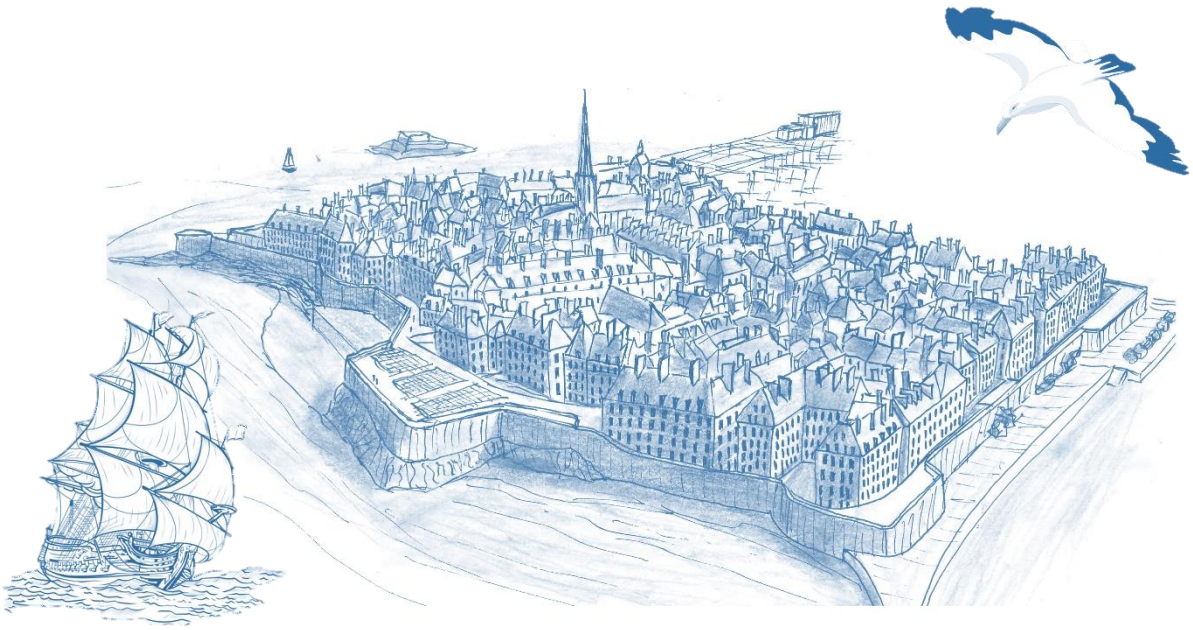
RÉSEAU FRANCOPHONE DE MÉTABOLOMIQUE ET FLUXOMIQUE

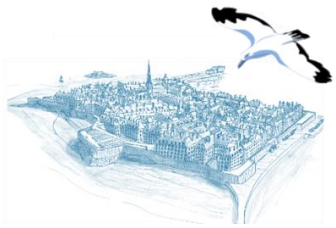
16èmes Journées Scientifiques

du Réseau Francophone de Métabolomique et de Fluxomique



3 au 6 juin 2024

Au Palais du Grand large de Saint-Malo (35)



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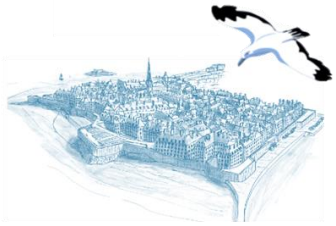


16 èmes Journées Scientifiques du RFMF

4-6 juin 2024, Saint-Malo

Partenaires institutionnels

des 16èmes Journées Scientifiques du RFMF

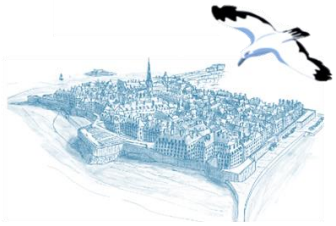
INRAE
la science pour la vie, l'humain, la terre

SIB
Swiss Institute of Bioinformatics

Université de Rennes

METABOHUB

RFMF
Réseau Francophone de Métabolomique et Fluxomique



16 èmes Journées Scientifiques du RFMF

4-6 juin 2024, Saint-Malo

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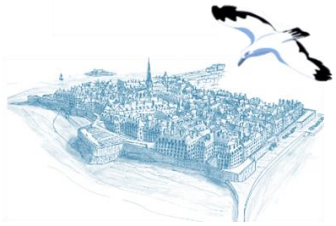


16 èmes Journées Scientifiques du RFMF

4-6 juin 2024, Saint-Malo

Partenaires Industriels Silver des 16èmes Journées Scientifiques du RFMF

BRUKER

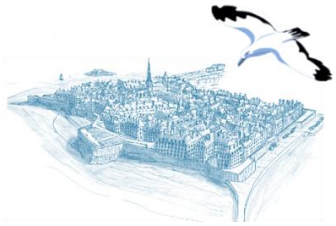
MERCK

LNI SWISSGAS

Partenaires Industriels Bronze des 16èmes Journées Scientifiques du RFMF

CHENOMX NMR Mixture Analysis

Eurisotop A CAMBRIDGE ISOTOPE LABORATORIES COMPANY

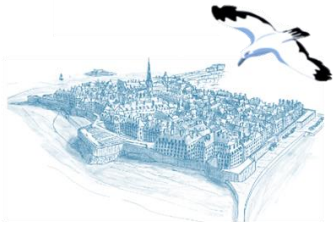


Le comité d'organisation

Le comité local

- Alain Bouchereau (PR Université de Rennes, IGEPP/P2M2)
- Pauline Le Boulch (Post-Doc IGEPP/P2M2)
- Sophie Charton (AI INRAE, IGEPP/P2M2)
- Sylvain Chereau (IE INRAE, IGEPP/P2M2)
- Mikaël Croyal (IR Nantes Université, CNRH)
- Thomas Delhaye (IE Université de Rennes, IETR/SM2)
- Younès Dellero (CR INRAE, IGEPP/P2M2)
- Olivier Filangi (IE INRAE, IGEPP/P2M2)
- Antoine Gravot (PR Université de Rennes, IGEPP/P2M2)
- Sylvain Guyot (DR INRAE, BIA/P2M2)
- Marine Letertre (MC Nantes Université/CEISAM)
- Anne Levrel (TR INRAE, IGEPP)
- Nathalie Marnet (IE INRAE, PRP/P2M2)
- David Rondeau (PR Université de Rennes, IETR/SM2)
- Mathieu Aubert (PhD IGEPP)
- Youcef Haddad (PhD IGEPP)
- Eloïse Ledoux (CDD AI, IGEPP/P2M2)
- Léo Andruszkow (CDD IE, IGEPP/P2M2)

IGEPP : Institut de Génétique, Environnement et Protection des Plantes - La Motte au Vicomte BAT 301, 35650 Le Rheu
P2M2 : Plateforme de Profilage Métabolique et de Métabolomique - La Motte au Vicomte BAT 301, 35650 Le Rheu
CNRH : Centre de Recherche en Nutrition Humaine Ouest (CRNH Ouest) - 5 allée de l'Ile Gloriette, 44093 - NANTES cedex 1
IETR : Institut d'Electronique et des Technologies du numéRique - 35700 Rennes
SM2 : Spectrométrie de Masse & Métabolomique - 35700 Rennes
BIA : Biopolymères Interactions Assemblages - La Motte au Vicomte BAT 305, 35650 Le Rheu
CEISAM : Chimie Et Interdisciplinarité, Synthèse, Analyse, Modélisation - 2 Chem. de la Houssinière, 44300 Nantes



Le conseil d'administration du RFMF

- Cédric Bertrand, Centre de Recherches Insulaires et Observatoire de l'Environnement, Université de Perpignan, Perpignan (66), France
- Samuel Bertrand, UFR des sciences pharmaceutiques et biologiques, Université de Nantes, Nantes (44), France
- Justine Bertrand-Michel, Plateforme MetaboHUB-MetaToul, I2MC Inserm Toulouse, Toulouse (31), France
- Benoit Colsch, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS) , MetaboHUB-IDF, Gif-sur-Yvette (91), France
- Cédric Delporte, Unité RD3-Pharmacognosie, bioanalyse et médicaments (RD3-PBM) & Plateforme analytique de la Faculté de Pharmacie (APFP), Université libre de Bruxelles (ULB) , Bruxelles, Belgique
- Corentine Goossens, Centre de Recherches Insulaires et Observatoire de l'Environnement, Université de Perpignan, Perpignan (66), France
- Audrey Le Gouellec, Université Grenoble Alpes et CHU Grenoble, Grenoble (38), France
- Florence Mehl, Institut Suisse de Bioinformatique, Lausanne, Suisse
- Pierre Petriacq, UMR1332 Biologie du Fruit et Pathologie/ Plateforme Bordeaux Metabolome, Villenave d'Ornon, Bordeaux (33), France
- Lindsay Peyriga, Plateforme MetaboHUB-MetaToul, TBI INSA Toulouse, Toulouse (31), France
- David Touboul, Institut de Chimie des Substances Naturelles, CNRS-ICSN, Gif-sur-Yvette (91), France

Le comité scientifique

Ce comité est constitué des membres des CA et CA junior du RFMF ainsi que d'un conseil scientifique local

Le conseil scientifique local

- Bouchereau Alain (PR Université de Rennes, IGEPP/P2M2)
- Croyal Mikaël (IR Nantes Université, CNRH)
- David Arthur (PR, EHESP)
- Dellero Younès (CR INRAE, IGEPP/P2M2)
- Giraudeau Patrick (PR Nantes Université, CEISAM)
- Gravot Antoine (PR Université de Rennes, IGEPP/P2M2)
- Guitton Yann (IR INRAE, Oniris/LABERCA)
- Guyot Sylvain, (DR INRAE, BIA/PRP)
- Le Bizec Bruno (PR Nantes Université, Oniris/LABERCA)
- Leblanc Catherine (DR, SB Roscoff, CNRS)
- Rondeau David (PR Université de Rennes, IETR/SM2)
- Soudant Philippe (DR CNRS, LEMAR/UBO)
- Derbre Séverine (MC Université d'Angers, SONAS)
- Lennon Sarah (MC Université de Rennes, IRSET)
- Tea Illa (MC Nantes Université, CEISAM)

IGEPP : Institut de Génétique, Environnement et Protection des Plantes, 35650 - Le Rheu
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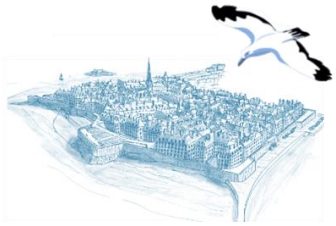
BIA : Biopolymères Interactions Assemblages, 35650 - Le Rheu
CEISAM : Chimie Et Interdisciplinarité, Synthèse, Analyse, Modélisation, 44300 - Nantes
LABERCA : Laboratoire d'Etude des Résidus et Contaminants dans les Aliments, Oniris, Ecole Nationale Vétérinaire, Agroalimentaire et de l'Alimentation Nantes Atlantique, 44300 - Nantes
LEMAR : Laboratoire des Sciences de l'Environnement Marin, UMR6539, Institut Universitaire Européen de la Mer (IUEM), Université de Bretagne Occidentale (UBO), 29238 - Brest
SONAS : Substances d'Origine Naturelle et Analogues Structuraux, Université d'Angers, Campus du végétal, 49070 – Beaucouzé
IRSET : Institut de recherche en santé, environnement et travail, 35700 – Rennes



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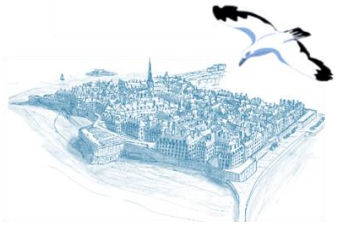
- David Touboul, Institut de Chimie des Substances Naturelles, CNRS-ICSN, Gif-sur-Yvette (91), France

Le conseil d'administration du RFMF junior

- Cécilia Bergès, Plateforme MetaboHub MetaToul – FluxoMet, Toulouse Biotechnology Institute, Toulouse, France
- Marine Letertre, Nantes Université, CNRS, CEISAM, UMR 6230, F-44000 Nantes, France
- Amandine Rocher, Plateforme MetaboHub MetaToul – FluxoMet, Toulouse Biotechnology Institute, Toulouse, France
- Thomas Brunet, Institut des Sciences Analytiques (ISA, UMR5280) – Equipe Anabio-MS, Villeurbanne, France
- Ghina Hajjar, Plateforme d'exploration du métabolisme (MetaboHUB Clermont-Ferrand), Unité de Nutrition Humaine, INRAE, Saint-Genès Champanelle (63), France
- Loic Mervant, Francis Crick Institute, Londres, UK
- Chloé Cloteau, IRS-UN, CNRH-Ouest, Plateforme spectrométrie de masse, 44300 Nantes, France
- Nathan Carriot, Université de Toulon, MAPIEM (EA 4323), Toulon (83), France
- Loïc Le Grégam, Plateforme MetaboHub MetaToul – FluxoMet, Toulouse Biotechnology Institute, Toulouse, France
- Téo Hebra, Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, République Tchèque



16 èmes Journées Scientifiques du RFMF

4-6 juin 2024, Saint-Malo

Ateliers du 3 juin 2024

Atelier n°1 : Atelier Junior Speed Networking

Personne(s) encadrant l'atelier :

Chloé Cloteau, chloe.cloteau@univ-nantes.fr

Ghina Hajjar, ghina.el.hajjar@gmail.com

Marine Letertre, marine.letertre@univ-nantes.fr

Public envisagé et les prérequis :

- Public : Jeunes chercheurs.euses en métabolomique et fluxomique
- Prérequis : Aucun

Objectif de l'atelier :

Cet atelier de Speed Networking a pour but de créer des liens scientifiques et sociaux entre les jeunes chercheurs.euses du RFMF junior afin de promouvoir et faciliter les échanges inter-laboratoires lors de l'atelier et tout au long des JS. Ainsi, plusieurs groupes de 6 à 10 personnes seront proposés selon les différentes thématiques de chacun.e permettant un Speed Networking qui se déroulera en 3 tours au cours de la pause déjeuner. Chaque tour aura une durée de 15 minutes comprenant une présentation rapide des participants au sein de chaque groupe suivie d'une discussion sous forme de brainstorming autour d'une thématique globale (*e.g.* science verte, choix de carrière). Des boissons ainsi qu'un déjeuner (du style Finger food) seront proposés. Au terme de l'atelier, un quizz sera distribué par groupe avec des questions sur les participants afin de favoriser les échanges convi*via*ux tout au long des journées scientifiques.

Durée de l'atelier : **1h15**

Date et lieu : Lundi 3 juin lors de la pause déjeuner de 12h30 à 13h45 au Dock à St Malo.

Nb de places maximum : 40 personnes

Atelier n°2 : Venez découvrir (ou redécouvrir) Workflow4Metabolomics

Personne(s) encadrant l'atelier : Binta Diémé (binta.dieme@uca.fr), Céline Dalle (celine.dalle@def.gouv.fr), Mélanie Petera (melanie.petera@inrae.fr), Cédric Delporte (cedric.delporte@ulb.be), Sylvain Chéreau (sylvain.chereau@inrae.fr), Yann Guitton (yann.guitton@oniris-nantes.fr)

Public envisagé et les prérequis :

- Public : Ouvert à tous – Utilisateurs débutants et confirmés de W4M - passionnés par le traitement de données en métabolomique ou simple curieux de découvrir W4M.
- Prérequis : Aucun! Nous accueillons tous les niveaux d'expérience.

Objectif de l'atelier :

Ne manquez pas cette occasion de développer vos compétences en métabolomique et d'explorer les dernières technologies avec des experts de renommée mondiale!

Vous ne connaissez pas W4M

- Découvrez Workflow4Metabolomics (W4M) et explorez les outils de traitement de données disponibles *via* Galaxy.

Vous utilisez déjà W4M

- Soyez à l'affût des dernières avancées de W4M, y compris les formations Galaxy, la spectrométrie de masse (MS/MS), la RMN 2D et bien plus encore!
- Faites-nous part de vos retours d'expériences : améliorations à apporter

Le programme sera le suivant :

1- Introduction à W4M et ses outils pour les débutants
2- Présentation des Nouveautés et Astuces d'Experts
3- Session Questions/Réponses Interactive

Durée de l'atelier : 1h30

Date et lieu : Lundi 3 juin au Palais des Congrès du Grand Large à Saint-Malo de 13h45 à 15h15

Nb de places maximum : 40 personnes

Atelier n°3 : Compétences et Métiers d'Avenir : Formater une Réponse à un Appel à Manifestation d'Intérêt pour un diagnostic national sur les formations en métabolomique, lipidomique et fluxomique

Personne(s) encadrant l'atelier :

- Cédric Bertrand, cédric.bertrand@univ-perp.fr, Pierre Pétriacq, pierre.petriacq@inrae.fr, Anne-Emmanuelle, hay@insa-toulouse.fr

Public envisagé et les prérequis :

- Public : les responsables de formation ou de modules
- Prérequis : être inscrit et avoir participer à la visio d'information qui sera proposée fin avril

Objectif de l'atelier :

Préciser les ambitions et fournir quelques éléments de contenus en une dizaine de lignes ….

L'appel à manifestation d'intérêt « Compétences et métiers d'avenir » (AMI CMA) s'inscrit dans le cadre des objectifs et leviers de France 2030. Il vise à répondre aux besoins des entreprises et des institutions publiques en matière de formation, d'ingénierie de formation, initiale et continue, et d'attractivité des formations, pour permettre l'acquisition des compétences nécessaires aux métiers d'avenir de France 2030. **L'objectif de cet atelier est de créer une *task force* pour répondre à cet appel à manifestation d'intérêt dans le cadre des domaines de la métabolomique, lipidomique et fluxomique.**

Il s'agira dans un 1er temps de définir les objectifs, d'identifier les acteurs actuels de la formation souhaitant participer, de définir et de valider la stratégie et le calendrier de réponse à l'AMI dans nos domaines. Puis nous rédigerons un document autour de nos différents objectifs au cours de l'atelier.

Info : Compétences et Métiers d'Avenir (CMA) – Appel à manifestation d'intérêt – 2021-2025 | ANR

Durée de l'atelier : 1h30

Date et lieu : Lundi 3 juin au Palais des Congrès du Grand Large à Saint-Malo de 13h45 à 15h15

Nb de places maximum : 22 personnes

Atelier n°4 : Modélisation prédictive par machine learning

Personne(s) encadrant l'atelier :

Sylvain Prigent sylvain.prigent@inrae.fr, Pierre Petriacq Pierre.Petriacq@inrae.fr, Millena Barros Santos millena.barros-santos@inrae.fr, Malo LeBoulch malo.le-boulch@inrae.fr

Public envisagé et les prérequis :

- Public : tout public (étudiant-e, personnel technique, enseignant-e, chercheur-e) intéressé par le machine learning appliqué à l'analyse de données métabolomiques
- Prérequis : R, RStudio

Objectif de l'atelier :

Destiné aux débutants et aux plus expérimentés en modélisation, cet atelier est conçu pour fournir aux participants une base théorique et pratique du machine learning (apprentissage automatique), en explorant les principes théoriques fondamentaux tout en vous offrant une expérience pratique. Rejoignez-nous pour découvrir ce que le machine learning peut apporter à vos études métabolomiques, et pour développer vos compétences en construction de modèles prédictifs.

Objectifs :

- Acquérir les bases théoriques de l'apprentissage automatisé : présentation de différents algorithmes et de workflows de prédiction.

- Construire des modèles prédictifs en utilisant R : acquisition d'une expérience pratique en utilisant le langage de programmation R pour construire des modèles prédictifs. À travers des exercices pratiques et des démonstrations, ils apprendront à prétraiter les données d'entrée, à sélectionner des variables pertinentes et à construire des modèles prédictifs.

