explore the roles of specific lipids like ceramides within the stratum corneum's layers and their contribution to overall skin function. However, the limitations of non-invasive sampling, restricted to the uppermost layers of the stratum corneum, make studying lipids in deeper layers, including those potentially involved in inflammatory processes like resolvins, challenging.

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Poster 97 - P97

RnmrQuant1D: a package dedicated to 1D proton NMR quantification

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Résumé

This package was initially developed as part of an ANR project on wine authenticity (ANR-21-CE21-0014). However, it is generic enough to be used on other biological and/or food matrices. This involves the implementation of an analytical protocol allowing quantification from an external standard.
The approach is based on 1) an internal peak peaking followed by a deconvolution, also called peak fitting, 2) an identification of the zones for each compound using a quantification profile, grouping all the parameters in the same file, 3) a calibration using external standards.

The quantification profile allows you to configure all the steps of spectrum processing, from preprocessing to identification and integration, then to quantification of the targeted chemical compounds in the sample. It is to be noted that this quantification profile strongly depends on analytical conditions, namely the NMR field and sequence used, but also on the type of biological samples (wine, fruits, ...).

This package was designed to fully automate absolute quantifications on well-targeted compounds and samples, according to a specific analytical protocol to be used routinely by non-experts (use of Jupyter notebooks or encapsulated in a Galaxy workflow).

To this end, it has been successfully used to quantify nearly 40 compounds (organic acids, amino acids, alcohol, polyols, polyphenols, sugars, esters and aldehydes) in wine (white and red) from two sequences (zgpr and noesy) according to a specific analytical protocol (Guillaume Leleu et al. 2024, https://doi.org/10.58233/IMGGSIME).
This package, written in R, is open source (https://github.com/djacob65/RnmrQuant1D) and a complete tutorial is available online (https://github.com/djacob65/RnmrQuant1D/wiki).

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Poster 98 - P98

New bacterial DMSP utilization pathways in terrestial environments

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Résumé

Acinetobacter baylyi ADP1 (ADP1) is an aerobic soil bacterium capable of metabolizing sulfur-containing compounds like dimethylsulfoniopropionate (DMSP), a precursor to the climatically active gas dimethylsulfide (DMS). Unlike marine microorganisms, which use well-characterized DMSP catabolic pathways-demethylation, which generates 3-methylthiopropionate (MTPA), and cleavage, producing acrylate and DMS- ADP1 lacks the genes encoding the enzymes responsible for these pathways, suggesting novel metabolic mechanisms.
This project aims to identify the genes, metabolites, and enzymes involved in ADP1's DMSP catabolism. Transcriptomic analysis under sulfate starvation revealed over 100 sulfate-starvation-induced (SSI) genes, including a striking abundance of flavin-dependent monooxygenases and dioxygenases, indicating oxidative metabolic reprogramming under sulfate limitation. Using genomic resources, such as a mutant library and cloned genes for protein expression, we combined transcriptomics and mass spectrometry-based metabolomics to explore ADP1 sulfur metabolism.

We identified two novel oxidative DMSP catabolic pathways in ADP1. One involves previously undescribed metabolites in a unique demethylation pathway, while the other introduces a cleavage pathway involving dimethylsulfoxonium propionate (DMSOP), a compound linked to oxidative stress protection and carbon-sulfur cycling in marine ecosystems. These metabolites were also detected in *Pseudomonas putida* KT2440, suggesting these pathways may be widespread among terrestrial bacteria. Enzymatic screening revealed functional redundancy, with multiple monooxygenases acting on shared substrates, and deletion mutants confirmed their in vivo roles.

This study provides new insights into the mechanisms by which bacteria adjust their metabolism in response to available sulfur sources, and opens new avenues for exploring the ecological roles of microbial sulfur metabolism in terrestrial environments.

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Poster 99 - P99

Exploring xenometabolome signature through liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) data

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Résumé

People are increasingly exposed to chemicals in their daily lives through various external sources (e.g. food and air). The xenometabolome is the set of metabolites originating from these external exposures and represents an important aspect to be explored to characterize these exposures.

In this study, an untargeted data analysis approach was developed to extract potential exposure markers without prior assumptions in large dataset generated by high-resolution liquid-coupled mass spectrometry (LC-HRMS) analysis of meconium samples (n> 300) of a cohort study. The workflow included: i) a signal-cleaning step by selecting features corresponding to the specific 12C/13C isotopes, ii) filtering based on characteristic signal pairs, such as conjugated and non-conjugated metabolite forms or halogenated species and iii) a metric to quantify the detection frequency of each feature across samples.

The application of this data mining strategy enabled a substantial reduction in dataset size during the cleaning step, achieving more than a sixfold decrease for data generated in both positive and negative ion modes. The mass defect plots were used to reveal the presence of halogenated species as well as conjugated and non-conjugated metabolite pairs. Common xenobiotic markers, such as paracetamol, caffeine and nicotine, were successfully detected in meconium, revealing early-life exposures. The proposed strategy shows great potential for exploring the chemical exposome in large, untargeted LC-HRMS datasets. It is applicable to various types of matrices and compatible with data generated by any HRMS platform.