Durée de l'atelier : 1h30

Date et lieu : Lundi 3 juin au Palais des Congrès du Grand Large à Saint-Malo de 15h30 à 17h00

Nb de places maximum : 20 personnes

Atelier n°5 : Comment rechercher, assembler et valider des biomarqueurs multiplexes pour prédire et classer : applications en santé, nutrition et dans la lutte contre la fraude

Personne(s) encadrant l'atelier :

- *Jean Charles Martin (jean-charles.martin@univ-amu.fr)*

Public envisagé et les prérequis :

- Public : Public intéressé par les applications de la métabolomique pour la prédiction (tout domaine d'applications)
- Prérequis : connaissance de base en statistiques (régression, stats multivariées)

Objectif de l'atelier :

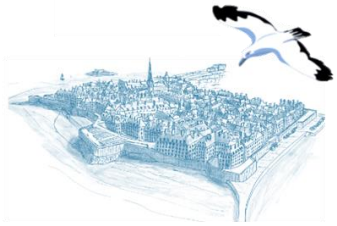
L'un des piliers de la métabolomique consiste à trouver des biomarqueurs pour classer des observations, ou pour prédire le comportement d'un système biologique. La recherche du biomarqueur unique est la plupart du temps voué à l'échec par manque de spécificité (interférence avec d'autres situations que celle qui est étudiée). L'assemblage de biomarqueurs dans une combinaison unique (une signature) parait plus avantageuse car caractéristique de la situation étudiée. Nous verrons comment rechercher cette signature, comment l'assembler en un score unique à l'aide d'algorithmes spécifiques, comment déterminer un seuil de décision permettant de prédire et de classer des observations, et comment valider cette signature multiplexe. Trois exemples seront développés en mode participatif, en lien avec la prédiction de l'évolution clinique de patients COVID19, de l'exposition nutritionnelle à la matière grasse laitière, ou de la lutte contre la fraude pour l'authentification de vins de terroir.

Mots clés : prédiction, biomarqueurs, régression PLS, régression logistique, C-Stat et courbe ROC.

Durée de l'atelier : **1h30**

Date et lieu : Lundi 3 juin au Palais des Congrès du Grand Large à Saint-Malo de 15h30 à 17h00

Nb de places maximum : 40 personnes



16 èmes Journées Scientifiques du RFMF

4-6 juin 2024, Saint-Malo

Programme détaillé par journée

Du mardi 4 juin au jeudi 6 juin 2024

Mardi 4 Juin 2024

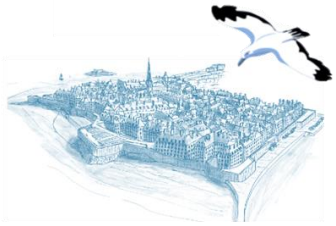
Chairs	Heures	Session		Intervenants	Titre
A.LE GOUELLEC / M. LETERTRE	9h00		B	Bienvenue	Pres. RFMF, VP Innovation UnivRen, Dir BiogenOuest , Organisation
	9h30	S1-Santé	**S1-CI1**	**J. IVANISEVIC**	(Unleashing the) Potential of Lipidomic Profiling for Population Health Research
	10h15		S1-O1	J-C. PORTAIS	Regulation of systemic lactate fluxes by brown and beige adipose tissues
	10h35		CS1	B. BRAHIM	**Shimadzu** - La spectrométrie de masse informatisée accélère la localisation des C=C pour la lipidomique non ciblée en utilisant la dissociation par attachement d'oxygène
	10h45			PAUSE	
	11h10		S1-O2	M. TREMBLAY-FRANCO	Integrative multi-modal metabolomics to early predict cognitive decline among Amyloid positive community-dwelling older adults
	11h30		S1-O3	J. CHAKER	Exploration des liens entre exposome chimique et santé cérébrale : application à large échelle d'une approche non-ciblée basée sur la LC-ESI-IMS-HRMS dans le projet Eglantine
	11h50		S1-O4	J-C. MARTIN	Early metabolic disruption and predictive biomarkers of delayed-cerebral ischemia in aneurysmal hemorrhage
	12h10		CS2	F. DROUYÉ	**Waters** - Pushing the boundaries of large-scale omics studies with Multi Reflecting Time-of-Flight (MRT) technology
	12h25		R1	REPAS	
J. BERTRAND MICHEL / G. HAJJAR	13h30		P1	**Posters Pairs**	Numéros Pairs
	14h30	S1-Santé	S1-O5	A. CIRILLO	How can metabolomics help the follow-up management of kidney transplantation recipients? An untargeted based-metabolomics multiplatform study.
	14H50		S1-O6	C. LE GOFF	Longitudinal NMR-based metabolomics analysis of mountain ultramarathon runners: new perspectives for athletes monitoring and injury prevention
	15H10		S1-F1	E. NICOL	Ex-vivo study of skin permeability and stability of a topical neurofibromatosis application using a combined LC-MS/MS and MALDI-FTICR imaging worklow
			S1-F2	M. GALMICHE	Integration of lipidomics and polar metabolomics for a molecular characterization of solvent neurotoxicity
			S1-F3	M-T. AVELLA	Early biomarkers of transition to psychosis detected by NMR IVDr technique: a pilot study
			S1-F4	C. PLAZY	Exploration of the metabolic impact of phenylketonuria by metabolomics on Dried Blood Spot
			S1-F5	A. DAVID	New markers for monitoring the elimination of the reactive N-Acetyl-p-benzoquinone imine after paracetamol/acetaminophen hepatotoxicity
	15H40	S5-Méthodo.	S5-F6	L. LE GREGAM	Développement d'un workflow computationnel pour la fluxomique ^{13}C
			S5-F7	H. GHOSSON	LC-HRMS-based metabolomics as a tool to develop analytical methods: How to choose the best extraction protocol when it comes to untargeted analysis?
			S5-F8	T. HEBRA	Discovery of the first archaeal terpene synthases: metabolic engineering meets untargeted metabolomics.
	16h00		CS3	C. MALPICA	**Proteigene (Biocrates)** - Enabling precision medicine with quantitative metabolomics by biocrates life sciences
C. LEBLANC / Y. HADDAD	16H15	S2-Sciences de la mer		PAUSE	
	16H40		**S2-CI2**	**S.PRADO**	Metabolomics unravels Chemical Signaling within marine and terrestrial holobionts
	17H25		S2-O7	B. DIEME	Analyse métabolomique des communautés microbiennes de la plastisphère dans le continuum fleuve-mer
	17h45		S2-O8	M. VALMORI	Complementary evaluation of lipid profiling of *Microchloropsis gaditana* by SFC and RPLC-HRMS/MS
	18h05		S2-O9	C. VIZON	Metabolomics and metabarcoding approaches reveal the importance of unseen players from benthic organisms in the behavior of coral larvae
	18h25		AG	**AG Sénior**	
	20h15		Cocktail	COCKTAIL	**FIN à 22h**

Mercredi 5 Juin 2024

Chairs	Heures	Session		Intervenants	Titre
P. PETRIACQ / T. HEBRA	9h00	S3-Agronomie	**S3-CI3**	**C. ANTONIO**	Mass Spectrometry-Based Forest Tree Metabolomics-metabolite responses to a changing climate
	9h45		S3-O10	M. CORSO	Multi-omic analyses elucidate specialized metabolites signature and distribution in developing seeds of the Brassicaceae species *Camelina* sativa
	10h05		S3-O11	K.MEKBEL	Message in the bottle: A Metabolomics Approach for Authenticating Provence Rosé Wines
	10h25		CS4	B. OMAIS	**Sciex -** A powerful single method for Metabolic Profiling and Characterization of cell culture media (CCM) components using ZenoTOF 7600
	10h35			PAUSE	
	11h00		S3-O12	S. COLOMBIE	Plant nitrogen metabolism in the growth-defence trade-off highlighted by constraint-based modelling
	11h20		S3-O13	N. SOMMERER	How to improve the resolution of molecular networks? By adding the Ion mobility dimension. An example based on cocoa polyphenol isomers
	11h40		S3-F9	S. PRIGENT	Unraveling *Brassica napus* leaf metabolic diversity: leveraging machine learning for agronomic traits prediction
			S3-F10	P. LE BOULCH	Diversité phytochimique de collections génétiques de Brassicacées pour la recherche de caractères d'intérêts agronomique et agroécologique
			S3-F11	L. FRETIER	Metabolomics approaches of seed-borne fungal endophytes for enhancing tomato seed performance in challenging environments
			S3-F12	M. LE BOULCH	The UNTWIST project: Unraveling Stress Response Mechanisms in *Camelina sativa* for Enhanced Crop Resilience in European Agriculture
	12h05		CS5	J. JEUDY	**Agilent Technologies** - Des solutions clef en main pour l'analyse ciblée multi-omique
	12h20		R2	REPAS	
	13h30		P2	**Posters impairs**	Numéros impairs
S. BERTRAND / C. BERGES	14h30	S4-Environnement	**S4-CI4**	**F. VERHEGGEN**	The smell of death: characterization and applications
	15h15		S4-O14	M-L. GODDARD	Impact de la mycorhization de la vigne sur son métabolisme primaire et ses réponses de défense face à l'infection par *Neofusicoccum parvum*, agent des dépérissements du bois
	15h35		S4-O15	A. MEJAIT	Evaluation of the environmental fate and impact of biopesticides using an innovative approach coupling high-throughput methods
	15h55		CS6	Y. THIRIET / M.P. PAVAGEAU	**ThermoFisher SCIENTIFIC** - Orbitrap Astral applied to Metabolomics
	16h10			PAUSE	
	16h45		S4-O16	Y. HADDAD	Unraveling the myth of Natural Deep Eutectic Solvents (NaDES) formation in desiccation tolerant seeds
	17h05		S4-O17	I. EL OUAR	Optimized LC-HRMS/MS workflow for molecular networking to explore the specialized metabolism of *Trichoderma reesei*
	17h25		S2+S4-F13	T. YON	Dealing with non-model organisms: Annotation of micro-algal metabolites using high-resolution mass spectrometry and advanced dereplication tools.
			S2+S4-F14	A.MEDINA	Insight on the relationship between the meta-metabolome, photosynthesis sensitivities and their natural fluctuation in freshwater microbial communities exposed to a model herbicide
			S2+S4-F15	E. PETI-JEAN	Metabolomics and functional genomics of halogenation mechanisms in brown algae
	17h45		MH	Membre d'Honneur RFMF 2024	
	18h30		AG-j	**AG RFMF Junior**	
	20h00		GALA	**DINER de GALA**	

Jeudi 6 Juin 2024

Chairs	Heures	Session		Intervenants	Titre
F. MEHL / A. ROCHER	9h00	S5-Méthodo.	**S5-CI5**	**O. YANES**	Decoding Metabolism: Navigating the Data Landscape of Metabolomics
	9h45		S5-O18	P. GIRAUDEAU	Hyperpolarized ^{13}C NMR metabolomics of urine samples at natural abundance applied to chronic kidney disease
	10h05		S5-O19	N. BUTIN	Data-driven ^{13}C-fluxomics towards ab initio reconstruction of metabolic networks
	10h25			PAUSE	
	10h45		S5-O20	D. SAUNIER	Workflow automatisé pour le traitement de données acquises par chromatographie liquide couplée à la spectrométrie de masse à haute résolution (LC-HRMS) pour caractériser l'exposome chimique
	11h05		S5-O21	L. QUIROS-GUERRERO	Improving prioritization methodologies for natural extracts: Integrating diverse data from metabolomics datasets and biological screenings into knowledge graphs
	11h25		CS7	E. RETHORE	**Groupe Roullier** - La métabolomique comme outil pour le développement de nouveaux produits de nutrition végétale
	11h40		PT	Prix de thèse Rolin-Portais 2024	
	12h10		FIN	Clôture des JS	
	12h30		R3	PANIERS REPAS	
	14h00		V	Visites	



Communications des conférenciers invités

Julijana IVANISEVIC, PhD, Senior Lecturer Head of Metabolomics Platform Faculty of biology and medicine UNIL | Université de Lausanne Quartier UNIL-CHUV Rue du Bugnon 19. Email : julijana.ivanisevic@unil.ch

Julijana Ivanisevic is a Metabolomics and Lipidomics group leader and Senior Lecturer at the Faculty of Biology and Medicine, University of Lausanne, Switzerland. Julijana joined UNIL in 2015 following a postdoctoral training at The Center for Metabolomics and Mass Spectrometry at The Scripps Research Institute in La Jolla, California (led by Prof. Gary Siuzdak). She received her PhD in chemical biology at the Aix-Marseille University, France, in 2011.

The aim of my team at UNIL is to develop new analytical approaches and apply them to biomedical and clinical research to advance our knowledge on cardiometabolic health and improve our healthspan with ageing. To this end, we are involved in longitudinal, metabolome-wide association studies of Swiss population (CoLaus, Complete Health and Heart cohort) in collaboration with clinicians and statistical geneticists.

SUMMARY: (Unleashing the) Potential of Lipidomic Profiling for Population Health Research

Lipid metabolism and circulatory lipid levels are tightly associated with our (cardio)metabolic health. Consequently, mass spectrometry (MS)-based lipidomics, able to measure thousands of individual lipid species, has emerged as a powerful phenotyping tool in epidemiological, human population, and in clinical intervention studies. However, ensuring high throughput and reproducible measurement of a wide panel of circulatory lipid species in large-scale studies poses a significant challenge. Even more, epidemiological studies require the acquisition of multiple sample cohorts over extended periods of time and are, therefore, subject to inherent variation in MS detection. In this talk, I will present our recently developed omics-scale targeted (LC-MS/MS) lipidomics platform and its application to the analysis of the first subset of thousand fasted plasma samples belonging to apparently healthy participants from prospective Lausanne population study (CoLaus). From the analytical point of view, I will highlight the importance of automation, stable isotope dilution, and the alternate analysis of reference material, as external quality control. From the biological point of view, I will show the results highlighting the individuality and sex-specificity of acquired lipid signatures, regardless of age. Finally, I will also touch upon the applicability of the established workflow for the investigation of the remodeling of lipid molecular landscape in response to exposure, such as high-fat diet for example.

Soizic PRADO. National Museum of Natural History. Unit “Molecules of Communication and Adaptation of Microorganisms”. UMR 7245 MNHN/CNRS, 57 rue Cuvier CP 54, 75005, Paris, France

Soizic Prado is a natural products chemist and deputy director of the "Molecules of Communication and Adaptation of Micro-organisms" unit at the Muséum national d'histoire naturelle in Paris. S. Prado's interests are chemical characterisation and ecological understanding of the molecular interaction involved between micro-organisms and their hosts. S. Prado has initiated and coordinated several national multi-partner projects focusing on the chemistry and chemical ecology of micro-organisms and has participated in several EU projects. She leads a working group within the COST Action "European Network In CHEmical Ecology : translating the language of life into sustainability (E-NICHE)" (CA22102, PI A-G Bagnères) and co-leads the new GDR ChemEcol. She is also heavily involved in research transfer and collaboration with industry.

Metabolomics unravels Chemical Signaling within marine and terrestrial holobionts

Complex interactions among host-associated microbes may influence numerous eukaryotic holobionts, encompassing both marine and terrestrial realms. Within plant and seaweed holobionts, these microbial interactions can yield benefits such as improved host health, enhanced growth, disease suppression, and adaptation to abiotic stressors. These intricate relationships are primarily driven by chemical signals that remain largely uncharacterized.

Through the integration of multidisciplinary approaches merging metabolomics and natural product chemistry, we aim to unveil new insights into the ecological roles of microbiota in both marine and terrestrial environments, emphasizing the pivotal role of chemical signaling. Additionally, we endeavor to showcase how deciphering this chemical communication enables the identification of diverse and original compounds with potent biological activities, particularly in the realms of agrochemicals and life sciences.

Carla ANTÓNIO ; Plant Metabolomics Lab Portugal, Forest Research Centre (CEF) School of Agriculture (ISA), University of Lisbon (ULisboa), Tapada da Ajuda, 1349-017 Lisboa, Portugal.
Email : plantmetabolomicslabpt@gmail.com

Dr. Carla António obtained her degree in Chemistry in 2003 from the Faculty of Sciences University of Lisbon (Portugal) and her PhD in Chemistry in 2008 from the University of York (UK). After completing her PhD, Dr. António joined the Max Planck Institute of Molecular Plant Physiology in Potsdam-Golm (Germany) to conduct postdoctoral research work. These past experiences were decisive to Dr. António's research independence and, since 2013, she is leading her independent Plant Metabolomics Lab in Portugal. In her Lab, Dr. António is interested in investigating plant responses to abiotic and biotic stress using MS-based metabolomics approaches. Unravelling signaling steps and metabolic pathways controlling abiotic and biotic stress tolerance of plants provides essential tools for coping with the adding negative effects of climate change.

Mass Spectrometry-Based Forest Tree Metabolomics — metabolite responses to a changing climate

Forests are of vital importance not only because they are a source of a wide range of economical valuable products but also because forest ecosystems provide crucial services to humankind, including preservation of biodiversity, soil and water quality, carbon cycling, climate regulation, and climate change mitigation. Metabolomics methods in forest tree research are particularly limited given the long-life cycle, large genome size and lack of genomic tools of forest tree species. However, since the major genomics breakthroughs in forest tree research (e.g., availability of the first ever tree genome sequence, Populus trichocarpa in 2006), metabolomics studies in forest tree species have generated increased interest. In this area, metabolomics represents a unique opportunity to explore the metabolic and developmental pathways of forest trees in response to critical damages associated with global climate change (abiotic/biotic). Abiotic stress events can devastate forests while biotic stress events, associated with forest insect pests and pathogen outbreaks, can seriously affect tree immune responses. Altogether, these environmental-stress factors promote tree disease spread and, eventually, lead to tree mortality. Digging deeper into the understanding of the defense mechanisms involved in the resilience of forest trees to climate change is, therefore, crucial to developing strategies that best secure the protection of our forests, their biodiversity, and ultimately, ourselves.

VERHEGGEN François. Laboratoire d'écologie chimique et comportementale, Gembloux Agro-Bio Tech, Université de Liège, 2b Avenue de la Faculté d'agronomie, 5030 Gembloux (Belgique), Email : fverheggen@uliege.be

François Verheggen is a Professor of Chemical Ecology at the University of Liege, Belgium. His team is specialized on the study of volatile organic compounds involved in the interactions between organisms, including animals, plants and microbes. He is specialized on insect pheromones, but he has always devoted a significant part of his research on the characterization of the volatiles released by dead animals.

THE SMELL OF DEATH: CHARACTERIZATION AND APPLICATIONS

After death, a body undergoes a complex decomposition process during which hundreds of volatile organic compounds (VOCs) are released. By leaving pig corpses to decompose in forests, meadows and inside houses, we highlighted the strong influence of the environment on the cadaveric odor profile, characterized by GC-MS and GCxGC-MS. Also, we demonstrated that the presence of scavenging insects on the corpse not only accelerates the decomposition process, but also modifies its VOC composition. These insects have specialized olfactory sensilla, allowing them to perceive the chemicals resulting from decomposition and use them to locate and colonize new cadavers. The same goes for parasitoids of scavenging species. By adapting our analytical method, we have also characterized the VOCs emitted by decomposing submerged and buried bodies. Thermodesorption (Tenax ta®) coupled with GC-MS allowed to list and quantify the cadaveric VOCs present in the soil layers located above and below buried rat corpses.

Studies examining human corpse odors are rare and often limited by their sample size. We therefore undertook the complete characterization of the volatilome of human corpses by periodically visiting a morgue. We collected, identified, and quantified the VOCs released by 20 human corpses at the fresh stage, using dynamic headspace collection. We also assessed the impact of skin temperature, gender, age, size, postmortem interval, presence of lividities or rigidities, on the volatilome.

All these results have led to a partnership with Belgian police officers specializing in the search for human corpses. Through behavioral analyzes, we demonstrated that police dogs responded positively to a mixture of the main molecules characterizing the odor of human corpses. This mixture is now used by dog handlers for the daily training of their animals. Optimizing its composition will make it possible to improve the performance of dogs in the field in the future.

YANES Oscar, Universitat Rovira i Virgili, Department of Electronic Engineering, IISPV, Tarragona (Spain). Biomedical Research Networking Centre of Diabetes and Metabolic Diseases (CIBERDEM), Instituto de Salud Carlos III, Madrid (Spain). Email : oscar.yanes@urv.cat

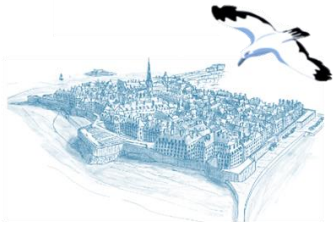
Oscar Yanes received his Ph.D. degree in Biochemistry (2006) from the Universitat Autonoma de Barcelona (Spain). In 2007 he became Research Associate in Gary Siuzdak's lab at The Scripps Research Institute (USA). Since 2011 he coordinates the Metabolomics Platform of the Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders (CIBERDEM), he is affiliated member at the IRB Barcelona and assistant professor at the Universitat Rovira i Virgili where he also leads his own research group (www.yaneslab.com). He has long experience in developing new technologies and methods, computational tools and applications in LC-MS, GC-MS and NMR-based metabolomics.

Decoding Metabolism: Navigating the Data Landscape of Metabolomics

Mass spectrometry (MS)-based metabolomics has revolutionized our ability to comprehensively analyze small molecules and metabolites in biological systems, with far-reaching applications in precision medicine, biomarker discovery, nutritional sciences, and environmental testing. Despite the vast amounts of data generated, current MS1- and MS2-based acquisition and data analysis strategies often yield low identification rates of metabolites.

In this presentation, I introduce novel experimental approaches aimed at optimizing MS2 acquisition to enhance biological specificity and significantly improve identification rates compared to traditional data-dependent acquisition (DDA) methods. Additionally, we address the challenge of unidentified MS/MS spectra by introducing innovative in silico fragmentation tools based on two novel deep learning models. These tools complement existing computational methods such as CFM-ID 4.0 and SIRIUS 4.0, facilitating metabolite annotation and identification.

Furthermore, I introduce Datoma, an innovative serverless cloud computing platform designed to streamline the processing of metabolomics data. Datoma boasts a comprehensive suite of curated software tools covering LC-MS/MS, GC-MS, and MS Imaging, all accessible via an intuitive web interface or programmatically through a dedicated Python package and API. With Datoma, users benefit from instant RAM and CPU resource provisioning and scalability, enabling the processing of large datasets at speeds exceeding 100 times faster than existing solutions. Its innovative cloud architecture enables easy and semi-automatic migration of new code and tools, empowering developers to create 'ready-to-use' data analysis tools for cloud computing while ensuring their scientific impact and visibility.



Communications orales

16 èmes Journées Scientifiques du RFMF
4-6 juin 2024, Saint-Malo

Oral 1 - O1

Regulation of systemic lactate fluxes by brown and beige adipose tissues

Rémi Montane[1], Yannick Jeanson[1], Damien Lagarde[1], Spiro Khoury[1,2], Léana Porcher-Bibes[1,2], Christophe Guissard[1], Anne Galinier[1], Edern Cahoreau[2,3], Luc Pellerin[4], Anne-Karine Bouzier Sore[5], Jean-Philippe Pradère[1], Louis Casteilla[1], Cedric Dray[1], **Jean-Charles Portais**[1,2,3], Isabelle Ader-Perarneau[1], and Audrey Carriere[1]

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[2] MetaboHUB-MetaToul, National Infrastructure of Metabolomics and Fluxomics, Toulouse, France – Institut National des Sciences Appliquées - Toulouse, Institut National des Sciences Appliquées, Toulouse – France
[3] Toulouse Biotechnology Institute, TBI-INSA de Toulouse INSA/CNRS 5504-UMR INSA/INRA 798, 5504 Toulouse, INSA - Institut National des Sciences Appliquées – France
[4] IRMETIST Inserm U1313 – Centre de Recherche Inserm, CHU Poitiers – France
[5] Centre de Résonance Magnétique des Systèmes Biologiques – CNRS : UMR5536, Université Victor Segalen - Bordeaux II – France

Activation of ”healthy” energy dissipating brown/beige adipose tissues represents an attractive therapeutic strategy against obesity and age-related metabolic disorders. Traditionally described for their ability to burn circulating glucose and lipids, these tissues can also use lactate - a highly abundant circulating metabolite playing critical roles in metabolic homeostasis - as an alternative substrate. Given their capacity to act as ”metabolite sinks”, we herein asked whether brown/beige adipose tissues impact systemic lactate clearance and inter-organ lactate fluxes. Our objective is to determine the role of brown/beige adipose tissues in systemic lactate clearance and the metabolic processes underlying such role. To address these questions, in vivo stable isotope tracing experiments with intra-peritoneal bolus injection of uniformly labeled 13C-lactate were performed in mice housed at different temperatures – conditions known to regulate brown/beige adipose tissues activation. Analysis of 13C label incorporation in metabolites by mass spectrometry enabled to trace lactate utilization and its metabolic fate in several organs including various adipose depots, liver, kidneys, muscle and brain. This work highlights that brown/beige adipocytes adipose tissues are involved in systemic lactate metabolism, both directly by using lactate as an oxidative substrate and indirectly, possibly through an inter-organ metabolic dialogue with the liver, this latter converting lactate into glucose during cold-induced activation of brown/beige adipocytes. These findings open novel perspectives for the role of brown/beige adipose tissues activity in systemic energy homeostasis. The regulation of systemic lactate metabolism may represent a novel mechanism by which brown/beige adipose tissues influence metabolic health.

16 èmes Journées Scientifiques du RFMF
4-6 juin 2024, Saint-Malo

Oral 2 – O2

Integrative multi-modal metabolomics to early predict cognitive decline among Amyloid positive community-dwelling older adults

Marie Tremblay-Franco[1,2], Cécile Canlet[2,3], Wan-Hsuan Lu[4,5], Jean-Charles Portais[4,6], Justine Bertrand Michel[7,8], Bruno Vellas[5,9], Louis Casteilla[4], and Isabelle Ader[4]

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[4] Gerontopole of Toulouse, Institute of Aging, Toulouse University Hospital – Institut National de la Santé et de la Recherche Médicale - INSERM, CHU Toulouse – France
[5] CERPOP UMR 1295, University of Toulouse III, INSERM, UPS, Toulouse – Institut National de la Santé et de la Recherche Médicale - INSERM – France
[6] FluxVivo Platform, MetaToul-MetaboHUB, National Infrastructure for Metabolomics and Fluxomics, Toulouse - France
[7] I2MC, Université Toulouse III - Paul Sabatier (UPS), Toulouse – Institut National de la Santé et de la Recherche Médicale - INSERM – France
[8] Lipidomic, MetaboHUB-MetaToul, National Infrastructure of Metabolomics and Fluxomics, Toulouse – France
[9] Gérontopole of Toulouse, Institute of Aging, Toulouse University Hospital – CHU Toulouse – France

Alzheimer's disease is strongly linked to metabolic abnormalities. We aimed to distinguish amyloid-positive people who progressed to cognitive decline from those who remained cognitively intact. We performed untargeted metabolomics of blood samples from amyloidpositive individuals, before any sign of cognitive decline, to distinguish individuals who progressed to cognitive decline from those who remained cognitively intact. A plasma-derived metabolite signature was developed from Supercritical Fluid chromatography coupled with high-resolution mass spectrometry (SFC-HRMS) and nuclear magnetic resonance (NMR) metabolomics. The two metabolomics datasets were analyzed by Data Integration Analysis for Biomarker discovery using Latent approaches for Omics studies (DIABLO), to identify a minimum set of metabolites that could describe cognitive decline status. NMR or SFC-HRMS data alone cannot predict cognitive decline. However, among the 320 metabolites identified, a statistical method that integrated the two datasets enabled identification of a minimal signature of 9 metabolites (3-hydroxybutyrate, citrate, succinate, acetone, methionine, glucose, serine, sphingomyelin d18:1/C26:0 and triglyceride C48:3) with a statistically significant ability to predict cognitive decline more than 3 years before decline. This metabolic fingerprint obtained during this exploratory study may help to predict amyloidpositive individuals who will develop cognitive decline. Due to the high prevalence of brain amyloid-positivity in older adults, identifying adults who will have cognitive decline will enable the development of personalized and early interventions.

16 èmes Journées Scientifiques du RFMF
4-6 juin 2024, Saint-Malo

Oral 3 – O3

Exploration des liens entre exposome chimique et santé cérébrale : application à large échelle d'une approche non-ciblée basée sur la LC-ESI-IMS-HRMS dans le projet Eglantine

Jade Chaker[1], Erwann Gilles[1], Sophie Lefevre-Arbogast[1,2], Sarah Lennon[1], Cécilia Samieri[2], and David Arthur[1]

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[2] Bordeaux population health – Université de Bordeaux, Institut de Santé Publique, d'épidémiologie et de Développement (ISPED), Institut National de la Santé et de la Recherche Médicale – France

Au cours des dernières décennies, de nombreux produits chimiques environnementaux auxquels nous sommes exposés ont été envisagés comme étant des facteurs de risque dans le développement et la progression des maladies neurodégénératives. Cependant, seules certaines familles de molécules ont été investiguées, telles que les pesticides organochlorés, tandis que les données sur d'autres familles de molécules telles que les retardateurs de flamme organophosphorés ou les additifs alimentaires restent parcellaires. Ainsi, dans le but d'étudier les liens entre expositions chimiques environnementales et santé cérébrale de manière exploratoire, plus de 700 échantillons sanguins de participants à la cohorte des Trois Cités (adultes de plus de 65 ans à l'inclusion) ont été analysés par une méthode non-ciblée basée sur la LC-ESI-IMS-HRMS. Plusieurs défis majeurs ont été relevés lors de cette étude ; dans un premier temps, la robustesse de la méthode analytique a pu être vérifiée. Les résultats de contrôle qualité sont conformes, malgré l'injection continue d'échantillons complexes pendant plus de deux mois. Dans un second temps, l'annotation de composés environnementaux souvent peu abondants en matrices biologiques est une tâche complexe puisque ces ions ne déclenchent pas toujours d'acquisition de données de fragmentation. Le logiciel Scannotation, développé au sein de l'équipe et disponible sur Github, a permis l'annotation efficace de composés environnementaux présents dans les échantillons, en utilisant uniquement les données acquises en mode Full Scan. Ainsi, cette étude a permis de caractériser l'exposome chimique d'une population âgée, ainsi que d'établir de premières associations entre expositions chimiques environnementales et déclin cognitif.

16 èmes Journées Scientifiques du RFMF
4-6 juin 2024, Saint-Malo

Oral 4 – O4

Early metabolic disruption and predictive biomarkers of delayed-cerebral ischemia in aneurysmal hemorrhage

Jean-Charles Martin[1], Karim Chikh[2], Marie-Christine Alessi[3], Catherine Defoort[3], and Nicolas Bruder[4,5]

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[2] Cardiovasculaire, métabolisme, diabétologie et nutrition – Université Claude Bernard Lyon 1, Hospices Civils de LInstitut National de la Santé et de la Recherche Médicale, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – France
[3] Centre recherche en CardioVasculaire et Nutrition – Aix Marseille Université, Institut National de la Santé et de la Recherche Médicale, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – France
[4] Service d'Anesthésie et Réanimation, Hôpital de La Timone [CHU - APHM], Marseille –France
[5] Service d'Anesthésie et Réanimation, INT (Institut de Neurosciences de La Timone), Hôpital de La Timone [CHU - APHM], Aix Marseille Université, Marseille – France

Delayed cerebral ischaemia (DCI) following aneurysmal haemorrhage (aSAH) is a major cause of complications and death. Here we set out to identify high-performance predictive biomarkers of DCI and its underlying metabolic disruptions using metabolomics and lipidomics approaches. This single-centre retrospective observational study enrolled 61 consecutive patients with severe aSAH, among them 22 experienced a DCI. Nine patients without aSAH were included as validation controls. Blood and cerebrospinal fluid (CSF) were sampled within the first 24h after admission. We identified a panel of 20 metabolites that together showed high predictive performance for DCI. This panel of metabolites included lactate, cotinine, salicylate, 6 phosphatidylcholines, and 4 sphingomyelins. Our prediction model was validated with external independent patients. We further enlarged the interplay of the metabolome and the lipidome between CSF and plasma in our patients to get mechanistical insights. We found that, aSAH and its associated DCI complications can extend beyond cerebral implications, with a peripheral dimension as well. As an illustration, early biological disruptions that might explain the subsequent DCI found systemic hypoxia driven mainly by higher blood lactate, arginine and proline metabolism likely associated to vascular NO, and disrupted ceramide/sphingolipid metabolism. We also found evidence, for the first time, pointing to a possible gut microbiota/brain DCI axis, and proposed the putative microorganisms involved. In conclusion, we found a robust multiplex biomarker predicting delayed cerebral ischemia. This signature is associated with significant peripheral and cerebral biological dysregulations, in which peripheral hypoxia could constitute an interesting target to prevent DCI.

16 èmes Journées Scientifiques du RFMF
4-6 juin 2024, Saint-Malo

Oral 5 – O5

How can metabolomics help the follow-up management of kidney transplantation recipients? An untargeted based-metabolomics multiplatform study.

Arianna Cirillo[1], Guillaume Resimont[2], Justine Massias[3], Emanuelle Vidal-Petiot[4], Francois Jouret[5], Martin Flamant[4], Yann Guitton[3], Pierre Delanaye[6], and Pascal De Tullio[1]

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In the context of chronic kidney disease, kidney transplantation (KTx) is considered the most favorable solution in terms of quality of life, morbidity, and mortality for patients with end-stage renal disease. However, KTx comes with its own set of risks, and monitoring kidney graft function is crucial for managing kidney transplantation recipients (KTRs). Because of limitations in the currently used techniques, there is a need for new biomarkers that can accurately reflect renal function or even predict its progression in KTRs, in order to improve patient management. This study aims to identify a new panel of biomarkers capable of predicting kidney function and the evolution of KTx. 56 patients from a well-phenotyped French cohort of KTRs were followed for one year post-transplantation. Urinary samples were collected at 3 and 12 months, and patients were stratified based on the evolution of kidney function. Untargeted NMR- and MS-based metabolomic approaches were applied to the cohort, followed by the integration of results from these dual methods. Multivariate analysis derived from both techniques allowed the identification of panels of biomarkers associated with the evolution of glomerular filtration rate (GFR) and used as predictive markers. Combining results from the dual approach increased discrimination and predictive performance, revealing a metabolomic signature that could forecast kidney function decline. In conclusion, this analysis shows the potential to predict GFR evolution at one year and these findings could represent an innovative and helpful tool for clinicians to improve patient care in the post-transplantation period.

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Oral 6 – O6

Longitudinal NMR-based metabolomics analysis of mountain ultramarathon runners: New perspectives for athletes monitoring and injury prevention

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This study aims to explore how metabolomic approach could provide valuable information about changes in athletes' metabolome occurring during a mountain ultramarathon race. To achieve this goal, we built a longitudinal cohort of athletes enrolled in "TOR des Géants". Using an 1H-NMR-based metabolomic approach, we evaluated metabolic changes that arise during both the race effort and the recovery phase and correlate them with functional muscles, cardiac, inflammatory, and kidney biomarkers already used in clinics. Processed data were analyzed with tools dedicated to longitudinal study design (ASCA+) and allowed us to assess specific changes in the metabolome and clinical biomarkers across the different time points. The data illustrated how the metabolism of athletes is impacted during the race and that 3-days recovery didn't allow a return to metabolic and functional baseline. Innovative pathway analysis such as single samples Pathway Analysis (ssPA) was employed to emphasize the signaling routes that play a crucial role in endurance effort and recovery. These analyses shed light to the metabolic shift that occurs during an extreme mountain ultramarathon race and how athletes recover from it after a 72h recovery period. Metabolomics-based analysis in the field of endurance sport is improving our understanding in the physiological responses to extreme effort. By its ability to provide valuable information about athletes' status in real condition, this methodology provide new tools for athletes' fitness evaluation, performance prediction, nutrient supplementation and the development of personalized follow-up, metabolomics offers the keys for a rationalized and healthy approach of extreme sport endurance practice.

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Oral 7 – O7

Analyse métabolomique des communautés microbiennes de la plastisphère dans le continuum fleuve-mer

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Les études des microorganismes qui vivent sur les déchets plastiques (la plastisphère) ont révélé un écosystème original qui suscite un intérêt croissant dans le contexte de la pollution plastique. Cette étude présente la première analyse métabolomique non ciblée de la plastisphère, afin de mieux caractériser les activités métaboliques des microorganismes qui s'y développent. Elle a été réalisée dans le cadre de l'expédition Tara Microplastics, où des granulés de polyéthylène ont été immergés pendant un mois dans cinq stations d'échantillonnage le long du continuum rivière-mer de neuf des principaux fleuves européens. Des techniques de chromatographie liquide couplée à la spectrométrie de masse haute résolution ont été utilisées pour analyser les extraits métaboliques des biofilms microbiens. Les résultats ont révélé une distinction claire entre les métabolomes des plastisphères d'eau douce et d'eau de mer. Pour la Loire, le Rhône, la Seine et la Tamise, les intensités d'un grand nombre de variables sont corrélées au gradient de salinité. Les métabolomes apparaissent similaires dans le Rhin et le Rhône, tout en étant différents du Tibre et de la Loire, qui présentaient une plus grande similarité avec la Tamise et la Seine. Les annotations putatives de 189 métabolites discriminants suggèrent que le métabolisme des lipides a été modulé de manière significative. Ces résultats confirment l'influence significative des facteurs environnementaux sur la diversité et les fonctions des microorganismes vivant sur les débris plastiques. Ces travaux confirment que l'approche métabolomique constitue un outil prometteur pour affiner notre compréhension du fonctionnement de l'écosystème complexe de la plastisphère.

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Oral 8 – O8

Complementary evaluation of lipid profiling of *Microchloropsis gaditana* by SFC and RPLC-HRMS/MS

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Characterization of the lipidome is generally achieved by reverse-phase liquid chromatography coupled to high-resolution mass spectrometry (HRMS/MS), which facilitates intraclass lipid separation. Alternatively, Supercritical Fluid Chromatography (SFC) coupled to HRMS/MS also enables broad lipidome profiling thanks to class separation[1]. The goal of this study is to combine lipid separation profiles for the complete lipidome characterization of microalgae and to explore the complementarity between these two approaches. A total lipid extract of Microchloropsis gaditana was analyzed in parallel on SFC-QTOF and RPLC-Orbitrap in electrospray ionization positive and negative modes and under datadependent acquisition (DDA) conditions. Resulting datasets were complementarily evaluated. The lipid annotation on both systems was achieved using the MS-DIAL software[2], thanks to the Lipidblast spectral library. Matched spectra were manually validated to further guarantee confident lipid annotation. Over 200 unique lipid species represented by glycerophospholipids (PC, PI, PG, PE), glycerolipids (TG, DG, MG), including glycolipids (DGTS, M/DGMG, SQDG) and fatty acids, were observed on both systems. The SFC lipid profiling enables the exploration of nofragmented or low-level confident annotations. While an iterative exclusion strategy was required on SFC-QTOF system for accessing to the low abundance lipid species eluting at the same retention time interval, intraclass separation on RPLC-Orbitrap improved the fragmentation of a large dynamic range of features in unique injection. This study demonstrates the complementary strengths of SFC and RPLC lipid profiling according two different mass analyzers in enhancing lipidome profiling of lipid extract.

(1) Lange M., et al., Chromatographia., 2019. (2) Tsugawa H., et al., Metabolites., 2015.

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Oral 9 – O9

Metabolomics and metabarcoding approaches reveal the importance of unseen players from benthic organisms in the behavior of coral larvae.

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Little is known about chemical and microbial landscape released by benthic organisms on coral larval behavior. Here we explored the effects of chemical and microbial exudates from different algal and cyanobacterial species on the swimming behavior and survival of Pocillopora acuta larvae in 10-minute videos and 24-hour survival assays. In response to exudates from the crustose coralline alga Porolithon onkodes, larvae were concentrated at the tank bottom, suggesting a pre-settlement behavior. However, in exudates from the macroalgae Dictyota bartayresiana and the cyanobacteria *Anabaena sp*.1, larvae swam in spiral or were metamorphosed without attachment, suggesting a stress behavior. Using metabolomics and metabarcoding, we identified infochemicals and microbial communities associated with the larval behavior. Chemical exudates were concentrated on reversed phase cartridge and analyzed in UHPLC-HRMS coupled with MS/MS-based molecular networking, while 16S rRNA gene was sequenced for microbial communities. Whereas exudates of P. onkodes were enriched in neutral sphingolipid and Nitrincolaceae bacterial family, diterpenoids and copiotroph bacteria were found in D. bartayresiana exudates, and lipocyclopeptides and bacterial communities linked to reef degradation were found in *Anabaena sp*.1 exudates. The use of metabolomics and metabarcoding has validated studies on isolated compounds and bacterial cultures involved in coral recruitment. These new insights show how waterborne metabolites and microbial communities released by benthic organisms may influence the behavior of coral larvae and show that the resilience of corals reef may be compromised with the increased of macroalgae and cyanobacteria.