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Poster 100 - P100

KMDSeeker : Un Nouvel Outil d'Aide à l'Annotation de Familles de Composés Chimiques

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Résumé

L'annotation des signaux dans le cadre d'analyse d'empreintes métaboliques reste un défi majeur. Pour y répondre, nous avons développé **KMDSeeker**, un package R basé sur le **Kendrick Mass Defect (KMD)**. Cette méthode de normalisation des masses permet d'aligner les composés partageant des motifs répétés en attribuant une masse entière à un groupe fonctionnel spécifique (ex. CH = 14,000 Da). Ainsi, des triglycérides aux chaînes de longueur variable présentent un même KMD, facilitant leur regroupement et l'annotation dans des mélanges complexes.
Intégré à une application **RShiny**, **KMDSeeker** permet une annotation interactive et efficace des données issues de spectrométrie de masse. Après un **peak-picking** via **Galaxy W4M**, les tableaux clés (variableMetadata, sampleMetadata et dataMatrix) sont exploités pour visualiser les pics détectés et leur distribution selon le KMD.

L'outil génère un **KMD plot**, affichant les valeurs de KMD en fonction de la masse nominale. L'utilisateur sélectionne un **cluster** (défini par **CAMERA** et **XCMS**) pour examiner les formules chimiques suggérées via **EnviPat**. Il peut ensuite valider ou non l'annotation et explorer les autres clusters partageant le même KMD, facilitant ainsi l'identification de motifs récurrents.
Enfin, **KMDSeeker** permet d'intégrer et d'enrichir une **base de données locale**, mettant en évidence les standards et composés connus. Cela améliore la rapidité et la précision de l'annotation. Cet outil constitue ainsi une solution performante pour l'analyse et l'annotation semi-automatique, structurée et approfondie des familles de composés en spectrométrie de masse.

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Poster 101 - P101

Simultaneous quantitation and discovery (SQUAD) metabolomics workflow on the Orbitrap IQ-X for the analysis of fecal bile acids

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Résumé

Bile Acids (BAs) are synthesized from cholesterol in the liver and are integral to lipid digestion and absorption. In the gastrointestinal tract, fecal bile acids serve as critical biomarkers and signaling molecules, interacting intricately with the gut microbiota. Perturbations in the gut microbiome can modify the composition and volume of the bile acid pool, resulting in the generation of various conjugated bile acids and structurally analogous metabolites, which may be implicated in pathophysiological conditions.
The SQUAD workflow for simultaneous quantitation and discovery of fecal BAs and BA conjugates have been successfully developed . This comprehensive metabolomics approach utilizes advanced instrumentation, including the Thermo Scientific Vanquish Horizon LC and Orbitrap IQ-X Tribrid mass spectrometer, and isotopically labeled standards, to achieve accurate quantification and confident annotation of unknowns. Incorporating AcquireX and RTLS enhances the detection and annotation of BA-related metabolites, potentially relevant. Calibration curves were generated using both unlabeled and labeled BA standards to achieve absolute quantitation. This approach enables accurate quantification using an ion trap across a broad dynamic range, spanning five orders of magnitude, for the targeted BAs in feces. Most of the targets exhibited a lower limit of quantification (LLOQ) of 5 pg on-column. In the mice dietary intervention study, the method successfully provided absolute quantification of the targeted BAs and their conjugates in fecal samples. Additionally, untargeted analysis was employed to detect and annotate relevant compounds in the different experimental groups.

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Poster 102 - P102

Curation of Metabolome-Metagenome Associations through Network Topology Analysis

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Résumé

Metabolic diseases such as obesity and type 2 diabetes are characterised by low-grade inflammation, persistent alterations in circulating metabolites and gut microbiome composition. Mapping microbial functions and metabolites associated with metabolic diseases onto metabolic networks, can use a promising strategy to unravel direct connectivity between microbiome functional potential and metabolic output, highlighting joint mechanistic contribution in disease-related biological processes.
In a study of healthy and obese patients from the *MetaCardis* cohort ($n = 859$), we identified 177 plasma metabolites and 412 KEGG Functional Orthologies (KOs) associated with obesity using Spearman correlations adjusted for demographics. We then constructed a directed, KEGG-based metabolic network using the R package *MetaboSignal*, including metabolites with a KEGG identifier ($n = 105$). We subsequently calculated topological metrics and performed network-based analysis.

Topological analysis showed that, the shortest path distance between obesity-associated metabolite and gut microbial function pairs was significantly shorter ($p = 0.0039$) than

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that of unrelated pairs. Centrality analysis identified strongly connected microbial functions, with 14 metabolites exhibiting high interconnectivity. Pathway analysis of this subset revealed significant overrepresentation ($p < 0.01$) of several microbial degradation pathways (*e.g.*, aminobenzoate degradation). Many of these plasma metabolites are direct degradation products of dietary or environmental aromatic compounds, such as polyphenols. These findings suggest that, in obesity, an altered gut microbiome drives specific xenobiotics degradation processes that generate bioactive metabolites, potentially contributing to metabolic dysregulation through inflammatory or endocrine effects.
Our integrated workflow for topological analysis of microbiome-host metabolic networks reveals disease-specific connectivity between the gut microbiome and metabolome.

Poster 103 - P103

La supplémentation maternelle en Saccharomyces boulardii améliore la croissance néonatale et module le métabolisme d'azote chez le chien

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Résumé

La programmation nutritionnelle périnatale permet d'influencer le développement du nouveau-né via l'alimentation maternelle. Chez le porc, la supplémentation en levures a montré des effets bénéfiques sur la santé des petits. La présente étude explore les effets d'une supplémentation en *Saccharomyces boulardii* (SB) chez des chiennes gestantes sur le métabolisme et la croissance précoce de leurs chiots.
Dix-sept chiennes et leurs 81 chiots ont été inclus. À partir du 28e jour de gestation, les femelles ont reçu quotidiennement deux capsules de placebo ou de SB (12,8 × 10 UFC), constituant deux groupes distincts. Le taux de croissance précoce (EGR) a été calculé entre la naissance (J0) et deux jours d'âge (J2). À J2, sera et urines ont été prélevés pour une analyse métabolomique par la chromatographie liquide couplé à la spectrométrie de masse à haute résolution (LC-MS). Les métabolites discriminants ont été sélectionnées par sPLS-DA (VIP $>$ 1) et corrélés à l'EGR via des tests de Pearson.

Les chiots du groupe SB ont présenté un EGR significativement plus élevé (p = 0,049). Leurs profils métabolomiques différaient nettement de ceux du groupe Placebo. Quatorze métabolites discriminants appartenaient à la voie du métabolisme d'azote. Notamment, la proline urinaire, impliquée dans la synthèse du collagène, était plus abondante et positivement corrélée à l'EGR.
Ces résultats suggèrent que la supplémentation maternelle en SB pourrait favoriser la croissance néonatale via la régulation du métabolisme d'azote. Des recherches supplémentaires sur la microbiote intestinale sont en cours pour explorer d'autres mécanismes d'action.

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Poster 104 - P104

Phenolic compounds profiling of honeys from French guiana

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Résumé

Honey is a delicious sweet natural matrix produced by honeybees from flower nectar or honeydew, and has been consumed by humans for centuries. This functional food is known for its advantageous effects on health such as antioxidant, anti-inflammatory or wound healing properties, thanks to its phenolic content and bioactive agents (1). These compounds are mainly linked to the botanical and geographical origin of the nectar sources (2). Previous work showed a high content in phenolic compounds in French Guiana honeys (3). Our study is the first to identify phenolic compounds of French Guiana honeys. For this purpose, 28 samples of honey from different areas were extracted by dispersive liquid-liquid microextraction (DLLME), and then analyzed by high-resolution mass spectrometry (HRMS) to obtain phenolic profiles. Results showed a high content of total phenols (between 30 and 90 mg GAE/100 g honey). In addition, comparison with MS library and honey compounds database led to 16 compounds such as *p*-coumaric acid, pinobanksin, pinocembrin, and chrysin (Figure 1). Dereplication highlighted abscisic acid derivatives as the four major peaks. Finally, 10 markers related to honey geographical origin were determined through untargeted screening and chemometric tools allowing to distinguish two groups: seaside forest honey and savanna forest honey. With this study, we have showed that metabolomics tools are reliable to assess honey authenticity and marker of geographical origin.

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Poster 105 - P105

Increasing plant metabolome coverage using Enhanced Dynamic Range (eDR) scan mode on a modified Orbitrap Hybrid mass spectrometer

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Abstract

High-confidence detection and identification of low to medium abundant metabolites in complex matrices pose significant challenges in LC-MS-based metabolomics. Segmenting the mass range into narrower windows, known as the BoxCar or spectral stitching approach, has shown to improve signal-to-noise ratios and increase the quality of detection of low-abundance molecules. We advanced these concepts by implementing intelligent MS1 multiplexing strategies with optimized injection times. This resulted in the Enhanced Dynamic Range (eDR) scanning mode on the modified Orbitrap™ Hybrid MS to increase metabolome coverage.
Enhanced Dynamic Range (eDR) refers to a scanning mode designed to extend the dynamic range of MS measurements. This is achieved by dividing the mass range into two separate Orbitrap MS1 subscans, each encompassing alternating mass range windows. For each subscan, quadrupole automatically isolates different m/z windows based on the user-defined settings, which are then transferred to Orbitrap in a single injection.
In this study, we analyzed tea extract samples on MS1 level to assess the increase in detected metabolome coverage resulting from eDR, and on MS2 level with DDA AcquireX Deep Scan mode to evaluate its impact on fragment-based annotation. The MS1 analysis, after background subtraction in eDR mode, resulted in more than a threefold increase in detected compounds (CVs $\leq$ 20%) compared to the legacy full scan. We also noticed an increase in spectral signal-to-noise ratios as well as lower CVs for selected low and medium intensity signals, some of which were not detected in the legacy full scan.