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Oral 10 – O10

Multi-omic analyses elucidate specialized metabolites signature and distribution in developing seeds of the Brassicaceae species *Camelina sativa*

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Seeds of Brassicaceae model and crop species accumulated a large diversity of Specialized Metabolites (SMs) with diversified structures and tremendous interest for agriculture, nutrition and health. Few information are available about SM modifications, tissue-distribution and accumulation during seed development and germination in Brassicaceae crop species, whose seeds are used for oil and protein extraction, and/or for valorisation of co-products, including many SMs. Among these species there is Camelina sativa (camelina), an oilseed Brassicaceae that is used for research purpose and cultivated for human and animal nutrition, and for industrial uses. While we previously explored in detail SM diversity and plasticity, no information are available about SM distribution and expression of SM-related proteins and/or genes in camelina seed tissues. In this study we used untargeted metabolomics (LC-MS/MS), proteomics (DIA) and transcriptomics (RNA-Seq) to analyse synthesis, transport, modifications and degradations of SMs that are accumulated in camelina seed coat and endosperm, and in the embryo at 6 developmental and 2 germination stages. Our results showed specific signatures for many SMs, and of related proteins or genes, during seed coat and embryo development. We also showed that, differently from Arabidopsis thaliana seeds, in C. sativa the defense and antinutritional glucosinolates (GSL) compounds were accumulated in the seed coat and endosperm, and the corresponding isothiocyanates degradation products were present at high level in the embryo of dry seeds. Characterizing seed SM spatial dynamics will help the development of crops with seeds of more balanced distribution of beneficial and antinutritional metabolites.

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Oral 11 – O11

Message in the bottle: A Metabolomics Approach for Authenticating Provence Rosé Wines

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The rosé wines of Provence have achieved remarkable commercial success in both France and globally. However, a concerning issue has emerged, with some producers outside of Provence fraudulously labeling their wines with the Provence appellation. This deceptive practice poses a threat to the reputation of authentic Provence rosés, as these imitations lack the distinctive organoleptic qualities of the genuine counterparts. To address this challenge, our study aimed to identify highly specific predictors of Provence rosé wines and establish a reliable method for detecting counterfeit products. We conducted a non-targeted metabolomics analysis on thirty samples of rosé wines categorized into three classes : Provence, non-Provence, and imitations. Utilizing UHPLC-MS-MS/MS, we captured the spectra of all detectable molecules through mass spectrometry. Employing advanced data analysis techniques such as machine learning (random forest), and C-Stat (logistic regression and ROC estimator), we successfully extracted and validated three confidently identified metabolites.
When combined into a composite score into a partial least-square model, these metabolites effectively differentiated Provence rosé wines from imitations or non-Provence rosés,demonstrating outstanding performance metrics (AUROC 1, sensitivity 1, specificity 1, error rate in prediction 0%).
This study provides a compelling proof-of-principle, establishing the existence of a specific molecular signature for Provence rosé wines that enables the confident identification of counterfeit products. For future investigations, it is imperative to validate the relevance of this approach across a larger and more diverse set of wines, encompassing various harvest years.

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Oral 12 – O12

Plant nitrogen metabolism in the growth-defence trade-off highlighted by constraint-based modelling

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Primary metabolism is well conserved across species but it is useful to explore its specificities to assess the extent to which some pathways may contribute to particular outcomes. Constraint-based metabolic modelling is an established framework for predicting metabolic fluxes and phenotypes. This modelling approach makes it possible to explore the metabolism to raise specific behaviours of developing fruit species or defence mechanisms of infected plants. We applied the constraint-based method with a knowledge-based metabolic model of heterotrophic cells describing a generic network of primary metabolism. This model was constrained using a large set of quantified metabolites and compounds. It was solved using the objective function of flux minimisation in agreement with the principle of 'minimal effort'. The calculated fluxes were first subjected to multivariate analyses and then looked in details within the considered pathways. When this method was applied to compare a panel of developmental series of eight fruit species, differences were highlighted between fast and slow growth. Only slow-growing fruits mobilize the tricarboxylic acid cycle in addition to glycolysis to promote high polyphenol accumulation at early stages of development. The virtual fruits, constructed by combining 12 of the major biomass compounds, confirmed this growth-defense trade-off. This same approach was used to investigate the fluxes involved in the defence of tomato stems against Botrytis cinerea. It showed that infection requires carbon and nitrogen resources whose distribution in the pathways depends on nitrogen availability. These two studies highlight the importance of nitrogen metabolism and protein in growth and defence mechanisms.

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Oral 13 – O13

How to improve the resolution of molecular networks? By adding the Ion mobility dimension. An example based on cocoa polyphenol isomers

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The objective of this study is to improve the feature-based molecular networking (FBMN) analysis by incorporating trapped ion mobility spectrometry (TIMS) into a UHPLC-HRMS/MS workflow in order to enhance the analysis of co-eluted polyphenol isomers from cocoa beans. Two types of cocoa beans (named black and brown cocoa beans), having the particularity to generate chocolates of black or brown colours, were analyzed by a UHPLC-QTOF or a UHPLC-TIMS-QTOF method. The processed HRMS and HRMS/MS data underwent uniand multivariate statistical analyses as well as FBMN analysis. The additional mobility dimension of TIMS improved the resolution of the molecular networks compared to the approach without TIMS, by the presence of additional nodes and clusters in the networks corresponding to isomeric compounds. Several types of isomers separated by TIMS were annotated based on their distinct MS/MS fragmentation patterns. They included polyphenol monomers ((epi)catechin-O-hexoside and (epi)catechin-C-hexoside), dimers (B-type procyanidin dimers and dehydrodicatechins B), trimers (B-type procyanidin trimers and dehydrotricatechins B), and tetramers (B-type procyanidin tetramers). Interestingly, statistical analyses revealed that the majority of the aforementioned isomers were found to be discriminating compounds for black or brown cocoa beans. Moreover, the accurate identification of TIMS separated isomers improves the understanding of cocoa bean metabolites. Dehydrodi(or tri)catechins B are oxidation products formed during cocoa processing, while their corresponding isomers, B-type procyanidin di(or tri)mers, are native compounds. This approach can be applied to other complex samples to enhance the resolution of molecular networks of plant specialized metabolites.

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Oral 14 – O14

Impact de la mycorhization de la vigne sur son métabolisme primaire et ses réponses de défense face à l'infection par *Neofusicoccum parvum*, agent des dépérissements du bois

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La vigne doit faire face à différents stress biotiques et abiotiques entraînants, dans un contexte de réchauffement climatique, l'apparition de plus en plus fréquente de maladies de dépérissements, avec des effets grandissants allant jusqu'à la mort des ceps. L'utilisation des pesticides est progressivement remplacée par des moyens plus respectueux de l'Homme et de l'environnement tels que les microorganismes bénéfiques. Nous nous intéressons ainsi à la symbiose avec des champignons mycorhiziens à arbuscules, aidant à la nutrition et à la tolérance à divers stress, dont le stress hydrique, et cherchons également à étudier la mycorhization comme moyen de lutte biologique face aux agresseurs.

Afin d'étudier l'effet de la mycorhization sur le métabolisme de la vigne, une expérimentation a été menée sur des plants du cépage Gewurztraminer de vigne préalablement mycorhizés par Rhizophagus irregularis. Nous avons réalisé des mesures agronomiques ainsi que des études transcriptomique et métabolomique comparatives sur des feuilles et des racines de plants mycorhizés ou non ainsi que des plants infectés ou non par le pathogène fongique *Neofusicoccum parvum*.

Une méthode d'extraction innovante des métabolites par extractions successives en polarité inversée a été mise en place ainsi que les méthodes analytiques non ciblées adaptées utilisant la GC-MS et la LC-MS/MS pour couvrir largement le métabolome. Nous avons ainsi pu mettre en évidence un impact significatif de la mycorhization sur le métabolisme foliaire et racinaire de la vigne, en particulier le métabolisme primaire ainsi que sur les voies de signalisation et les réponses de défense (Goddard et al., 2021).

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Oral 15 – O15

Evaluation of the environmental fate and impact of biopesticides using an innovative approach coupling high-throughput methods

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Biopesticides are complex substances that are derived from natural sources, and offer a promising alternative to traditional pesticides, but it is still unknown how long biopesticides and their residues remain in the environment then how long they impact organisms living in soil. In this context, we conducted an experiment to evaluate the environmental fate and impact of Beloukha (Bioherbicide). A kinetics study was performed over 57 days in soil microcosms comparing treated and non-treated conditions. The samples were analyzed using high-throughput omics techniques (metabolomics UHPLC-HRMS and 16S and 18S metabarcoding). Thanks to metabolomics data (12246 metabolites), we could determine the dissipation time. It's the time required for the dissipation of the biopesticides compounds. For that, a statistical method was developed which uses the occurrence and the intensities of each metabolite throughout the kinetics. It allowed the separation of the soil metabolites and the by-products from the pesticide components. Through subsequent kinetic modeling using exponential degradation function, we found that 99% of the biopesticide compounds exhibit a half-life below 38 days. Metabarcoding analyses revealed biodiversity changes over time and the impact of the biopesticide and its by-products on bacteria. In particular, we found that bacterial assemblages were only impacted at the first-time steps of the experiment. Moreover, correlation analyses revealed high correlations between biopesticide compounds and bacteria genera that play important roles in plant growth and azote fixation. In essence, this work establishes a robust workflow applicable for studying the dissipation of biopesticides in the environment and their impact on biodiversity.

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Oral 16 – O16

Unraveling the myth of Natural Deep Eutectic Solvents (NaDES) formation in desiccation tolerant seeds

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To ensure their continuity on earth, plant species must transfer their genetic heritage to the next generation *via* the reproductive propagules, i.e., pollen grains and seeds. As part of their development, these tissues undergo programmed desiccation and enter into dormancy to cope with various stresses during dispersal. Although the complete process of acquiring their desiccation tolerance is not fully understood, the accumulation of compatible metabolite solutes and molecular chaperones, capable of exchanging hydrogen bonds, is a key mechanism for stabilizing macromolecules during dehydration. At the same time, natural deep eutectic solvents (NaDES) are emerging classes of green solvents formed by biobased hydrogen bond donor and acceptor compounds. Although they are much studied for their various applications such as solvation or biocatalysis, their presence as a liquid phase in living dehydrated cells remains to be proven. The aim of this work is to provide tangible arguments regarding NaDES formation in oilseed rape (Brassica napus L.) and their role in desiccation tolerance. The presence of reliable NaDES ingredients in seeds and anthers was provided by metabolic profiling techniques. Their colocalization and molecular interaction was also established by mass spectrometry imaging (DESI). The study of affinity between these ingredients by cold spray ionization mass spectrometry (CSI-MS) and the prediction of their capacity to form NaDES, with Cosmo-RS software, were used to select suitable mixtures. Based on these results, some NaDES were prepared in-vitro and their physicochemical properties studied. Finally, their ability to protect antioxidant enzymes against thermal stress and desiccation were assessed.

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Oral 17 – O17

Optimized LC-HRMS/MS workflow for molecular networking to explore the specialized metabolism of *Trichoderma reesei*

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Filamentous fungi, such as *Trichoderma reesei*, are subject to a range of biotic and abiotic stresses throughout their lifecycle. Environmental factors stimulate the production of specialized metabolites for survival. Although *T. reesei* is widely studied for its cellulase production, its specialized metabolic pathways remain significantly underexplored and could provide highly valuable metabolites. The *T. reesei* hyperproducer cellulase strain RUT-C30 was studied through an ethyl acetate/butanol extract followed by an untargeted metabolomic analysis based on high-performance liquid chromatography (HPLC) coupled with tandem high-resolution mass spectrometry (HRMS/MS). The resulting data were then used to generate a molecular network using MetGem (1), to chemically profile the sample and highlights the presents molecular families. Through this methodology, multiple known *T. reesei*'s constituent species were annotated. Among these, 30 sorbicillinoids and 5 paracelsins, which are notable bioactive families (2,3), were successfully annotated in the molecular network. The proximity of mono, di, and tri-sorbicillinoids within the network facilitated their annotation. Moreover, our strategy led to the discovery of previously undescribed compounds, including novel peptaibol sequences and potential coumarin derivatives. In order to significantly improve the annotation, an in silico MS/MS database of peptaibol was generated and interrogated. This work showcases the potential of a comprehensive untargeted LC-HRMS/MS approach for the characterization of *T. reesei*'s understudied metabolome.

(1) Olivon F et al., Analytical Chemistry, 2018, 90, 23, 13900-13908
(2) Guo Q et al., Journal of agricultural and food chemistry, 2023, 71, 37, 13612-13632
(3) Daniel J.F et al., Natural product reports, 2007, 24, 5, 1128-1141.

16 èmes Journées Scientifiques du RFMF
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Oral 18 – O18

Hyperpolarized ^{13}C NMR metabolomics of urine samples at natural abundance applied to chronic kidney disease

Victor Ribay[1], Benoît Charrier[1], Mikaël Croyal[2,3], Bertrand Cariou[4], Samy Hadjadj[2,4], Julien Boccard[5], Jean-Nicolas Dumez[1], Marine Letertre[1], and **Patrick Giraudeau**[1]

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NMR-based metabolomics mostly relies on 1D 1H experiments for sensitivity reasons, which often results in significant peak overlap and limits the analysis of inherently complex samples. 13C NMR benefits from wider spectral dispersion and narrower signal linewidth but is barely used in metabolomics due to its low sensitivity. In this context, Dissolution Dynamic Nuclear Polarization (d-DNP) provides an efficient opportunity to significantly improve the sensitivity of 13C NMR. We initially incorporated 13C d-DNP into an untargeted metabolomics workflow applied to model plant extracts. Recently, thanks to a meticulous optimization of the experimental workflow, we reported the first hyperpolarized 13C NMR experiments on urine samples at natural abundance in conditions compatible with metabolomics studies.

In this presentation, we will report the first application of this promising hyperpolarization approach to a clinical metabolomics study. The analysis of urine samples from patients with different stages of chronic kidney disease (CKD) was performed using 13C d-DNP NMR and conventional 1H NMR metabolomics to explore the complementarity between the two methods. Data were meticulously acquired and processed according to metabolomics standard guidelines to ensure trustful results. Supervised analysis of the 13C d-DNP NMR dataset provided a valid statistical model separating patients with CKD stages 3 and 4 from patients with CKD stage 1. Results from 13C d-DNP NMR highlighted several biomarkers known to be biologically relevant, but also showed interesting complementarity with conventional 1H NMR, while raising exciting challenges associated with the analysis of spectral fingerprints stemming from this new approach.

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Oral 19 – O19

Data-driven ^{13}C-fluxomics towards ab initio reconstruction of metabolic networks

Noémie Butin[1,2,3], Pierre Millard[2,3], Clément Frainay[4], Loïc Le Grégam[2,3], Fabien Jourdan[3,4], Uwe Schmitt[5], Floriant Bellvert[2,3], Patrick Kiefer[5], and Jean-Charles Portais[1,2,3]

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Metabolic networks reconstruction is a valuable tool for understanding biological systems, by identifying the link between the structure of the network, its organization and its functional properties. The reconstruction process, usually based on the genome, provide a comprehensive model that represents an organism's entire set of biochemical reactions. Alternative experimental reconstruction approaches, aim at reconstructing the active metabolic networks directly from metabolome observation, in a way that is orthogonal and complementary to in silico approaches. In this work, we have developed a new data-driven approach for ab initio metabolic reconstruction based on isotopic tracing experiments. Isotopic labeling, especially with 13C isotopic tracers, has long been used to study the structure and the activity of metabolic pathways. Our approach, IsoMet for Isotopic driven Metabolic reconstruction, is based on the interpretation of untargeted metabolomics data collected in 13C-labeling dynamic experiments. It aims at providing direct access to the active metabolic network of an organism, specific to a cell type in a given context, without prior considerations. IsoMet shares concepts with non-stationary 13C-fluxomics approaches, such as label propagation simulation and flux calculation. Its basic principle consists to construct and test the ability of different network topologies to explain observed labeling dynamic data, leading to the construction of active metabolic subnetworks. IsoMet is generic and can be applied to various stable isotope tracers (13C, 15N, etc) and different type of isotopic measurement (MS, MS/MS, NMR, etc). It is intended to be applicable to various biological models, ranging from poorly known organisms to complex systems.

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Oral 20 – O20

Workflow automatisé pour le traitement de données acquises par chromatographie liquide couplée à la spectrométrie de masse à haute résolution (LC-HRMS) pour caractériser l'exposome chimique

Dylan Saunier[1], Eric Venot[1], Sylvain Dechaumet[1], Nihel Bekhti[1], Blanche Guillon[1], Florence Castelli[1], Etienne Thévenot[1], François Fenaille[1], Blandine De Lauzon-Guillian[2], Karine Adel-Patient[1], and Estelle Rathaho-Paris[1]

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La détection et la caractérisation de xénobiotiques au sein de matrices complexes constituent un enjeu majeur en chimie analytique. Le développement d'outils analytiques de plus en plus sensibles tels que la spectrométrie de masse à haute résolution couplée à la chromatographie liquide (LC-HRMS) a permis la détection d'un grand nombre de composés par analyse. Cependant l'augmentation résultante de données produites dans le cadre de projets toujours plus ambitieux rend cet exercice impossible à effectuer manuellement. Ainsi, nous avons conçu un workflow automatisé sous le langage R permettant le traitement d'un grand nombre de jeux de données produits par LC-HRMS afin d'extraire des signaux répondant à des critères assurant leur fiabilité ainsi que des caractéristiques de xénobiotiques. Il comprend séquentiellement : i) des filtres isotopiques afin d'identifier les composés contenant des isotopes tels que : 12C/13C, 79Br/81Br, 35Cl/37Cl ou 32S/34S ii) un filtre métabolique détectant les signaux possédant des différences de masses correspondant à des biotransformations connues (glucuronidation, sulfatation, conjugaison au glutathion) iii) un filtre exogène sélectionnant les signaux dont la fréquence de détection dans les échantillons est en dessous d'un certain seuil iv) une proposition d'annotation prenant en compte les informations des filtres isotopiques v) une recherche automatique sur la banque de données Pubchem.

L'application aux jeux de données LC-HRMS générés à partir d'échantillons de méconium (n = 308) et de lait maternel (n = 320) provenant de la cohorte EDEN a permis d'identifier des composés exogènes comme l'acétaminophène, la caféine et la nicotine, permettant ainsi de valider notre approche.

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Oral 21 – O21

Improving prioritization methodologies for natural extracts: Integrating diverse data from metabolomics datasets and biological screenings into knowledge graphs.

Luis Quiros-Guerrero [1,2], Frederic Burdet[3], Olivier Kirchhoffer[1,2], Paola Haemmerli[1,2], Louis-Felix Nothias[4], Pierre-Marie Allard[5], Arnaud Gaudry[1,2], Jahn Nitschke[6], Nabil Hanna[6], Florence Mehl[3], Antonio Grondin[7], Bruno David[7], Chunyan Wu[8], Erick Carreira[9], Thie Soldati[6], Christian Wulfrum[8], Marco Pagni[3], and Jean-Luc Wolfender[1,2]

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[7] Green Mission Department, Herbal Products Laboratory, Pierre Fabre Research Institute, Toulouse – France
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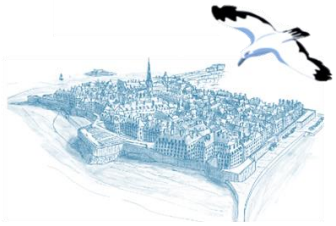
Integrating diverse datasets in natural products (NPs) research, such as metabolite profiling (UHPLC-HRMS2), annotations, and biological screening results, presents significant challenges. Typically, data is processed uniformly to generate feature tables across samples for techniques like Molecular Networking, enabling precise comparisons within similar groups. However, integration of new samples and tcomparing them across different batches face obstacles due to experimental variations. To overcome these challenges, innovative sample-centric approaches have emerged to explore varied datasets of Natural Extracts (NEs) over time (Gaudry et al. 2022). Additionally, there is a growing need for a comprehensive knowledge driven frameworks that integrates all types of data, leading to the adoption of Knowledge Graphs (KG) in metabolomic projects (Gaudry et al. 2023). KGs provide structured representations of complex datasets through RDF semantic web data standardization (RDFSemanticWebStandards 2014), facilitating exploration and connecting information (Caufield et al. 2023). For instance, they allow linking a sample's taxonomy, spectral annotations, and bioactivity with existing knowledge. In this context, a multidimensional KG comprising more than 250 million triples was generated as part of a collaborative project, incorporating UHPLC-HRMS2 data from over 3,000 NEs, fractions, and pure compounds, alongside taxonomical information, chemo-informatics results, and bioassay outcomes for tuberculosis, obesity, anticancer, and antiviral models. We will outline the key elements for constructing the KG based on NEs experimental data and demonstrate the potential of using SPARQL queries (SPARQLQueryLanguageforRDF 2018) to explore the KG, illustrating their effectiveness in guiding sample selection and expediting NP discovery.