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Poster 106 - P106

Impact of Pre-analytical Variability on Metabolomic Stability: Toward Standardized Clinical Applications

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Résumé

Clinical metabolomics is emerging as a powerful tool in laboratory medicine for diagnostic and prognostic purposes. However, integrating this approach into clinical practice requires standardized pre-analytical protocols, aligned with ISO 15189 standards. This study explores the impact of pre-analytical conditions on blood metabolome stability across different matrices.
Initial experiments assessed metabolomic stability in four matrices (serum, EDTA plasma, heparin plasma, fluoride plasma) collected from four healthy donors. NMR spectroscopy was used to quantify metabolites after immediate centrifugation and after a 4-hour delay. Serum, showing the highest stability, was selected for further analysis on samples from four additional donors. These samples were subjected to varied conditions: centrifuged immediately and frozen after 0, 4, or 24 hours at 4∘C or 20∘C; or stored uncentrifuged at 4∘C or 20∘C for 1, 4, or 24 hours before processing. Metabolic variations were evaluated using PCA, PLS-DA, and repeated-measures ANOVA.

Outliers were excluded via PCA, while PLS-DA and ANOVA confirmed significant differences in metabolite stability under different conditions. Serum consistently showed better stability than plasma. Immediate centrifugation best preserved the metabolome, but when not possible, storage at 4∘C was clearly preferable to room temperature.
These findings underline the critical influence of pre-analytical factors on metabolomic profiles and support serum as the matrix of choice. This work offers a foundation for developing standardized protocols for sample handling, crucial for implementing metabolomics in routine clinical diagnostics and personalized medicine.

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Poster 107 - P107

New R Shiny applications designed to improve the metabolomics workflow have highlighted the impact of Neofusicoccum parvum on grapevine metabolism

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Résumé

In the context of global warming, grapevines have to cope with a variety of biotic and abiotic stresses, leading to an increase in the incidence of decline diseases caused by pathogenic fungi, which can even lead to the death of the vine. Since the ban on sodium arsenite, vine growers have no means of controlling the disease. In order to determine the health status of the grapevines and to propose new means of control, we need to understand the interaction between these pathogenic fungi and the plant. Therefore, we are carrying out metabolomic studies under different experimental conditions in order to identify markers of infection as well as markers of grapevine resistance.

To optimize our metabolomics workflow to highlight these biomarkers as efficiently as possible, we have developed several tools with R Shiny: Cinderella cleans our GC-MS and LC-MS/MS data, MetaboStat performs statistical analyses to highlight biomarkers of interest, and DBOmics compiles all the results from the different experiments to provide an overview of each biomolecule considered.

These tools will be presented through our study of the effects of infection of grapevines by the fungal pathogen *Neofusicoccum parvum* strains Bourgogne and Bt67, for which we performed GC-MS and LC-MS/MS analyses on extracts of wood, leaves and roots from Chardonnay and Gewurztraminer plants infected or not with the fungal pathogen.

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Poster 108 - P108

logiciel de traitement des données métabolomiques basé sur segmentation des données par méthodes multi-blocs

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Résumé

MetaboSmart, un logiciel pour faciliter l'analyse et la représentation des données de métabolomiques et multi-échelles.
La métabolomique génère des données complexes qu'il est parfois difficile d'interpréter. Nous avons développé un logiciel permettant de stratifier l'information métabolique en blocs (fonctionnels ou statistiques), réduisant ainsi la dimensionalité et facilitant l'interprétation et la représentation des systèmes métaboliques.

Le logiciel utilise des scripts R et l'interface utilisateur R Shiny.

Les métabolites annotés sont regroupés par l'opérateur en blocs selon des critères d'ontologies fonctionnelles qu'il choisit, ou de grappes statistiques (méthode WGCNA). Pour chaque observation, une valeur combinatoire (score) est calculée pour chaque bloc à l'aide d'une méthode d'ACP, de PLS ou d'OPLS hiérarchiques. Les matrices de scores générées par chaque modèle sont comparées pour déterminer celle qui correspond le mieux à l'ACP des données initiales. Enfin, des techniques de classification, notamment PLS-DA, OPLS-DA, régression logistique, Random forest sont appliquées à la matrice retenue afin de déterminer les fonctions biologiques les plus déterminantes dans la classification des individus. Les interactions entre fonctions (blocs) peuvent être visualisées sous forme de réseau d'interactions. L'outil vise à optimiser l'interprétation des données métabolomiques en exploitant des approches avancées de modélisation statistique.
Cette méthode est également applicable à la lipidomique et aux données multi-échelles regroupées selon des critères fonctionnelles, permettant de les représenter dans le même espace normalisé, de les assembler et de les comparer.

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Poster 109 - P109

COSMECUR : COSmétique et MEtabolomique comparative du CURcuma de Mayotte

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Résumé

Le curcuma (*Curcuma longa*) est reconnu pour ses propriétés antioxydantes, anti-inflammatoires et colorantes, largement exploitées en agroalimentaire et cosmétique. Toutefois, la richesse et la diversité de son métabolome varient selon l'origine géographique, influençant son potentiel d'application. Dans ce contexte, nous avons comparé les empreintes chimiques d'extraits de curcuma provenant de Mayotte et du Pérou, afin d'identifier des métabolites à forte valeur ajoutée pour l'industrie cosmétique.
Les échantillons ont été analysés par LC-HRMS Q-Exactive Plus, générant des données haute résolution soumises à une exploration métabolomique non ciblée. Les réseaux de similarités spectrales ont permis de regrouper les signaux en clusters structuraux, facilitant l'annotation automatisée et manuelle des composants. L'utilisation de FBMN et de SIRIUS ainsi qu'un travail bibliographique approfondi a complété l'identification et l'annotation, en mettant en regard les fonctions bioactives rapportées et les applications potentielles.

Nos analyses ont mis en évidence plusieurs familles de composés, notamment des curcuminoïdes dont la concentration relative est significativement plus élevée dans le curcuma de Mayotte. Parmi eux, des dérivés hydroxylés et glycosylés présentent un intérêt marqué pour leurs activités antioxydantes et hydratantes, suggérant une utilisation comme agents photoprotecteurs et actifs anti-âge.
Ce poster présentera l'ensemble de ces résultats soulignant la spécificité métabolique du curcuma de Mayotte et ouvrent des perspectives de valorisation ciblée en cosmétique, via l'isolement de molécules bioactives et le développement de formulations innovantes.

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Metabolomic insights into Mindfulness: exploring the impact of non-judgmental self-awareness meditation on Healthcare Student well-being

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Résumé

Today, there is growing evidence that healthcare workers are experiencing high levels of stress and psychological distress. This can alter their prosocial abilities, such as empathy and the capacity to support others, ultimately affecting their daily performance and the quality of care provided to patients. Although this issue is beginning to gain recognition, little has been done to formally address it – particularly during their training, when students already face considerable stress and mental strain. In this context, Mindfulness-Based Interventions (MBIs) have shown effectiveness in various clinical settings. This work presents an extensive metabolomic study involving multiple data blocks to explore the potential of Mindfulness-Based Cognitive Therapy for Life (MBCT-L) for reducing anxiety and stress while enhancing the overall well-being of healthcare students. First, clinical scores obtained from questionnaires were investigated using dimensionality reduction and hierarchical clustering (PCA-HCPC) to stratify the cohort into three distinct groups, namely "High Improvement", "Moderate Improvement", and "Low to No Improvement". Then these groups were further used to drive chemometric analyses on several metabolomic data blocks to deconvolute the biological modulations underlying the effects of MBCT-L and link these signatures to the observed clinical outcomes. The preliminary results obtained make it possible to discuss biological hypotheses to describe the beneficial effects of the intervention on participants' well-being. This work opens new perspectives for a better understanding of the benefits associated with mindfulness practice in the studied population.

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Poster 111 - P111

IS WEIGHING ENOUGH TO DETERMINE THE AMOUNT OF STRATUM CORNEUM REMOVED BY TAPE STRIPPING? AN ALTERNATIVE APPROACH USING CERAMIDE QUANTIFICATION

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Résumé

This study presents a novel analytical method for quantifying stratum corneum (SC) removal during tape stripping based on SC marker quantification using UHPLC-MS/MS. An initial untargeted LC-HRMS analysis identified potential SC markers, leading to a refined targeted approach for quantifying specific SC markers, such as ceramides. The method involved analyzing fourteen successive tape strips from thirty skin samples and correlating the quantified SC marker levels with the SC weight removed. Results demonstrated a strong correlation between specific SC marker concentrations and the measured SC weight. This highly specific and sensitive analytical method offers significant advantages over traditional gravimetric and imaging techniques by minimizing potential artifacts. Applicable to human skin models, this quantitative method can be combined with drug analysis to improve the assessment of dermatopharmacokinetic parameters and topical formulation input rates by enabling accurate measurement of chemical concentration profiles within the SC.