Communications orales sponsors gold

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Oral constructeur 1 – CS1

Shimadzu

La spectrométrie de masse informatisée accélère la localisation des C=C pour la lipidomique non ciblée en utilisant la dissociation par attachement d'oxygène

Bessem Brahim – Ingénieur Application, Shimadzu France

Les lipides constituent un groupe de composés très divers, tant du point de vue de leur structure que de leurs fonctions dans les cellules, tissus et organes. Une part importante du lipidome est composée de lipides insaturés, c'est-à-dire de lipides qui présentent au moins une double liaison (C=C) dans un ou plusieurs de leurs constituants hydrophobes avec au moins une double liaison (C=C) dans une ou plusieurs de leurs chaînes lipidiques hydrophobes constitutives (ce qui inclut les acyles gras et les éthers gras) chaînes d'acyle et d'éther gras ainsi que les bases sphingoïdes). Par conséquent, les isomères résultant de différentes positions C=C contribuent de manière significative à la diversité structurelle des lipides.

La lipidomique non-ciblée basée sur la spectrométrie de masse a permis l'élaboration de l'atlas du lipidome des organismes vivants au niveau moléculaire. Bien que la position des C=C soit un facteur crucial dans les systèmes biologiques, les structures définies par C=C n'ont pas encore été caractérisées de manière exhaustive. Nous présentons une approche de lipidomique non-ciblée permettant la localisation des C=C, via une combinaison de dissociation par attachement d'oxygène et de spectrométrie de masse informatisée afin d'augmenter le taux d'annotation.

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Oral constructeur 2 – CS2

Waters

Pushing the boundaries of large-scale omics studies with Multi Reflecting Time-of-Flight (MRT) technology

Freddy Drouyé, Jodie Melin – Mass Spectrometry Sales Specialist, Waters Corporation

In this fast-paced scientific talk we'll look at the benefits of a prototype benchtop multi-reflecting time-of-flight (MRT) mass spectrometer for multi-omics analyses, and how high resolving power and sub ppm mass accuracy, achieved independent of acquisition rate, provides high quality data for exceptional scientific interpretation.

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Oral constructeur 3 – CS3

Proteigene (Biocrates)

Enabling precision medicine with quantitative metabolomics by biocrates life sciences

Carlos Malpica, PhD, MBA - Senior Business Consultant

Over the years biocrates life sciences has enabled epidemiology research teams to generate metabolomics data from cohorts subject to study for disease onset and progression. Most studies included healthy control individuals whose data is now compiled in a reference database, QMDB, which is available to our collaborators (https://biocrates.com/quantitative-metabolomics-database).

Data generated with biocrates tools, including the latest MxP Quant 500 XL, provide the broadest commercially available coverage of metabolic pathways with quantitative measurements for more than 1000 metabolites (https://biocrates.com/mxp-quant-500-xl). In addition to this data, we have developed MetaboIndicator, an add-on to our WebIDQ software, which provides clinically relevant parameters (https://biocrates.com/metaboindicator).

A single blood sample can provide valuable clinical data and the above referred tools are now in routine use at translational medicine units and precision medicine clinics around the world.

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Oral constructeur 4 – CS4

Sciex

A powerful single method for Metabolic Profiling and Characterization of cell culture media (CCM) components using ZenoTOF 7600

Omais Badaoui, Ph.D., Senior Sales Manager, SCIEX

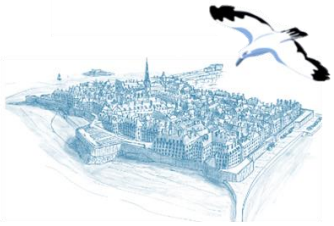
Biopharmaceutical production involves various media systems, and the components, levels, and consumption of critical cell culture media (CCM) can vary depending on the specific product, cell type, and cell line. It is crucial to have both qualitative understanding and quantitative tracking of these variations to meet quality requirements and improve manufacturing efficiency.

The analytical requirements include monitoring and identifying numerous metabolites with diverse chemical properties, analyzing complex matrices with wide natural abundance and chemical properties, and achieving sensitive quantitation and targeted/non-targeted identification simultaneously.

To address these challenges, a highly sensitive LC-MS/MS method has been developed and optimized. By combining data-independent acquisition (DIA) with the Zeno trap, this method enables quantitation at very low levels with great repeatability for all concentrations. Moreover, using Zeno SWATH DIA instead of SWATH DIA approaches allows the detection of even very low abundant targeted metabolites. The high mass accuracy achieved at both MS and MS/MS levels enables confident putative identifications compared to the CCM library. Additionally, the method utilizes Electron-Activated Dissociation (EAD) to precisely characterize components.

In summary, the optimized LC-MS/MS method offers highly sensitive and accurate mass measurements, allowing for improved monitoring, identification, and quantitation of metabolites in biopharmaceutical production.

RÉSEAU FRANCOPHONE DE MÉTABOLOMIQUE ET FLUXOMIQUE



16 èmes Journées Scientifiques du RFMF

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Oral constructeur 5 – CS5

Agilent Technologies

Des solutions clef en main pour l'analyse ciblée multi-omique

Jérémy Jeudy - LC/MS Product Specialist France

La recherche moderne nécessite des approches ciblées et non ciblées pour mieux comprendre la biologie, les maladies et les mécanismes observés lors d'interventions thérapeutiques potentielles.

A travers cette courte présentation, venez découvrir les dernières solutions d'Agilent en analyses multi-omiques, conçues pour répondre aux besoins actuels des laboratoires en biologie et recherche médicale. Cette session vous donnera un aperçu d'une approche ciblée et polyvalente, permettant de profiler et comparer divers types d'échantillons et de molécules biologiques à travers un workflow avancé, complet, et extrêmement reproductible.

Cette plateforme intégrée pourra vous guider à chaque étape : préparation des échantillons, analyse LC/MS, et traitement des données, révélant ainsi des connaissances biologiques essentielles. En outre, elle s'étend aux analyses lipidomiques et protéomiques, offrant une flexibilité exceptionnelle pour vos projets de recherche.

Vous pourrez découvrir comment cette approche peut accélérer vos études, faciliter l'adoption des techniques omiques dans votre laboratoire, et améliorer significativement l'efficacité et la précision de vos analyses.

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Oral constructeur 6 – CS6

Thermo Fisher Scientific

Orbitrap Astral applied to Metabolomics

Yannick Thiriet and **Marie-Pierre Pavageau**, Thermo Fisher Scientific

The new Orbitrap Astral mass spectrometer provides a unique opportunity to perform a high-throughput SQUAD analysis without compromising the sensitivity and coverage utilizing a parallel orbitrap astral analysis.

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Oral constructeur 7 – CS7

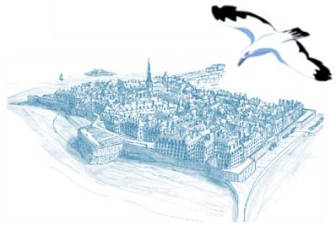
Centre Mondial de l'Innovation, Groupe Roullier

La métabolomique comme outil pour le développement de nouveaux produits de nutrition végétale

Elise Rethoré - R&D Nutrition Végétale - Chef de Projet - Gestion des Stress Abiotiques chez les plantes

Le groupe Roullier est un acteur majeur international dans le domaine de la nutrition végétale et animale. Son Centre Mondial de la Recherche (CMI) développe de nouvelles solutions innovantes pour répondre aux enjeux agronomiques de demain. La métabolomique constitue un des outils nécessaires à la caractérisation des matières premières et à l'optimisation des processus d'extraction des molécules d'intérêt. Dans un second temps, le profilage métabolique chez la plante ou l'animal permet de mieux comprendre l'effet des solutions technologiques sur leur performance agronomique. Au travers de quelques exemples, nous illustrerons ces différentes étapes du processus R&D et expliquerons pourquoi la compréhension fine des mécanismes d'action des produits développés est essentiel pour répondre au mieux aux besoins des agriculteurs.

RÉSEAU FRANCOPHONE DE MÉTABOLOMIQUE ET FLUXOMIQUE



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4-6 juin 2024, Saint-Malo

Communications flash

16 èmes Journées Scientifiques du RFMF
4-6 juin 2024, Saint-Malo

Oral Flash 1 – F1

Ex-vivo study of skin permeability and stability of a topical neurofibromatosis application using a combined LC-MS/MS and MALDI-FTICR imaging worklow

Edith Nicol[1], Bernard Do[2,3], Marina Vignes[4], Maxime Annereau[2,4], Muriel Paul[3,5], Pierre Wolkenstein[6,7], Ruxandra Gref[2], David Touboul[1], and Philippe-Henri Secretan[8]

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[2] Inst. des Sciences Moléculaires d'Orsay – Univ. Paris-Saclay, Centre Nat. de la Recherche Scientifique – France
[3] Department of Pharmacy – AP-HP, Hôpital Henri Mondor, Créteil – France
[4] Clinical Pharmacy Department – Gustave Roussy Cancer Campus – France
[5] Epidemiology in Dermatology and Evaluation in Therapeutics – Univ. Paris-Est Créteil Val-de-Marne - Paris 12 – France
[6] Department of Dermatology – National Referral Center for Neurofibromatosis, Henri Mondor University Hospital, Assistance Publique – Hôpitaux de Paris, Creteil – France
[7] Clinical Investigation Center – Inserm 1430, Henri Mondor University Hospital, Assistance Publique – Hôpitaux de Paris, Creteil – France
[8] Université Paris-Saclay – Matériaux et Santé, Orsay – France

Neurofibromatosis type 1 (NT1) is a genetic disorder affecting the nervous system leading to the appearance of neurofibromas, which are treated by surgical removal, leading to regrowth and significant surgical risks (1). Recent studies have identified Selumetinib as a promising candidate for clinical trials in NT1-related plexiform neurofibromas (2). To propose a topical formulation of selumetinib, a prerequisite is to characterize its behaviour in the skin, and all the more since it has been shown that selumetinib is amenable to degradation in the presence of sunlight (3). In this study we have investigated the fate of selumetinib when administered to skin explants. The use of MALDI-FTICR analysis showed the permeation of selumetinib and the detection of metabolites from photodegradation processes within the different skin layers. Ultrahigh mass resolution allowed specific distinction of selumetinib from endogenous molecules causing interferences. LC-MS/MS study was carried out on tissue extracts to quantify selumetinib and its degradation product in the different skin layers. The results show limited permeation, often restricted to the epidermis and *via* skin annexes to the dermis. Both techniques conclude that selumetinib is partially photodegradable in the skin, leading to the same compound as identified in solution. This study enabled a better understanding of selumetinib's permeation for topical treatments, and demonstrated the need to obtain a formulation protecting selumetinib and improving its' permeation.

(1) Wilson BN, et al. J Am Acad Dermatol. 2021;84(6):1667-76 ; (2) Gross AM, et al. N Engl J Med. 2020;382(15):1430-42 ; (3) Bouchema TS, et al. Pharmaceutics. 2022;14(12):2651

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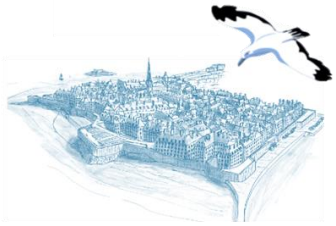

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4-6 juin 2024, Saint-Malo

Oral Flash 2 – F2

Integration of lipidomics and polar metabolomics for a molecular characterization of solvent neurotoxicity

Mathieu Galmiche[1,2,3], Isabel Meister[1,2,3], David Pamies[3,4], David Lopez Rodriguez[3,4], Marie-Gabrielle Zurich[3,4], Julien Boccard[1,2,3], and Serge Rudaz[1,2,3]

[1] Université de Genève – Suisse
[2] Institut des Sciences Pharmaceutiques de Suisse Occidentale (ISPSO) – Suisse
[3] Swiss Centre for Applied Human Toxicology (SCAHT) – Suisse
[4] Université de Lausanne (UNIL) – Suisse

Glycol ethers such as propylene glycol butyl ether (PGBE) are organic solvents used in many industrial processes and their ubiquitarian presence constitutes a source of human exposure. Associations have been found between occupational solvent exposure and neurogenerative diseases, but neurotoxicity is still not systematically assessed. To investigate neurotoxicity at the molecular level, in vitro human 3D brain spheroids comprising neurons and glial cells were exposed to 5, 10 and 20 mM PGBE and its main metabolite 2-BPA for 2 days and 1 week, respectively. Untargeted chemical profiling was achieved using a double extraction strategy, where the remaining pellet obtained after polar metabolites extraction was re-extracted for lipidomics. Polar metabolomics (zwitterionic Hilic) and lipidomics (RPLC) were acquired in HRMS in data-dependent mode. Polar metabolomics led to 120 manually curated level 1 identifications and 86 relevant level 3/4 annotations. OPLS data analysis showed alterations of the levels of ribonucleotides and carbohydrate phosphates of the glycolysis pathway, suggesting increased energy metabolism after acute exposure (48 h) to the solvents, followed by a strong decrease after prolonged treatment (1 week). Based on the high-quality MS/MS annotation of 713 lipid species, lipidomic analysis revealed that exposures to PGBE and 2-BPA caused significant decreases of phosphatidylethanolamines and hexosylceramides. These lipid alterations may indicate the disruption of the myelin sheath, the multilayered membrane produced by oligodendrocytes which is critical for neuronal signal transmission in axons. These results, complemented by other omics analyses, pave the way for a better understanding of glycol ethers' neurotoxic modes of action.

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Oral Flash 3 – F3

Early biomarkers of transition to psychosis detected by NMR IVDr technique: a pilot study

Maria Teresa Avella[1,2], Cédric Caradeuc[3,4], Oussama Kebir[1,2,5], Marie-Odile Krebs[1,2], Nicolas Giraud[3,4], Boris Chaumette[1,2,5,6], Ariel Frajerman[7,8], and Gildas Bertho[3,4]

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[2] Institute of Psychiatry, Paris – GDR 3557 of Psychiatry – France
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[5] PEPIT, Paris – GHU Paris Psychiatrie et Neurosciences – France
[6] Department of Psychiatry, McGill University, Montreal – Canada
[7] MOODS Team, Le Kremlin Bicêtre – INSERM, CESP, Univ. Paris-Saclay, Faculté de Médecine Paris-Saclay – France
[8] Service Hospitalo-Universitaire de Psychiatrie de Bicêtre, Assistance Publique-Hôpitaux de Paris, Hôpitaux Universitaires Paris-Saclay, Hôpital de Bicêtre, F-94275 – Mood Center Paris Saclay – France

Introduction : Schizophrenia is a severe mental illness whose onset is frequently preceded by a prodromal phase, with subjects experiencing it being defined as ultra-high risk of psychosis (UHR). Psychotic transition occurs in 25% of cases within a year from the diagnosis, and it has been associated to metabolic dysregulation.

Aim of the study : Detect serum biomarkers predictive of psychosis conversion among UHR patients.

Materials and methods : 14 UHR subjects converting to psychosis (UHR-C) and 21 not converting (UHR-NC) were selected from the project ICAAR (Centre Hospitalier Sainte-Anne, Paris). Serum samples were collected at baseline (M0), after six (M6) and twelvemonths (M12). Serum were analysed using the NMR high-throughput technique IVDr (In Vitro Diagnostic for research) which provided an automated report of 112 lipoprotein parameters and 32 metabolites parameters with absolute quantifications. Statistical analysis were conducted with SIMCA 17.0, MetaboAnalyst 6.0 and R Studio 4.3.0.

Results : Lipoprotein parameters related to high-density lipoprotein subgroup 4 (H4FC, H4A1) and low-density lipoprotein subgroup 4 (L4FC) are reduced in UHR-C compared to UHR-NC at M0. A good discrimination capability at ROC analysis is observed with negative correlations relative to symptoms. Differences in biomarkers is observed due to treatment and according to sex.

Conclusions : This pilot study suggested that NMR could be a useful technique for better understanding the pathophysiology of early psychosis and detecting biomarkers of the psychotic transition. These results need to be validated with a larger cohort, and to find significant biomarkers at follow-up.

16 èmes Journées Scientifiques du RFMF
4-6 juin 2024, Saint-Malo

Oral Flash 4 – F4

Exploration of the metabolic impact of phenylketonuria by metabolomics on Dried Blood Spot

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Phenylketonuria is a hereditary metabolic disease that causes a variety of damage in patients, including neurological signs and developmental disorders. This disease is detected by the Guthrie test, which determines the blood concentration of phenylalanine on Dried Blood Spot (DBS) collected on the 3rd day of life. The aim of this project is to study the metabolic profiles of patients with phenylketonuria or permanent moderate hyperphenylalaninemia and to compare them with the metabolic profiles of controls. Metabolomics analysis was perfomed by non-targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS). Different protocols were investigated in order to optimize the extraction method and collect a maximum amount of metabolites. The chosen protocol was applied to samples from 30 patients and 30 controls. The metabolic profiles of patients and controls were compared using multivariate statistical approaches. This enabled us to identify significantly different metabolic fingerprints between patients and controls, but also according to disease severity or control by treatment. A number of metabolites and metabolic pathways were shown to be deregulated in patients. This work has validated an extraction method for metabolites on DBS, applicable to other pathologies relevant for newborn screening. It has also highlighted the value of non-targeted metabolomics approaches for studying metabolic disturbances in patients with phenylketonuria and more generally in the context of metabolic diseases.

16 èmes Journées Scientifiques du RFMF
4-6 juin 2024, Saint-Malo

Oral Flash 5 – F5

New markers for monitoring the elimination of the reactive N-Acetyl-p-benzoquinone imine after paracetamol/acetaminophen hepatotoxicity

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Paracetamol/acetaminophen (N-acetyl-p-aminophenol, APAP) overdose is one of the most important causes of drug-induced liver injury worldwide. Hepatotoxicity induced by APAP is mainly caused by the production of N-acetyl-p-benzoquinone imine (NAPQI), a highly reactive intermediate formed predominantly *via* the cytochrome P450 2E1. Here, we used human studies and in vitro models to demonstrate that NAPQI-glutathione derived thiomethyl metabolites (i.e., S-methyl-3-thioacetaminophen sulfate and S-methyl-3-thioacetaminophen sulphoxide sulfate) identified using high-resolution mass spectrometry could serve to monitor NAPQI detoxification and elimination in patients (after intake at recommended dose or after intoxication). In addition to improve APAP intoxication diagnosis, these biomarkers could also serve to detect inter-individual variability in NAPQI production to assess susceptibility to APAP-induced hepatotoxicity. Using in vitro human models, we showed that these thiomethyl metabolites are directly linked to NAPQI detoxification since they are mainly formed after exposure to glutathione-derived conjugates *via* an overlooked pathway called the thiomethyl shunt. These delayed thiomethyl metabolites have great potential in future clinical studies in order to provide a more reliable history of APAP ingestion in case of acute intoxication or to study underlying causes involved in APAPinduced hepatotoxicity. Further toxicological investigations would also help to understand if the thiomethyl shunt acts only as a pathway for NAPQI detoxification and excretion, or if it contributes to liver or renal toxicity in case of APAP overdose through the generation of the newly identified intermediates with highly reactive sulfur-containing fragment as observed with environmental contaminants.

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Oral Flash 6 – F6

Développement d'un workflow computationnel pour la fluxomique ^{13}C

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En biologie des systèmes, l'étude des flux métaboliques est cruciale pour comprendre le phénotype des organismes vivants. Dans les domaines de la santé et de la biotechnologie, elle facilite la découverte des mécanismes régulateurs clés des maladies ou l'optimisation des processus biologiques pour la production de molécules d'intérêt. Une des techniques majeures utilisée est l'Analyse des Flux Métaboliques (MFA), qui consiste à étudier et quantifier les vitesses de réactions biochimiques au sein des réseaux métaboliques grâce à des méthodes de modélisation mathématique basée sur contraintes. En 13C-MFA, l'utilisation d'isotopes stables du carbone dans des expériences de marquage isotopique (ILE) fournis des données expérimentales permettant de contraindre les modèles et ainsi augmenter la précision des flux calculés. Récemment, un nombre croissant d'études démontrent qu'il est possible d'augmenter le débit des ILE et des étapes analytiques. Cependant, le traitement des données et la modélisation des flux reste un goulot d'étranglement en 13C-MFA. Ceci est dû à la complexité qui découle de la multiplication des logiciels disponibles et du manque d'interopérabilité entre ces derniers. Cette partie reste donc majoritairement manuelle et sujette aux erreurs humaines, diminuant la reproductibilité et augmentant les coûts. Pour répondre à ce problème, nous avons intégré et/ou développé plusieurs outils/modules pour effectuer les différentes parties du workflow global (approche en microservices). Disponibles sur la plateformeWorkflow4metabolomics et rendus interopérables, nous les avons implémentés au sein d'un workflow computationnel automatisé, flexible, reproductible, et haut débit pour la 13C-MFA.