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Poster 112 - P112

Investigating the Accelerated UV Ageing of Dyes Using Untargeted LC-MS-based Metabolomics

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Résumé

The degradation of natural dyes is a primary challenge for their identification in historical textiles. Flavonoids, the main natural yellow dye compounds, have a poor light fastness that not only changes the perceived colour of an historical fabric, but can also bias the identification of the plants that were used to dye it. In this context, an untargeted LC-MS-based metabolomics strategy has been developed to analyse dye compounds and other molecules found in dyed fabrics. This process will allow a better comprehension of the UV-induced degradation of molecules both from the dye extract and the fabric.

A series of cotton fabric samples have been dyed with European buckthorn berries (*Rhamnus cathartica* L.) and then subjected to accelerated UV ageing corresponding to 1, 10 or 100 years of natural sunlight. First, CIEL*a*b* colorimetric measurements and E calculation were used to assess the colour variations. Next, a soft extraction method was used to recover the dye compounds and the resulting extracts were analysed by UPLC-DAD/HRMS.

A correlation between UV exposure and colour fading was observed using CIEL*a*b* data and quickly explained by the degradation of the main dye molecules. This untargeted metabolomics approach highlighted an ageing-related discrimination between samples that was related both to buckthorn chemomarkers, and to compounds coming from the fabric itself. Dye compounds and degradation products were putatively annotated. These results will be used to better understand light-induced ageing of dyed fabrics to assist future research on historical textiles.

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Poster 113 - P113

Untargeted NMR-based metabolomics identifies a metabolic signature depending on the type of kidney transplant donor

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Résumé

Background: Kidney transplant outcomes differ between kidneys from brain-dead donors (DBD) and circulatory-dead donors (DCD). While DCD grafts are more often associated with delayed graft function (DGF), DBD grafts that develop DGF tend to show poorer long-term survival. These differences suggest distinct underlying injury mechanisms depending on donor type. In this context, identifying metabolic biomarkers may offer valuable insights into graft quality and help refine pre-transplant evaluation to improve both short- and long-term outcomes.
Objective: This study applies untargeted NMR-based metabolomics to investigate metabolic alterations between DBD and DCD kidney tissues.

Methods: Kidney samples from rats (7 controls, 7 DCD, 9 DBD) were collected. Tissue powders were analysed by 1H-NMR spectroscopy, followed by multivariate OPLS-DA and univariate statistical analyses (ANOVA, t-tests).

Results:

- OPLS-DA revealed distinct metabolic profiles between DBD and DCD and controls.
- ANOVA identified 34 significantly different metabolites across groups.
- T-tests (DBD versus DCD) identified 19 metabolites, including increased levels of pyridoxine, 2-hydroxybutyrate and 2-oxocaproate in DCD kidneys, while glutamate, lysine, and pyroglutamate were decreased.
- Metabolomic signatures were partly consistent between kidney tissue and previously analysed perfusate samples.

Conclusion: This study demonstrates that NMR-based metabolomics can differentiate DBD from DCD kidneys and identify specific metabolic patterns. Further validation in larger human or animal cohorts could establish metabolomics as a tool for pre-transplant graft assessment, improving decision-making.

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Poster 114 - P114

Validation d'une méthode de quantification large échelle couvrant un large panel de biomarqueurs nutritionnels

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Résumé

La quantification d'une grande diversité de métabolites reste le défi majeur en métabolomique. Elle permettrait d'obtenir des résultats plus précis, inter-comparables, et de proposer des combinaisons de plusieurs biomarqueurs, plutôt que de se limiter à des biomarqueurs uniques. Dans le cadre de recherches en nutrition, étudiant l'impact des comportements alimentaires sur la santé, une méthode de quantification d'un large panel de biomarqueurs nutritionnels permettrait de considérablement améliorer l'évaluation de la consommation de certains aliments, pour à terme permettre de meilleures recommandations nutritionnelles.

La quantification simultanée d'un grand nombre de métabolites présente néanmoins plusieurs défis, notamment en termes de complexité de calibration, de validation, d'analyse, de retraitement, d'où la nécessité d'automatiser certaines étapes. Des questions se posent également quant à la transférabilité d'une telle méthode entre différents laboratoires. Concernant la précision, un défi supplémentaire est à relever car à ce jour il n'existe pas de recommandations consensus pour la validation d'une méthode ciblée large échelle.
Pour répondre à ces enjeux, nous avons développé une méthode de quantification d'une centaine de biomarqueurs nutritionnels dans des échantillons d'urine humaine. Un essai interlaboratoire a été mis en place afin d'évaluer la fiabilité de notre méthode, mais également de déterminer la dilution optimale à utiliser pour les échantillons d'urines. Deux modes de dilution ont été testés : une dilution basée sur l'osmolalité et une dilution fixée à un ratio $\frac{1}{2}$.
L'objectif est de garantir une utilisation efficace de notre méthode sur différentes cohortes, contenant près de 1000 échantillons, dans le cadre de recherches nutritionnelles variées.