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Oral Flash 7 – F7

LC-HRMS-based metabolomics as a tool to develop analytical methods : How to choose the best extraction protocol when it comes to untargeted analysis ?

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Among lots of exigencies, two major requirements are necessary to assure the best performance of untargeted metabolomics-based approaches: (1) covering the maximum number of compounds present in the studied samples, and (2) determining the "optimal" analytical conditions to do so.
The present work suggests an approach to address these exigencies. This approach is based on LC-HRMS untargeted analyses and metabolomics computational tools. It will be developed and applied to assess the optimal extraction protocol dedicated to analyze a wide range of pesticides formulates and microbial metabolites at once, all hidden in a complex environmental matrix: agricultural soil.

Therefore, to compare five different extraction protocols applied on two types of soil and two formulated herbicides, four criteria were selected: (1) the coverage of compounds in term of polarity, (2) the quantitative performance, (3) the repeatability, and (4) the capacity to discriminate between contaminated and non-contaminated soils. To assess each criterion, different data analysis and visualization tools were used (e.g. hierarchically-clustered and polarity-segmented Heatmaps, van Krevelen diagrams, Euclidean Distances, RSD density plots, and OPLS-DA). They will be presented, explained and discussed in order to show their advantages, limitations and complementarities, as well as giving the best practices and tricks to render their exploration optimal.
These tools finally allowed for the selection of the best extraction protocol. They are thus suggested to assess the performance of analytical methods when it comes to untargeted metabolomics analyses.

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Oral Flash 8 – F8

Discovery of the first archaeal terpene synthases: metabolic engineering meets untargeted metabolomics

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Our lab developed a machine learning model that detects terpene synthases. Our model predicted terpene synthase activity for 7,000 ”dark matter” protein sequences from UniRef50 with no InterProScan signatures (even ”domain of unknown function”).
We expressed 17 ”dark matter” proteins in Saccharomyces cerevisiae JWY501, genetically modified to overproduce sesquiterpenes (C15H24) and diterpenes (C20H32). To discover their terpene synthase activity, we had to integrate untargeted metabolomics into the metabolic engineering workflow.

Metabolic engineering for terpene synthesis discovery relies on GC-EI-MS as a detector. In most cases, the product is known or guessed based on literature. Therefore, the major drawback of electronic impact (low or no signal from molecular ion) is circumvented by searching diagnostic fragments and NIST-EI library queries. This strategy only confirmed 3 enzymes as terpene synthases.

Electrospray is not commonly used for terpenes, but we found it can ionize terpene scaffolds surprisingly well. Thus, we took advantage of untargeted metabolomics strategies (MetaCorrelate module in MZmine 3, introduced for the Ion Identity Networking workflow) to search for possible chemical variations of terpene scaffolds and discovered that 4 additional enzymes were producing terpenes. We went back to GC-EI-MS data confirmed that 2 of the enzymes discovered using LC-HRMS were missed in our first analysis. Out of 7 enzymes we confirmed activity, 3 were detected by GC-MS and LC-HRMS, 2 by GC-MS and 2 by LC-HRMS. Among the 7 confirmed terpene synthases, 3 are of archaeal origin. This work constitutes the first experimental evidence of active terpene biosynthesis in the Archaea kingdom.

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Oral Flash 9 – F9

Unraveling *Brassica napus* leaf metabolic diversity: leveraging machine learning for agronomic traits prediction

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Rapeseed (*Brassica napus*) emerged through interspecific hybridization between Brassica rapa and Brassica oleracea. Subsequent genetic breeding efforts focused on reducing grain erucic acid and glucosinolates due to their toxicity, reflecting modern accessions. Creating crops with agroecological relevant metabolic profiles requires a chemical diversity characterization. Despite recent reports on vegetative parts involving glucosinolates and phenolics, intraspecific phytochemical diversity in leaves is understudied. Moreover, leveraging vegetative metabolomics to predict agronomic traits holds breeding potential. This study aimed to analyze the leaf metabolome across 304 brassica accessions and associate metabolome with agronomic traits using machine learning-based predictive approaches. LC-MS/MS metabolomics was performed on ethanolic extracts. MS-signals were processed by MSDIAL, resulting in 16,192 features, and annotations were performed using an in-house database. Predictive metabolomics based on LASSO, Ridge, and Elastic-Net models were employed to predict qualitative and quantitative traits using 4,919 curated features. Classification analyses achieved high accuracies (> 95%, p< 2e-16), with perfect accuracy (100%) for species prediction, revealing 261 predictive biomarkers, including some glucosinolates, and pointing out the modernity of accessions. Regression results showed r2-values above 0.75 for phenolic, glucosinolates, and erucic leaf content, and r2-values under 0.6 for protein and lipid leaf content, and leaf surface and area. Future predictions will incorporate root metabolome to associate with these agronomic traits, and a smaller panel of spring accessions (170) to predict grain yield under different conditions. Overall, predictive modeling facilitates understanding metabolic-phenotypic associations, aiding genotype selection for plant performance, including yield and flowering. This metabolomics approach holds promise for future breeding endeavors.

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Oral Flash 10 – F10

Diversité phytochimique de collections génétiques de Brassicacées pour la recherche de caractères d'intérêt agronomiques et agroécologiques

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Le métabolisme spécialisé représente une dimension essentielle des interactions entre les plantes et leurs bioagresseurs. Sa caractérisation au sein des espèces cultivées est donc un enjeu majeur, en particulier dans des programmes de sélection variétale. Néanmoins, chez les Brassicaceae et plus particulièrement chez le colza (Brassica napus), ce métabolisme est encore trop partiellement connu. Notre objectif est de caractériser la diversité phytochimique qui existe au sein de collections génétiques chez des espèces du genre Brassica et d'identifier les déterminismes génétiques qui contrôlent ce métabolisme spécialisé par le biais d'approches combinées de génétique, de génomique et de métabolomique. Les projets de recherche menés ces dernières années nous ont permis de (1) mettre en évidence des liens entre la résistance à un agent pathogène racinaire et des variations alléliques sur plusieurs loci, affectant les teneurs en certains métabolites spécialisés, (2) démontrer que l'architecture génétique globale du métabolisme spécialisé est organisée chez le colza sous forme de petits réseaux de QTL métaboliques qui contrôlent indépendamment des sous catégories de composés et (3) montrer qu'il était possible d'enrichir le répertoire métabolique du colza et d'élucider des voies de biosynthèse encore inexplorées, en s'appuyant sur des stratégies innovantes de croisement et d'introgression. Ces travaux ouvrent la voie vers une meilleure compréhension du métabolisme spécialisé chez les plantes de la famille des Brassicaceae. Ils ont également permis de générer des ressources destinées à soutenir les efforts de recherche pour l'étude des interactions entre les plantes du genre Brassica avec différents bioagresseurs et avec le microbiote racinaire.

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Oral Flash 11 – F11

Metabolomics approaches of seed-borne fungal endophytes for enhancing tomato seed performance in challenging environments

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Seed germination is drastically decreased by biotic and abiotic stresses. The improvement of seed germination efficiency is, hence, a strategic priority to increase food production. In order to substitute chemical treatments currently used, there is a need to develop environmentally-friendly solutions. A way to enhance seed performance is to exploit the beneficial impact of endophytes on plant fitness. The current view is that such phenomenon relies on chemical mediations using the large variety of molecules produced by endophytes. Tomato is one of the most important crops worldwide undergoing important periodic losses due to abiotic and biotic stresses. Tomato seed germination and seedling establishment are particularly sensitive to salt and water stress as well as fungal pathogens. Recent studies support the potential of endophytes to improve tomato tolerance to salt stress or pathogens. Here, we explore the potential of metabolites from tomato seed-borne fungal endophytes to improve tomato seed germination under stress. We thus isolated and identified 63 cultivable fungal strains from a core collection of tomato seeds. We have initiated bioactivity screening to evaluate their efficacy in combating pathogens and enhancing seed germination under salt/drought stress conditions. Furthermore, the metabolome of the most promising strains is being investigated using LC-MS/MS and molecular networking techniques to dereplicate extracts and identify the most active compounds. The next step will be to isolate the most active compounds in order to generate new high-value molecules capable of providing alternative and environmentally friendly solutions to the currently used pesticides.

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Oral Flash 12 – F12

The UNTWIST project : Unraveling Stress Response Mechanisms in *Camelina sativa* for Enhanced Crop Resilience in European Agriculture

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Climate change, particularly variability, challenges European agriculture, causing drought and high-temperature stress, reducing productivity and yield. Camelina sativa, a native European oilseed crop, has regained attention for its adaptability, yield stability, and high performance in variable environments. The UNTWIST project aims to understand the stress response mechanisms of Camelina sativa using a multidisciplinary approach, creating an unprecedented dataset from a core collection of 54 Camelina lines grown under various stresses and locations across Europe.
Employing top-down modeling, we predict phenotypic field traits from metabolic data using machine learning. A predictive model was developed by combining phenotypic field data with targeted and LCMS based untargeted metabolomics data from early-stage leaves of Camelina lines grown under control, water, or thermal stress conditions. This model enables the prediction of Thousand Kernel Weight (TKW) and fatty acid content under various stresses. Current efforts involve comparing these results with those of genomic prediction based on the same data.

The bottom-up approach focuses on the growth and development of Camelina fruit by reconstructing four compartmentalized genome-scale metabolic networks of diverse stress responsive lines. Metabolic data is being utilized to calculate fluxes for each model, constraining networks to provide insights into drought and heat tolerance mechanisms. These networks will be further refined with omics from the focus lines.
The UNTWIST project's comprehensive analysis of Camelina sativa's stress response mechanisms will advance our understanding of crop resilience, ultimately contributing to the development of more sustainable and climate-resilient farming practices.

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Oral Flash 13 – F13

Dealing with non-model organisms : Annotation of micro-algal metabolites using high-resolution mass spectrometry and advanced dereplication tools

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Over the millennia, the particularly harsh conditions prevailing in the oceans have forced marine micro-organisms to produce a wide variety of bioactive molecules in order to survive. These marine natural products (MNPs) represent a reservoir of bioactive compounds with promising pharmaceutical prospects and economic potential. The development of highthroughput metabolite profiling and bioinformatic tools to process large datasets is now accelerating the discovery of new MNPs. The current challenge lies in the annotation of new natural products in complex extracts. The project CEVAMAP focuses on the research of high-value-added metabolites produced by a selection of micro-algae and cyanobacteria strains isolated from French Polynesian lagoons. The 99 micro-algae strains were cultivated over a 6-month period to ensure sufficient biomass for metabolite profiling and bioactivity testing. Extraction conditions (number of cycles, solvent composition, etc.) and purification methods (elimination of salts and polar primary metabolites) were optimized to obtain comprehensive extracts enriched in secondary metabolites. Untargeted analyses were then performed using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS, Obitrap Exploris 120). The resulting dataset was processed with MzMine 3 and annotated through molecular networking (GNPS, cytoscape). The annotation coverage was then improved using SIRIUS and Tima-r tools in addition to in-house database obtained from purified compounds. Antiviral, antibacterial and anticancer activity assays were also carried out on a selection of strains, followed by a micro-fractionation approach to study the families of molecules responsible for these activities and their presence in the dataset.

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Oral Flash 14 – F14

Insight on the relationship between the meta-metabolome, photosynthesis sensitivities and their natural fluctuation in freshwater microbial communities exposed to a model herbicide

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Facing aquatic chemical pollution, the study of microbial communities improves the ecological dimension of ecotoxicology. Despite growing knowledge on the effects of contaminants on biofilms, there is still a paucity of information about the natural fluctuation of the sensitivity of these communities to chemical stress. This is particularly the case of periphyton's photosynthesis sensitivity, for which the molecular/biochemical processes underlying its response remains partly described. To tackle this issue, untargeted meta-metabolomics can provide a comprehensive picture of the molecular/biochemical response prior physiological/functional impairment. In this context, the present study aims to characterize the monthly changes of periphyton's sensitivity over a year at the physiological and molecular levels by measuring the photosynthetic yield and the meta-metabolome. Periphyton were monthly-colonized in-situ and acutely exposed in controlled conditions to terbuthylazine. Sensitivity fluctuation was assessed by determination of benchmark-dose-1SD for the photosynthetic yield and the whole meta-metabolome. The results showed the strong sensitivity shift of the metametabolome vs the lesser one of the photosynthetic yield. Moreover, this study confirmed the higher sensitivity of the meta-metabolome, as most of signals responded prior to the photosynthesis. Further annotation of metabolites underlined the response of various classes including alkaloids and fatty acids. Among them, oxylipins were identified as part of the response to the oxidative stress enhanced by terbuthylazine exposure. Overall, this study highlighted the need to take into account of the natural fluctuation of microbial sensitivity in order to get better mechanistic understanding of the periphyton's meta-metabolome response to chemical stress.

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Oral Flash 15 – F15

Metabolomics and functional genomics of halogenation mechanisms in brown algae

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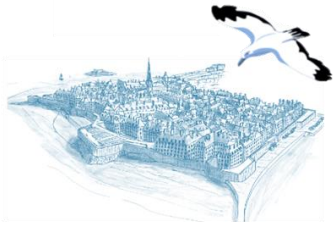
Some brown algae are known to concentrate halogens such as bromine and iodine to produce halogenated metabolites. Volatile or not, they are still poorly described. In addition the processes and function of halogenation remain uncertain in these marine organisms. Those compounds may have important roles in signalling and/or defense during biotic interactions and physiological responses to environnemental changes. My thesis work under HALOGENE project, aims to explore the production of halogenated metabolites and the role of halogen metabolism in brown algae through functional genomics and metabolomics studies. One of the putative halogenating key enzymes, vanadium-dependent haloperoxidase (vHPO), was inactivated using the CRISPR-Cas9 method in the model brown alga Ectocarpus siliculosus. The extinction of vHPO activity has been validated for 3 independent knock-out mutants. Chemical extractions using different polar and non-polar solvents for metabolomic analysis were carried out. Several analytical optimisations led to the detection of nearly 1,000 features in the wild-type strains under standard culture conditions. A workflow, based on LC-HRMS data and utilizing analytical tools like MZmine3, Sirius, HaloSeeker, and molecular networking, will enable the comparison and description of entire metabolomes between strains, as well as the specific characterization of the halometabolome. The current study will provide new knowledge about the chemical diversity of halogenated metabolites and their biosynthesis in brown algae.

RÉSEAU FRANCOPHONE DE MÉTABOLOMIQUE ET FLUXOMIQUE



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Communications posters

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Section 1 - Poster 1 – S1-P1

A machine learning analytical pipeline of breast cancer metabolomic profiles in West African patients, showing oxidative stress and deregulated serotonin metabolism

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A targeted metabolomic analysis was performed on plasma samples from a cohort of West African patients with breast cancer, compared with healthy controls, using the MxP500 kit (Biocrates) measuring 1063 metabolites. Univariate and multivariate analyses, through Mann-Whitney and OPLS-DA, respectively, were performed as the primary method. Despite achieving a good prediction with Q2cum of 0.55 and an AUC of 0.84, we sought to enhance the predictive model performance using a machine learning approach. Various algorithms were tested and compared using AUC, computed with repeated nested cross validation. Finally, ridge logistic regression, coupled with Boruta feature selection, yielded the most discriminating results with an AUC of 0.88. To facilitate interpretation of the model, SHAP values were chosen to extract the most important metabolites which were then compared with best metabolites (VIP > 1) obtained from the best OPLS-DA model. The combined analysis of univariate, OPLS-DA and ridge logistic regression predictions revealed a metabolomic profile of breast cancer comprising metabolites with higher blood concentrations, such as GABA, ADMA, triglycerides, an increased oxidative stress (increased Met-SO / Met ratio), and metabolites with lower concentrations, such as serotonin, threonine or xanthine. Most of these metabolites have been reported in dispersed manner throughout the literature in different breast cancer models and situations. In conclusion, this machine learning approach offers an interesting alternative to OPLS-DA for metabolomic data analysis and presents a global picture of metabolite signatures in breast cancer among West African patients.

Abbrev*ia*tions:
GABA: Gamma-aminobutyric acid
ADMA: Asymmetric dimethylarginine
MetSO: Methionine sulfoxide
Met: Methionine

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Section 1 - Poster 2 – S1-P2

A non-targeted metabolomics profile of ischemic stroke in West African patients

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In African populations, stroke exhibits higher prevalence, severity, and mortality rates compared to European or United States populations. Despite this, the majority of research studies have predominantly focused on Caucasian populations. To address this gap, we conducted a non-targeted metabolomics study involving West African patients who recently suffered from ischemic stroke (n = 79), comparing them to a healthy control group (n = 40). Our aim was to uncover new physiological and pathological insights. Among the 136 detected metabolites, we constructed a highly discriminant OPLS-DA model consisting of 125 molecules, 23 of which showed a VIP score exceeding 1, a fold change greater than 1.2, and still significant after Bonferroni correction. Our findings strongly indicate mitochondrial impairment and oxidative stress in the stroke patients. Moreover, within the ischemic stroke group, 39 cases had a clearly identified cause (17 atherothrombotic, 13 lacunar, 8 thromboembolic, and 1 embolic cardiopathy), while 40 cases were cryptogenic (without any identified etiology). Although we were unable to develop a robust predictive model considering the four identified classes, we successfully established a model capable of discriminating between atherothrombotic and lacunar stroke patients using only 8 molecules. Notably, this model identified 40% of our cryptogenic cases as more closely related to atherothrombotic patients and 32.5% as more affiliated to the lacunar group. While these results are preliminary, they bring attention to an underrepresented population and provide potential avenues for stratification.

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Section 1 - Poster 3 – S1-P3

An examination of the metabolomic profile of A315T TDP-43 mice

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Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by the degeneration of upper and lower motor neurons. Our aim was to explore an ALS mouse models (TDP-43 A315T) on metabolipidomics profiles. This data could serve to identify new markers of progression for ALS, including therapeutic evaluation. Brain, muscle, liver and serum samples were collected from wild-type (WT) and transgenic (Tg) mice (n=10 per strain). Samples underwent metabolite extraction and targeted acquisition using C18 and HILIC modes. Univariate analysis included the volcano plot of the False Discovery Rate. Multivariate analysis included PCA, OPLS-DA, and VIP scores. The quality of the models was characterized by : CV-ANOVA, R2 and Q2 values, and the permutation plot. We obtained good model robustness and predictive capability for all tissue and serum data examined. OPLS-DA score plots showed two distinct clusters between WT and Tg groups, corresponding to specific metabolomic profiles for each group. For these scores, we observed CV-ANOVA determined pvalues < 0.01, Q2 values > 0.8, and validated models using permutation tests. Analysis performed on extracted metabolites from the brain demonstrated a significant difference between levels of taurine, alpha-d-glucose and d-xylose. These three metabolites had VIP scores higher than 1, and a p-value < 0.05 suggesting them to be discriminatory molecules in the brain of ALS mice. KEGG enrichment analysis demonstrated changes in pathways associated with glycolysis/gluconeogenesis, carbohydrate metabolism, pentose and glucuronate interconversions. These findings reveal potential biomarkers of ALS related to the TDP-43 mutation, and provide further insights into the pathogenesis of ALS.

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Section 1 - Poster 4 – S1-P4

Apport de la métabolomique quantitative dans la compréhension des mécanismes physiopathologiques induits par les régimes obésogènes

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Introduction : L'obésité représente un défi majeur pour la santé publique à l'échelle mondiale. Elle s'associe à un risque accru de développer diverses maladies chroniques telles que le diabète de type 2, les maladies cardiovasculaires et est également liée à des complications respiratoires.
Méthode : deux études ont été récemment menées sur des souris C57Bl/6J, soumises à un régime obésogène riche en gras et en sucre suivi d'une analyse quantitative du métabolome par spectroscopie RMN du proton sur six organes (le foie, le poumon, le coeur, les muscles squelettiques, les reins, le cerveau) et le serum.
Résultats : Ces études montrent une perturbation de l'homéostasie métabolique dans la plupart des organes, notamment des altérations des métabolites appartenant au cycle des donneurs de méthyl (métabolisme du 1 carbone). Des changements précoces sont observés dès 6 semaines de régime, tandis que d'autres changements métabolites apparaissent significatives à 14 semaine, coïncidant avec l'installation d'une intolérance au glucose et à l'insuline. Le poumon est particulièrement affecté, présentant des dépôts lipidiques et des caractéristiques communes avec le foie.
Conclusion : Ces données permettent de pointer l'impact systémique des régimes obésogènes sur l'organisme mais également la complexité de déterminer des biomarqueurs de ces régimes selon l'interaction possible avec le temps. Une catégorisation plus fine des biomarqueurs est ici proposée.