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Poster 115 - P115

Développement d'une méthode LC-MS/MS de dosage simultané des hormones thyroïdiennes (T4, T3 et rT3) dans différentes matrices biologiques

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Résumé

Les êtres vivants sont exposés à diverses substances avérées ou suspectées d'être des perturbateurs endocriniens, tels que les bisphénol, les phtalates présents dans les plastiques et certains pesticides.
Ces substances altèrent le fonctionnement hormonal et induisent des dysfonctionnements physiologiques. La perturbation du fonctionnement des hormones thyroïdiennes (HT) entraine des conséquences sur le développement du cerveau chez les mammifères et impacte la métamorphose des amphibiens.

Le dosage de ces hormones (T4, T3 et rT3) est un indicateur des effets possibles des perturbateurs endocriniens sur les organismes vivants.

Le dosage des HT par LC-MS/MS est une technique encore peu répandue mais tend à remplacer le dosage radio-immunologique actuel, car elle peut allier sensibilité de détection et reproductibilité tout en ciblant simultanément plusieurs analytes à quantifier.

La méthode d'extraction, utilisant un échangeur de cations, reprend celle mise en place par l'Amsterdam Institute for Life and Environment (1,2). Les performances analytiques ont été évaluées sur différentes matrices : sérum, urine et tissus.

Les résultats obtenus avec le sérum montre une limite de détection de 2,5 pg/μL pour T4

*Intervenant

(_˜3,2nM) limite comparable pour T3 et rT3 (_˜3,8nM). Les essais sur têtards entiers sont en cours d'optimisation. L'ajout d'hormones marquées en début de protocole permet de suivre les rendements d'extraction, actuellement estimés à environ 70 %.

Cette méthode promet d'être un outil fiable, sensible et robuste pour évaluer les perturbations du système thyroïdien dans divers contextes d'exposition environnementale.

1. Hansen et al, Anal. Bional. Chem. (2016)
2. Pannetier et al, Environ. Toxicol. Chem. (2023)

Optimization of a Soil Metabolite Extraction Protocol for High-Throughput Screening of Truffle-Associated Biomarkers

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Résumé

Mycorrhization, a complex symbiosis between fungi and plant roots, is critical to ecosystem functioning and truffle cultivation. However, successful mycorrhization and consistent truffle production depends on multiple poorly understood factors, complicating its control in agricultural settings. The soil metabolome offers a promising but underexploited avenue for understanding and predicting mycorrhization. Metabolite profiling may enable predictive metabolomics as a tool to predict and explain variability in symbiosis success and enhance truffle production. However, although current extraction methods are effective they are hindered by low-throughput, limiting their scalability.
This project aims to optimise and automate a metabolite extraction protocol for high-throughput soil analysis without compromising efficiency. A two-step ethanol extraction was implemented: first with 80% ethanol, then 50%, followed by filtration, SpeedVac concentration, freeze-drying, and resuspension in 80% ethanol. The protocol was tested on two soil types from the Aquitaine region (Cestas and Sarlande), each sampled under two ectomycorrhizal host trees: Acer pseudoplatanus and Calocedrus decurrens. Soil input masses of 50, 100, 200, and 400 mg were compared to find the best trade-off between efficiency and throughput.

Metabolite profiling via LC-HRMS is ongoing to evaluate extraction quality and metabolic diversity. The best-performing conditions will be scaled up using robotic phenotyping on hundreds of samples.
This optimised protocol will enable the realisation of large-scale soil metabolomic studies, support biomarker discovery, and contribute to better understanding of ectomycorrhizal interactions, with the aim of ultimately improving truffle cultivation strategies.

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Poster 117 - P117

Analyse ciblée de métabolites : Comparaison entre DBS et Sérum par LC-MRM

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Résumé

Les échantillons de type *Dried Blood Spot* (DBS) constituent une méthode d'échantillonnage et de conservation à la fois pratique et polyvalente. Cette matrice est devenue un outil incontournable dans de nombreuses applications telles que le dépistage néonatal, les tests de maladies infectieuses, l'analyse génétique, etc. Le DBS est désormais largement utilisé comme alternative au prélèvement sanguin, en raison de son caractère peu invasif, de sa facilité de transport et de son coût réduit.
La plateforme dispose d'une méthode métabolomique ciblée validée pour l'analyse du sérum humain. Face à l'intérêt croissant de l'utilisation de la matrice DBS, nous prévoyons d'adapter et de mettre en œuvre une méthode, transférée depuis la plateforme de Tours, afin d'élargir le catalogue d'analyses disponibles sur la plateforme. Nous proposons une méthode analytique permettant de quantifier plusieurs familles de métabolites : les acides aminés, dérivés du tryptophane, acides organiques et acides biliaires. Les échantillons sont préparés sur des plaques 96 puits comprenant deux dérivatisations distinctes. L'analyse est ensuite réalisée par LC-MRM à l'aide d'un système Agilent 6495d fonctionnant en mode positif pour les acides aminés et les dérivés du tryptophane, et en mode négatif pour les acides organiques et les acides biliaires. Le traitement de données est assuré par le logiciel Skyline.
Cette étude va permettre de mettre en place une méthode d'analyse spécifique à la matrice DBS. Une comparaison des profils métaboliques ciblés et quantitatifs entre les matrices DBS et sérum sera réalisée afin d'identifier les différences et similitudes pour chaque famille de composés analysés.

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Poster 118 - P118

Study of the uptake of labelled glucose in the biosyntehsis pathway of polyphenols using cell suspension cultures

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Résumé

Plant cell suspension cultures have proven to be an interesting tool for the production of secondary metabolites thanks to several advantages such as rapid growth (exponential phases in less than a week) and easily modulable controlled cultivation conditions. Coupled with metabolomic and fluxomic studies, they can serve for analysing the modulation of compounds of interest biosynthesis and better understanding the effects of elicitors, biotic and abiotic stresses on plant metabolome. In our study, we used a cell suspension culture of *Vitis vinifera* cv *Gamay Teinturier,* well known for its ability to produce a wide range of phenolic compounds (anthocyanins, stilbenes and catechins). The nutrient medium was replaced at the exponential phase by fresh medium containing labelled glucose and a kinetic study was conducted on the following 4 days (9 points of analysis). We analyzed the metabolome under a targeted approach by UHPLC-HRMS (Q Exactive, Thermo). We could observe an early incorporation of the labelling to the main polyphenols, with a different rate of accumulation for some isotopologues. Interestingly, we did not find for the glycosylated polyphenols a priority incorporation of the labelling in either the glucoside moiety or in the aglycon form.

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Poster 119 - P119

Unveiling the Metabolic Impact of Growth Hormone in Dairy Cows: An Integrated Metabolomics Analysis of Milk and Urine

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Résumé

Recombinant bovine growth hormone (rbGH) is a synthetic analogue of natural somatotropin, used to promote growth and milk production in dairy cattle. While permitted in certain countries, its use is strictly prohibited in Europe due to animal welfare and consumer safety concerns. Consequently, reliable analytical methods-such as immunoassays and liquid chromatography-tandem mass spectrometry (LC-MS/MS)-are essential for monitoring its illicit use.

In this study, urine and milk samples were collected from cows treated with rbGH at multiple time points, up to 219 days after initial administration. A longitudinal analysis was conducted using advanced metabolomics and lipidomics approaches based on liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). This untargeted strategy enabled the detection of subtle and time-resolved molecular changes associated with hormonal exposure. Urine analysis revealed comprehensive metabolic alterations, while lipidomics profiling of milk highlighted dynamic shifts in lipid composition over time. To further elucidate the biological impact of rbGH, the most discriminant molecular features were subjected to targeted fragmentation (PRM) on an orthogonal mass spectrometry platform, providing structural information and aiding in the identification of affected metabolic pathways.

By leveraging state-of-the-art LC-HRMS technologies, this work provides valuable insights into the systemic effects of rbGH and supports the discovery of novel, yet-to-be-identified molecular biomarkers for improved monitoring. These findings enhance the analytical toolbox available for regulatory surveillance and contribute to a deeper understanding of the biological impact of rbGH administration in livestock.

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Poster 120 - P120

Caractérisation de la biomasse lignocellulosique prétraitée sous forme de pastilles par spectrométrie de masse MALDI-FT-ICR.

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Résumé

Dans le cadre du projet Amaretto (PEPR B-BEST), l'objectif est d'identifier des marqueurs et de prédire la réactivité de la biomasse lignocellulosique lors de l'hydrolyse enzymatique durant le procédé de production du bioéthanol. Pour cela une caractérisation de fraction solide de la biomasse lignocellulosique prétraitée est mise en œuvre.
Des pastilles d'échantillons solides sont préparées, avec ou sans mélange de matrice, et analysées directement par spectrométrie de masse par résonance cyclotronique ionique à transformée de Fourier (FT-ICR) couplée avec une source de désorption/ionisation par laser assistée par matrice (MALDI). L'optimisation de la méthode a porté sur le choix de la matrice (9AA, DHB, HCCA), sa concentration massique, le mode de préparation (mélange homogène par broyage, ou dépôt par spray en surface de pastille), ainsi que sur l'optimisation des paramètres d'analyse (puissance du laser, mode d'ionisation positif et négatif, et gamme de m/z).

Les analyses LDI en mode négatif ont permis de détecter des petites molécules de m/z $<$ 250 correspondants à des monomères issus de la lignine et des hémicelluloses, sans nécessité d'ajout de matrice. En parallèle, des analyses sont menés en mode positif MALDI afin d'optimiser la détection des composés de plus haute masse ($m/z >$ 250).
Ces résultats montrent l'intérêt de la combinaison LDI/MALDI-FTICR pour cartographier la fraction chimique des biomasses prétraitées. L'étape suivante portera sur l'annotation systématique des ions détectés et l'analyse statistique des ces résultats.

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