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Section 1 - Poster 5 – S1-P5

Approche métabolomique appliquée aux larmes basales de patients atteints de sclérose latérale amyotrophique : une étude comparative prospective

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CONTEXTE : La sclérose latérale amyotrophique (SLA) est une maladie neurodégénérative progressive caractérisée par la dégénérescence des neurones moteurs. **OBJECTIF** : Cette étude vise à étudier le métabolome des larmes basales de patients atteints de SLA à la recherche de biomarqueurs diagnostiques et pronostiques.

MéTHODES : Il s'agit d'une étude cas-témoins prospective menée entre 2021 et 2023, incluant des patients atteints de SLA diagnostiqués selon les critères révisés d'EI Escorial. Les larmes basales ont été recueillies à l'aide de tubes en verre microcapillaires et analysées à l'aide de la chromatographie ultra haute performance couplée à la spectrométrie de masse.

RéSULTATS : Vingt-cinq patients atteints de SLA et 30 témoins ont été inclus. Aucune différence significative dans l'expression des métabolites n'a été observée entre les patients atteints de SLA et les témoins ($p > 0,05$). Cependant, le métabolome des larmes basales différenciait les formes bulbaire et spinale de SLA avec cinq métabolites sous-exprimés dans la forme bulbaire (aniline, trigonelline, caféine, théophylline, méthyl beta-D-galactoside) et un métabolite sous-exprimé dans la forme spinale (acide dodéc-2-ènedioïque).

CONCLUSION : Bien que cette étude n'ait pas identifiée de signatures métaboliques capables de distinguer les patients atteints de SLA des témoins, elle met en évidence l'existence de métabotypes lacrymaux spécifiques des formes bulbaire et spinale de la maladie. Cela ouvre des perspectives de recherche pour l'identification de biomarqueurs des maladies neurodégénératives dans leur ensemble.

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Section 1 - Poster 6 – S1-P6

Assessing the Impact of Post-Mortem Interval on Metabolomic Profiles : Preclinical Study of Post-Mortem Brain Tissue

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Neurodevelopmental and neurodegenerative disorders represent significant challenges to medical research. Post-mortem studies offer valuable information on underlying mechanisms or molecular markers linked to these disorders. However, the timing of tissue collection postmortem is a critical factor that can influence the metabolome. This study aims to explore the impact of post-mortem interval on brain metabolomic. Due to the heterogeneity of causes of death and the timing of clinical brain sample collection, interpreting post-mortem analyses is more challenging. Therefore, the use of an animal model is relevant.
For this purpose, samples from 5 brain regions (cortex, striatum, hippocampus, thalamus, and cerebellum) are collected at 7 distinct time points (t0, t2h, t6h, t12h, t24h, t36h, and t48h). All rats are sacrificed simultaneously time, and at time t0, brain regions samples are collected immediately after sacrifice. The remaining samples are kept at room temperature for 2 hours before being dissected (t2h group), or placed at 4◦C before the dissection at different time points. Each time-group includes six rats, resulting in a total of 210 samples, which are analyzed by LC-HRMS. The obtained data are processed using multivariate analyses.

The global PCA score plot indicates that the metabolome of each brain region is significantly affected by the time elapsed between tissue collection and sacrifice.
This research sheds light on the intricacies of post-mortem tissue analysis. Given that the timing of human brain sample collection is not always controllable, future statistical analyses will be required to identify the affected metabolic pathways and those that remain unaffected.

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Section 1 - Poster 7 – S1-P7

Breast cancer risk and the social, nutritional and chemical exposome

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Breast cancer (BC) is the most common cancer in women, and a major cause of womendeath in mid-life. In France, BC incidence has increased in 60-70 year-old women in the last decade. Multiple factors are associated with BC including lifestyles and reproductive patterns. Evidence suggests that environmental factors are determinants of BC, and their impacts are documented over lifespan. However, further research that understands the interactions between chemical and nonchemical factors, including individual factors, nutrition and the social environment, is needed. The aim of this interdisciplinary study is to map the chemical, nutritional and social exposome and provide preliminary data on associations between exposome and BC risk and aggressiveness. The selected cohorts of BC include two longitudinal cohorts established by the ICO in Nantes and one retrospective cohort in Tours. The cohorts have collected epidemiological and clinical data and have archived breast tissue and blood serum samples. The contribution of our team in this study is the characterization of the chemical exposome by using non-targeted approaches based on LC-ESI-HRMS in blood serum samples (N= 216) from BC cases, extracted by protein precipitation and phospholipid removal. This approach aims to expand the targeted approach based on the characterization of POPs, nutritional lipids, PUFAs, phytosterols, cholesterols, and inflammatory markers. Moreover, the social, psychosocial, and dietary factors related to BC are evaluated. Overall, our study provides the foundation for a long-term research program targeting the influence of the environment on BC in the Grand Ouest and offers new opportunities for prevention.

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Section 1 - Poster 8 – S1-P8

Characterization of diffuse large B-cell lymphoma subgroups by metabolomic analyses

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Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive form of lymphoma and characterized by a high heterogeneity. Despite recent advances, 40% of patients do not respond to conventional treatment and relapse, leading to death. Research has shown that the dysregulation of cancer cell metabolism can be targeted to overcome treatment resistance and lead to a personalized medicine. In this context, and in order to better characterize DLBCL cell lines, the first results of metabolomic analyses using high-resolution mass spectrometry coupled to liquid chromatography (LC-HRMS) will be presented. Metabolic extracts from the intra- and extra-cellular compartments of seven DLBCL cell lines were obtained and subjected to LC-HRMS profiling using an Orbitrap Fusion coupled to HILIC column separation. Among the 2,500 robust metabolic variables obtained in the LC-HRMS datasets, over 180 metabolites were identified, and some 20 of these proved significantly modulated specifically to the metabolism of the different lines. With the broader aim of developing and validating approaches combining mass spectrometry and ultra-high-resolution NMR to define new subgroups of DLBCL patients, the first MS / NMR cross-observations will be presented to highlight the points of convergence and the complementary nature of both techniques. These preliminary results, which will be refined and extended to the analysis of cell metabolism in the presence of drugs in the remainder of the project, pave the way for new diagnostic and then therapeutic options.

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Section 1 - Poster 9 – S1-P9

Chimie analytique et recherche translationnelle en santé : glycation de l'apoB100, métabolisme des LDL et risque cardiovasculaire dans le diabète

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Introduction – Les patients diabétiques présentent un risque cardiovasculaire (CV) élevé que les marqueurs cliniques traditionnels n'expliquent pas totalement. L'hyperglycémie chronique sous-jacente au diabète induisant la glycation des LDL et exacerbant leurs propriétés pro-athérogènes, nous avons cherché à identifier un biomarqueur de glycation de la protéine fonctionnelle des LDL, l'apoB100.

Méthodes – L'identification et la quantification du biomarqueur ont été réalisées par LCMS/MS *via* une approche protéomique ascendante. Les profils métabolomiques ont été obtenus par 1H-RMN IVDr (n=16). La fonctionnalité des LDL a été étudiée in-vitro et ex-vivo sur des hépatocytes humains, puis in-vivo chez la souris (n=40) et l'Homme (n=30) *via* l'utilisation de traceurs détectables par LC-MS/MS. L'association entre les concentrations plasmatiques du biomarqueur et le risque d'évènements CV a été évaluée dans une cohorte prospective de 1425 patients diabétiques.

Résultats – Nous avons identifié un peptide signature de l'apoB100 glyquée, augmenté dans le plasma des patients diabétiques et associé aux altérations des profils lipoprotéiques et métaboliques déterminés par 1H-RMN. Une réduction dose-dépendante de l'internalisation hépatique des LDL glyquées est observée in-vitro et ex-vivo, et une diminution de leur clairance plasmatique est mesurée chez la souris et l'Homme, en lien avec les concentrations du biomarqueur. Cliniquement, des concentrations plasmatiques élevées du biomarqueursont significativement et indépendamment associées au risque de coronaropathie ischémique.

Conclusion – Les différentes techniques de la chimie analytique ont permis l'identification d'un biomarqueur associé aux anomalies du métabolisme des LDL athérogènes et au risque CV dans le diabète, ouvrant de nouvelles perspectives pour son utilisation clinique.

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Section 1 - Poster 10 – S1-P10

Développement d'une méthode GC-C-IRMS pour l'analyse isotopomique des acides gras dans des échantillons biologiques de patientes atteintes du cancer du sein

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Le développement de nouvelles méthodes de diagnostic précoce et de stratégies thérapeutiques repose sur la découverte de biomarqueurs. Dans ce cadre, des premiers résultats brevetés[1] ont démontré que l'analyse isotopomique semble être un outil prometteur[2,3]. Afin d'identifier des biomarqueurs dans des échantillons biologiques provenant de patientes atteintes du cancer du sein, une méthode GC-C-IRMS est ici développée pour l'analyse quantitative et isotopique (13C) en abondance naturelle des acides gras (FA). Pour cela, les lipides sont extraits des échantillons biologiques (sérum, tissus adjacents ou tumoraux), puis soumis à une réaction de saponification pour obtenir des acides gras libres (FFA), qui sont ensuite analysés en 13C par GC-C-IRMS. Ici, l'étape de dérivation des FA en ester méthylique est exclue, réduisant ainsi la consommation de solvants polluants et évitant l'introduction d'un carbone exogène susceptible de modifier les valeurs isotopiques (13C).

L'acide myristique, palmitique, stéarique, oléique, palmitoléique et linoléique ont été identifiés, quantifiés et leurs compositions isotopiques (δ13C) ont été déterminées dans les matrices biologiques étudiées. La méthode a été validée dans les mélanges standards et les échantillons biologiques. Cette méthode a été ensuite appliqué sur des échantillons issus de patientes atteintes cancer du sein. La concentration et la composition isotopique des FFA issues des différentes familles de cette pathologie ont été comparées.

Ce travail ouvre la voie à l'identification de biomarqueurs spécifiques qui pourraient avoir un potentiel diagnostic, pronostique ou thérapeutique dans la prise en charge de cette maladie.

[1]Tea I, International patent WO2012/123886(A1),2012 ; [2]Tea I, Sci Rep. 2016;6:34251. ; [3]Tea I, Metabolites. 2021;11:370.

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Section 1 - Poster 11 – S1-P11

Dimorphisme sexuel sur les réponses métaboliques à une intervention de 12 semaines combinant supplémentation en citrulline et entraînement par intervalles à haute intensité (HIIT) chez les personnes âgées

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Introduction : Le vieillissement est associé à un déclin progressif de la masse et de la force musculaires squelettiques ainsi qu'à une augmentation de l'adiposité altérant la santé et la qualité de vie. Nous avons suggéré que le HIIT combiné à une supplémentation en citrulline (CIT) était une intervention efficace pour améliorer l'état de santé des personnes âgées obèses. En effet, l'ajout de CIT au HIIT entraîne une augmentation plus importante de la force musculaire et une diminution significative de la graisse corporelle. **Objectif** : Déterminer s'il existe un dimorphisme sexuel dans la réponse à la CIT associée au HIIT chez les personnes âgées obèses.
Méthode : Des individus obèses âgés ont suivi un programme HIIT de 12 semaines avec ou sans CIT. Des échantillons de sang ont été prélevés avant et après l'intervention. Les profils métabolomiques et lipoprotéiques ont été évalués par RMN-IVDr (Résonance Magnétique Nucléaire - Diagnostic In Vitro pour la recherche).
Résultats : Les profils lipoprotéiques sont différents chez les femmes et les hommes, avant et après l'intervention. Le cholestérol total et le cholestérol LDL diminuent chez les femmes, alors que le VLDL augmente. Chez les hommes, nous observons une augmentation du HDL. **Conclusion** : Nos résultats suggèrent une amélioration de la santé des participants âgés obèses qui ont suivi le programme. Néanmoins, les adaptations induites semblent affecter différemment les femmes et les hommes. D'autres études sont nécessaires pour confirmer ces résultats et comprendre les mécanismes impliqués dans la différence entre les sexes dans les réponses à la citrulline.

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Section 1 - Poster 12 – S1-P12

Etude de l'impact de la chirurgie sur le profil métabolomique des animaux dans le cadre de l'implantation d'un dispositif EEG

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Un modèle animal original permettant l'étude de la vulnérabilité au Trouble de Stress Post-Traumatique (TSPT) a été développé. Le traitement des données a mis en évidence la présence d'un biomarqueur électroencéphalographique (EEG) de vulnérabilité, c'est-à-dire présent avant exposition au stress (Desnouveaux et al., 2023). Par ailleurs, la vulnérabilité au TSPT est corrélée aux capacités antioxydantes de chaque individu. Ainsi, les travaux présentés ici s'inscrivent dans le cadre de l'étude de l'effet de l'administration d'une molécule anti-oxydante vs placebo, sur un modèle animal préalablement implanté d'un capteur EEG. Soixante-sept rats ont été implantés chirurgicalement par des capteurs EEG. Après 10 à 13 jours de récupération, les sujets ont été soumis à un stress multi-sensoriel ou une condition contrôle tout en recevant soit un antioxydant soit un placebo durant 5 jours (1 dose/j) avant réexpositions au stress. Durant l'expérimentation, 4 prélèvements plasmatiques ont été effectués. Les échantillons furent préparés puis analysés sur un appareil UHPLC-QToF en phase inverse et HILIC, en ionisation positive et négative. Les données ont été extraites sur Galaxy-Workflow4metabolomics et des analyses statistiques multivariées ont été effectuées (SIMCA v18).
L'effet de la chirurgie a été mis en évidence par des modèles discriminants avec 37 ions significatifs (score VIP> 2). Leur profil au cours du temps a montré que l'impact de cette intervention est bien supérieur au nombre de jours de récupération. Quel que soit le modèle expérimental, il est indispensable, au-delà de l'observation comportementale, de prendre en compte ce phénomène afin d'en limiter l'impact sur l'issue des études métabolomiques.

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Section 1 - Poster 13 – S1-P13

Evaluation of pre-analytical and analytical conditions for the metabo-lipidomic approach on human vitreous body

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The vitreous body, situated behind the lens, is pivotal for ocular metabolism and is linked to pathologies such as retinal detachment (RD), epiretinal membranes (ERM), and macular holes (MH). This study aimed to provide a standardised workflow using the metabo-lipidomic high throughput method to investigate vitreous body metabolism in patients with vitreoretinal interface pathologies.
Nineteen patient samples were pooled and divided into several volumes (15 µl, 25 µl, 50 µl, 125 µl). Utilizing ultra-high-performance liquid chromatography coupled with highresolution mass spectrometry (UHPLC-HRMS) and nuclear magnetic resonance spectroscopy (1H-NMR), we explored metabo-lipidomic profiles.

For UHPLC-HRMS-based metabolomic analysis, we assessed 10 samples each of 15 µl, 25 µl, and 50 µl, extracted with acetonitrile (ACN) or methanol (MeOH). Lipidomic analysis included 10 samples of each volume, extracted using isopropanol (IPA), methyl tert-butylether (MTBE), or MeOH/chloroform (MeOH/CHCl3), and 5 samples of 125 µl extracted *via* MeOH/CHCl3. Additionally, 1H-NMR analysis was performed on 5 samples of 125 µl under three conditions : native state, filtration tube (30 kDa), and after extraction using MeOH/CHCl3.

From UHPLC-HRMS analyses, extraction solvent and volume were selected based on identified metabolites with a coefficient of variation of normalized peak areas below 30% and metabolic pathways. Method selection for 1H-NMR depended on identified and quantifiable metabolites.
This study will contribute to the standardisation of metabo-lipidomic investigations of human vitreous body, providing a basis for understanding the pathophysiological processes involved in vitreoretinal interface pathologies. Furthermore, this strategy will aid in discovering prognostic factors to identify patients at risk for visual loss.

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Section 1 - Poster 14 – S1-P14

Impact de levure vivante *Saccharomyces cerevisiae* var. boulardii CNCM I-1079 sur le microbiote des chiennes gestantes et le phénotype immunométabolique des chiots

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La période entourant la mise-bas représente un défi pour les chiennes et leurs chiots. L'objectif de cette étude était d'évaluer l'effet d'une supplémentation des chiennes avec le probiotique S. boulardii CNCM I-1079 (SB-1079). Au total, 36 chiennes et leurs 254 chiots ont été inclus. Les mères ont été divisées en deux groupes (SB-1079 vs Contrôle) et supplémentées du 28ème jour de gestation (G28) à la fin de la lactation (J56). Les performances de reproduction, la croissance des chiots, le microbiote fécal et la métabolomique par la LC-HRMS/MS du plasma ont été analysés.
Chez les chiennes, SB-1079 a conduit à une stabilisation du microbiote juste après la misebas. Une réduction du nombre de chiots de faible poids à la naissance, une amélioration des qualités nutritionnelles du colostrum et du lait et une augmentation des taux de croissance entre J21 et J56 ont été observés dans le groupe SB-1079. La métabolomique a quant à elle permis l'identification de 228 métabolites parmi lesquels 23 ont été sélectionnés comme discriminant les deux groupes de chiennes. Parmi eux, la créatine était augmentée dans le groupe SB-1079. Chez l'Homme, une augmentation des besoins en créatine au cours du 3ème trimestre de grossesse du fait des besoins métaboliques accrus du foetus a été décrite. De plus, des altérations de son niveau circulant pourraient être indicatives de mauvaise santé périnatale. Ces résultats suggèrent l'intérêt d'une supplémentation en probiotique sur leur santé des chiennes et de leurs chiots.

*Garrigues et al. 10.3389/fnut.2024.1366256

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Section 1 - Poster 15 – S1-P15

Impact of quaternary ammoniums on permeability and intestinal microbiota : a study to detect biomarkers of exposure in rats

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The use of biocides (e.g. quaternary ammoniums) has been widespread due to their disinfectant, virucidal and bactericidal properties. The risk of exposure to these substances is all the greater important as the cases of use are multiple.

The aim of this study was to assess the impact of oral administration of biocide, a benzalkonium chloride (BAC-C12), on intestinal health. To do this, an impact study was conducted on rats associated with human flora to simulate human oral exposure to BAC-C12 and help understand the intestinal effects.

This study was carried out by daily gavage for 25 days on three groups of 6 rats (i.e. highdose, low-dose and control). Faeces and plasma were collected daily and at the end of the study, respectively.

The non-targeted approach involved global chemical fingerprint comparisons, acquired by High Resolution Mass Spectrometry (LC-HRMS) to highlight discriminants signals related to the rat exposure to quaternary ammoniums. Data were then processed using the Galaxy tools available on the W4M platform. Multivariate analyses (PCA and PLS-DA) performed on the results obtained from plasma and faeces samples showed that there was a distinction between the high-dose and the control groups. Exposure of rats to BAC-C12 seems to have a dual impact on endogenous compounds and on the xenobiotic by generating specific metabolites. These metabolites have yet to be identified.
These elements will complement the results obtained using other approaches to assess whether or not quaternary ammoniums have an impact on permeability and intestinal microbiota.

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Section 1 - Poster 16 – S1-P16

Longitudinal NMR-based metabolomics analysis of mountain ultramarathon runners : New perspectives for athletes monitoring and injury prevention

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This study aims to explore how metabolomic approach could provide valuable information about changes in athletes' metabolome occurring during a mountain ultramarathon race. To achieve this goal, we built a longitudinal cohort of athletes enrolled in "TOR des Géants". Using an 1H-NMR-based metabolomic approach, we evaluated metabolic changes that arise during both the race effort and the recovery phase and correlate them with functional muscles, cardiac, inflammatory, and kidney biomarkers already used in clinics. Processed data were analyzed with tools dedicated to longitudinal study design (ASCA+) and allowed us to assess specific changes in the metabolome and clinical biomarkers across the different time points. The data illustrated how the metabolism of athletes is impacted during the race and that 3-days recovery didn't allow a return to metabolic and functional baseline. Innovative pathway analysis such as single samples Pathway Analysis (ssPA) was employed to emphasize the signaling routes that play a crucial role in endurance effort and recovery. These analyses shed light to the metabolic shift that occurs during an extreme mountain ultramarathon race and how athletes recover from it after a 72h recovery period. Metabolomics-based analysis in the field of endurance sport is improving our understanding in the physiological responses to extreme effort. By its ability to provide valuable information about athletes' status in real condition, this methodology provide new tools for athletes' fitness evaluation, performance prediction, nutrient supplementation and the development of personalized follow-up, metabolomics offers the keys for a rationalized and healthy approach of extreme sport endurance practice.

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Section 1 - Poster 17 – S1-P17

Metabolomic Profiling of Osteocyte Extracellular Vesicles and Matrix Bound Vesicles using a Prototype Benchtop Multi Reflecting Time-of-Flight (MRT) Mass Spectrometer

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Extracellular vesicles (EVs) are lipid delimited nanoparticles that function in development and intercellular signaling events throughout the body. Within bone there exists a subset of EVs, known as matrix binding vesicles (MBVs), which have been long proposed to associate with the underlying collagenous extracellular matrix to drive early mineralization events during bone development. However, the precise relationship between EVs and MBVs and their differential roles in bone development and metabolism remains a point of contention.

EVs and MBV's were extracted from MC3T3 osteocyte cells and analysed by both HILIC and Reversed-Phase chromatographic methods on a prototype benchtop MRT mass spectrometer. Chromatographic separation was investigated using 'conventional' scale and also a higher throughput methodology which utilized a 3.5 minute gradient, using a 1x50mm column. Data were acquired using a data independent analysis (DIA) strategy, across the mass range 50-1200 Da, and the instrument consistently produced a mass spectral resolution in the region of 100,000 FWHM.

This feasibility dataset has putatively identified several significant biologically relevant polar metabolites, including amino acids, carboxylic acids, fatty acids, phenols, pyridines and indoles being present within both the EV and MBV extracts. LC-MS data were processed using MARS (Mass Analytica). The data were peak picked, normalized and putative compound identifications were gained with database searches conducted using a library consisting of the human metabolome (HMDB) database. A mass tolerance of +/- 1ppm was used as a tolerance for compound matching, which resulted in mass accuracies being returned at the sub-ppm level.

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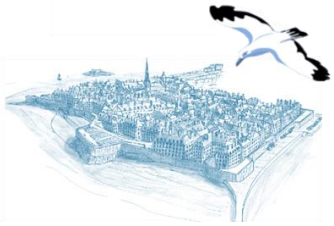

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Section 1 - Poster 18 – S1-P18

Metabolomic signature of two major respiratory pathogen in human airway epithelium

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SARS-CoV-2 and influenza virus cause asymptomatic to severe respiratory infections with frequently confused initial symptoms. Many studies have already been conducted on these viruses but host-pathogen interactions, including host metabolic responses to infection, are still unclear. While patient samples are great models to study human diseases as closely as possible, their obtention and available experimental condition tests are limited. Human airway epithelium (HAE) is a primary in vitro 3D model biologically closer to a real airway epithelium than immortalized cell lines. This model enables biologically relevant analysis with available tools to monitor infection status. This work combines HAE model to reproduce SARS-CoV-2 or influenza infections at various progressions with comparative LC-HRMS to better understand the impact of viral infections and their status on metabolic responses. SARS-CoV-2 and influenza virus infection induce a decrease of integrity and a rise in IL-6 secretion by HAE, while increasing viral replication. Those measures allow the classification of infections into early, intermediate or late groups. Preliminary results obtained with an exactive orbitrap MS show the feasibility of untargeted intracellular metabolomic analysis on HAE model. This work demonstrates that SARS-CoV-2 and influenza induce progressive and measurable infection in HAE, inducing metabolic responses assessable with metabolomics. In further experiments, intracellular metabolomics will be performed. ” Metabotypes ” obtained will be compared with infection status to investigate the impact of infections and their progression on the metabolism disruption. This work will allow the determination of host-specific responses induced by each virus and potentially new specific biomarkers.

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Section 1 - Poster 19 – S1-P19

Metabolomic study of Myeloperoxidase-oxidized LDLs effects on endothelial cells – overview from an intra- and extracellular perspective

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In the field of atherosclerosis, myeloperoxidase (MPO)-oxidized LDLs have been extensively studied and described as pro-atherogenic. LDLs oxidized by MPO are known as Mox-LDLs. We found that these Mox-LDLs interact with vascular endothelial cells. Among the effects already observed on endothelial cells, we know that Mox-LDLs induce a secretion in the supernatant of mediators linked to the control of inflammation (IL-8, resolvin D1), a disruption of endothelial barrier permeability and a decrease in cell mobility and migration. It is important to note that we do not know how Mox-LDLs produced these effects and what other metabolic pathways might be activated by Mox-LDLs. This is why we used an untargeted LC-MS (QTOF) metabolomic approach that analyzes cell supernatants and lysates from HUVECs exposed to different combinations of Mox-LDLs and native unoxidized LDLs (nat-LDLs). These results (C18 column, negative mode) reveal about 40 potential metabolites of interest (26 metabolites in cell lysate and 16 in the supernatant). It is noteworthy that in the supernatant, we observed a synergistic effect for certain metabolites in the condition combining Mox-LDLs with nat-LDLs (versus Mox-LDLs alone or nat-LDLs alone), which had already been demonstrated previously. This is not observed in cell lysates, where Mox-LDLs alone have the strongest effect. Among these features, one ion (267.1965 m/z) is detected only in the supernatant of stimulated cells and demonstrate a strong synergic effect. The next step will be to identify all these features of interest with MS/MS data and tools such as Sirius or MetGem (molecular networks).

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Section 1 - Poster 20 – S1-P20

Metabolomics and lipidomics to characterize synergistic effects of the anti-cancer drugs erlotinib-HCl and tubacin in 3D cell model of kidney cancer

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Single-drug cancer chemotherapies may lead to the rapid emergence of tumor resistance. Low-dose synergistic optimized drug combinations (ODC) can improve antitumoral activity by targeting various signalling pathways while minimizing resistance and side effects. Synergistic drug-drug interactions of erlotinib-HCl and tubacin were identified in a proprietary four-drug ODC containing histone deacetylase inhibitors (tacedinaline and tubacin) and tyrosine kinase inhibitors (erlotinib-HCl and dasatinib). Metabolomic and lipidomic analyses were conducted to evidence the potential pathways involved using human kidney homotypic 3-dimensional spheroids exposed to erlotinib-HCl, tubacin or ODC for 72 hours. Polar metabolomics was obtained with a zHilic stationary phase (pH=9.3) coupled to an Orbitrap Exploris 120 in negative mode, while lipidomics was obtained on a C18 column and positive mode, resulting in 118 (97 level 1) metabolites and 543 MS2-confirmed lipids, respectively. Multivariate analysis showed distinct alteration patterns following exposure to tubacin and erlotinib-HCl. Polar metabolomics revealed increased levels of metabolites linked to the purine pathway in tubacin-treated spheroids or to glycolysis and Krebs cycle pathways in erlotinib-HCl-treated cells, while the leucine/isoleucine degradation pathway was shifted by both drugs. Lipidomics exhibited other compound-specific signatures. For erlotinib-HCl, triglycerides were increased, while most phospholipids (phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, and phosphatidylserines) decreased. Conversely, for tubacin, several monohexosylceramides were increased, while trihexosylceramides decreased.

This preliminary study provides indications on the biochemical events that occur in kidney cancer cells following exposure to erlotinib-HCl and tubacin and highlights their different mechanisms of action and their potential synergistic effect within the tested mixture.

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Section 1 - Poster 21 – S1-P21

Microbial-host signalling

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Metabolic phenotyping (ie metabotyping) by 1H NMR spectroscopy and Mass Spectrometry (MS) has identified a series of microbial-mammalian co-metabolites associated with various cardiometabolic diseases. In particular, microbial metabolite trimethylamine (TMA), a bacterial degradation product of dietary choline and its detoxification metabolites trimethylamine-N-oxide (TMAO) and dimethylamine (DMA) were associated with insulin resistance and fatty liver disease. TMAO was subsequently shown to be involved in cardiovascular disease, which triggered a quest for disease mechanisms. To tackle the complexity of the microbial-mammalian metabolic axis, we highlighted that gut microbial metabolites and their mammalian detoxification products impact various mechanisms ranging from regulation of energy homeostasis to signaling and epigenetics beyond simple metabolism. To identify novel mechanisms for TMA and TMAO, we have screened them against a panel of 456 kinases to identify potential pharmacological targets. We show that TMA impacts a key pathway involved in the regulation of innate immunity and metabolic homeostasis. Altogether, the methylamine pathway illustrates complexity of the microbial-host metabolic and signalling crosstalk and opens up avenues to translate ”bioactivity metabolomics” findings into health benefits.

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Section 1 - Poster 22 – S1-P22

Parkinson's disease modelled in Drosophila : study of gut-brain interactions using NMR metabolomics analysis

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The gut-brain axis is a major bidirectional communication system now recognized as being involved in the initiation and propagation of neuronal pathologies, including Parkinson's disease. Our NMR metabolomic study aims to provide a better understanding of brain-gut interactions in Parkinson's disease. The fruit fly Drosophila melanogaster is a model of choice for the study of human disorders. This organism possesses homolog genes for about 70% of known human genes causing diseases. It is relatively cost and time effective, and the manipulation of gene expression in the fly is easily achieved.

Here we studied Parkinson's disease models developed in Drosophila, by the in vivo expression of a mutant pathogenic form of human α-synuclein (α-synA30P). In order to uncover reciprocal influences of these two organs during the development of the pathology, we expressed α-synA30P either in neurons and/or the gut in Drosophila.

We analyzed by NMR metabolomic modifications in the brain and gut, at early (10 day-old) and late (30 day-old) stages of the disease. Metabolites were extracted from the head and body of Drosophila. 1D 1H-NMR spectra were acquired on a 700 MHz spectrometer equipped with a cryoprobe. Statistical analyzes were performed to differentiate diseased Drosophila from their controls and detect reliable biomarkers. Our results demonstrate interestingly that the two organs interact and influence each other at both stages of the disease. α-synA30P expression in the brain leads to rapid metabolic defects, visible in both organs, while the effects of its expression in the gut appear to be slower.

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Section 1 - Poster 23 – S1-P23

Pathology detection in high level endurancetrained athletes during an hypoxia training camp

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Introduction : High level athletes regularly use altitude training camp in their preparation. Such camps involve important physiological stresses that should be carefully calibrated and monitored to achieve an optimal stress dose in order to induce adaptations. Pathologies in such environment can be tricky to manage as a stressed athlete will be more susceptible to infections and in the other way, the pathology will induce a supplementary stress. Given those important impacts it seems interesting to be able to detect a pathological athlete as soon as possible.

Methods : 27 high level rowers performed an altitude training camp. Among them 8 had pathologies involving the respiratory system (COVID, bronchitis, etc.). For each of them, urine samples where retrieved each morning before any meal was taken. NMR analysis were carried out using a 500MHz spectrometer. The resulting spectra where pre-processed with NMRProcFlow and statistics where done with MetaboAnalyst. A PCA and a PLS-DA where performed on the processed data.

Results : Preliminary results showed that the PCA didn't discriminated pathological subjects. The PLS-DA on the other hand achieved this discrimination with a 100% accuracy, a 85 R2 and a 0.49 Q2 between the first day and the day on which the subject had symptoms. When compared to healthy subjects on the same day we obtained an accuracy of 73%, a 97 R2 and a 0.25 Q2.

Conclusion : Using PLS-DA, we were able to detect pathological athletes. Such early detection can have huge impacts in athletes training, especially during training camps preceding major competitions.

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Section 1 - Poster 24 – S1-P24

Perinatal chemical exposome and micro-sampling: from targeted to non-targeted analysis

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Several chronic diseases and developmental and reproductive disorders are suspected of being caused by exposure to chemicals mixtures in utero. It is therefore important to be able to assess the chemical exposome at birth and conduct biomonitoring (HBM) studies for the perinatal period. Micro-sampling techniques, such as dried blood spots (DBS), are innovative because of their non-invasive nature, however, many challenges remain before DBS can be used in HBM studies.

The main objective of the thesis project presented here is to develop protocols for DBS sampling and sensitive and robust analytical methods for analysing mixtures of micropollutants from DBS. Following on from and in parallel with a systematic review of this subject, sample preparation methods are being developed with a view to quantify PFAS and POPs from DBS, respectively by liquid and gas chromatography mass spectrometry. The problems associated with sampling contamination are currently being studied.

As a next step, suspect screening and non-targeted analyses using LC-ESI-IMS-HRMS will allow to considerably increase the number of exogenous compounds that can be analysed in mixtures. Finally, after studying the comparability of these methods with those used for serum analysis, and setting up standard operating procedures, the ultimate aim is to be able to apply these methods to the study of associations between chemical exposome and health events, as well as comparing exposures between mothers and their children, in order to gain a better understanding of the extent to which mixtures of chemicals we are exposed at birth.

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Section 1 - Poster 25 – S1-P25

Pharmacometabolomics applied to low dose interleukin-2 treatment in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a devastating motor neuron disease. The immunosuppressive functions of regulatory T lymphocytes (Treg) are impaired in ALS, which correlates to disease progression. The phase 2a IMODALS trial reported an increase in Treg number in ALS patients following the administration of low-dose (ld) interleukin-2 (IL-2). We propose a pharmaco-metabolomics approach to decipher metabolic modifications occurring in patients treated with ld-IL-2 and its relationship with Treg response. Blood metabolomic profiles were determined on Days (D)1, D64 and D85 from patients receiving 2MIU of IL-2 (group T, n=12) and patients receiving a placebo (Group P, n=12). We discriminated the three timepoints within group T (average error rate of 42%). Among the important metabolites, kynurenine increased between D1 and D64, followed by a reduction at D85. The percentage increase of Treg number from D1 to D64, as predicted by the metabolome at D1, was highly correlated with the observed value. This study provided a proof of concept of metabolic characterization of the effect of ld-IL-2 in ALS. These data could present advances towards a personalized medicine approach and present pharmacometabolomics as a key tool to complement genomic and transcriptional data to assess drug characterization, leading to systems pharmacology.

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Section 1 - Poster 26 – S1-P26

Quantitative dose-response modelling in metabolomics to highlight hepatotoxic effects of enniatin B in heparg cells

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Metabolomics approaches lead to mechanistic information and early warning signals, which is very usefull to highlight biomarkers when organisms are exposed to xenobiotics(i). One of the challenges is the detection and modelling of significant diversity of responses and effects concentrations (EC) derivation(ii). The biomarkers trends responses can be valuable way to a biological interpretation of biomarkers(iii). The aim of this study was to apply doseresponse modellisation to disclose the hepatotoxic effects of the emerging mycotoxin Enniatin B (ENNB). ENNB is commonly found in cereals and raising concern for food and feed safety. Due to the lack of toxicokinetic and toxicodynamic data to derive Health-Based Guidance Values, ENNB is not regulated at the European level. Dose-response frameworks such as DRomics can derive a benchmark dose (BMD) from continuous responses, which is the concentration or dose that is associated with a benchmark response(iv)(v). Therefore, HepaRG cells were treated with a range of 10 concentrations (0-4 µM) of ENNB for 48h. The intracellular metabolome was extracted by cold methanol/water (75:25). The cell extracts were analysed by untargeted data-dependent acquisition (DDA) using ultra-high-performance liquid chromatography–mass spectrometry (UHPLC–MS/MS) with a Compound Discoverer data acquisition workflow (Thermo Fisher Scientific). The responses of intracellular metabolites were modelled with the DRomics tool. The trends of responses (monotonic or bi-phasic) from biomarkers of hepatotoxicity are still studied as well as the BMC of the pathways involved.

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Section 1 - Poster 27 – S1-P27

Quantitative Metabolomics Following the Ketogenic Diet in Rats Using Nuclear Magnetic Resonance Spectroscopy: Potential Mechanisms of Neuroprotection

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The antiepileptic effects of the ketogenic diet (KD) have been well-documented ; however, the underlying mechanisms of this phenomenon remain unclear. One hypothesis suggests the reduction of excitotoxicity *via* mediation of the balance of glutamate, glutamine, and y-aminobutyrate (GABA) to favor the inhibition of neurotransmission. Here, we perform targeted quantification of these key metabolites among others in the serum, cerebrospinal fluid (CSF), brain tissue, and urine of male and female Sprague Dawley rats under the KD condition. We selected 1D 1H-NMR for this study because of its superior reproducibility and suitability for targeted identification and quantification of many metabolites in complex mixtures. Providing additional support for the excitotoxicity hypothesis, we observe an increase in GABA, a critical inhibitory neurotransmitter, in the CSF among other localized alterations in serum, and CSF, but not brain tissue as a result of the administration of the KD. To determine the rate at which these changes occur, we repeated metabolite quantification on urine samples collected over a 6-week period from the start of the diet. Thus, we characterize KD induced changes in the endo and exo metabolomes, with specific focus on modulation in the balance of metabolites involved in the TCA cycle, ketone body synthesis, glycolysis, the glutamate decarboxylase pathway, and the GABA shunt.

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Section 1 - Poster 28 – S1-P28

Search for markers involved in lymph node remodeling in the pre-metastatic stage by metabolomic analysis

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Background : Our laboratory has provided evidence for the existence of a premetastatic niche in the sentinel lymph node (LN) draining a human cervical neoplasm, which is characterized by a specific lymphangiogenic, immune, and extracellular matrix profile. Metabolomics is a promising approach that provides an opportunity to link the metabolome with physiological or pathological status.

Aim : The aim of this project is to identify new predictive markers of the arrival of metastases in the LN by applying a metabolomic approach to human and murine samples.

Methods : To identify metabolic pathways modulated in LN, we benefit from a preclinical mouse model, the ”ear sponge assay”, which reproduces each step of the metastatic cascade. The mice’s lymph nodes are analyzed by NMR-based metabolomics and histological procedure. Furthermore, we will use a cohort of patients suffering from advanced cervical cancer and we will analyze different samples issued from the same patient.

Results : Different analyses performed on mice LNs highlight a clear discrimination between the cervical LN (draining the tumor) and the other LNs (mandibular, sub-draining LN, and axillary/inguinal, control LN) issued from mice bearing a tumor. Our data show that the tumor development impacts the environment of the draining LN, but not that of distant LNs.

Conclusion : Our results highlight metabolomics’s interest in investigating LN remodeling during the metastatic process. Indeed, the tumor microenvironment impacts the cervical lymph node at a metabolite level. These results will be confirmed by additional experiments in order to perform further analysis on the discriminating metabolites.

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Section 1 - Poster 29 – S1-P29

Study on the connection between mycotoxins and Kashin-Beck Disease in China

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Kashin-Beck disease (KBD), also known as the 'Big Bone Disease', is a chronic, disabling disease characterized by multiple deformed bones and joints in all parts of the body. The disease is developing in childhood. Microscopically, the degenerative changes in the KBD patient's cartilage are characterized by deep zone chondrocyte necrosis, middle zone with adjacent necroptosis, and apoptosis. KBD is endemic in Asia, from the South-East of Siberia to Tibet, affecting the northern and central provinces of China, the north of North Korea, also some areas in Russia. It mainly affects remote, rural populations. However, the etiology of KBD remains unknown. In the last decade, fungal contamination, mainly T2 toxin derivates, was considered one of the multifactorial environmental risk factors. Since 2002, a Tibetan team and international experts established the KBD Foundation to investigate the role of cereal fungal contamination, mainly Alternaria species, in KBD. They sampled cereals in different areas, some were KDB Endemic-Areas (EA) and others Non-Endemic-Areas (NEA).

We took advantages of this cereal collection to identify compounds specifically present in the cereals from the KBD-related regions. Metabolomics analysis of the data obtained by LC-HRMS(/MS) was performed to analyze compound profiles between EA and NEA. Several metabolites were highlighted as statistically increased in EA. Molecular network analyses of the data further allowed us to identify one of them as enniatin B (ENN B) as a specific compound in the KBD endemic regions samples. However, the latter have shown low chondrocyte toxicity. Other metabolites highlighted are currently under further identification.

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Section 1 - Poster 30 – S1-P30

Targeted and non-targeted lipidomic analysis of adipose tissue from general population to identify metabolic perturbations related to phthalate exposure

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Adipose tissue dysfunction plays a relevant role in metabolic disorders, however few studies have applied lipidomic analysis to explore the potential associations between environmental chemicals and dysfunctional phenotypes. The objective of this study was to develop and apply a lipidomic workflow to explore the associations between exposure to phthalates and altered lipid profiles in adipose tissue of general population. For the present study, 81 individuals undergoing non-cancer related surgery from the GRAMO cohort (Spain) were included. Adipose tissue metabolites and lipids were extracted and two approaches were performed : a targeted study with Biocrates p180 kit and an untargeted lipidomic using a LC-Q-Exactive. Data were processed with Galaxy W4M. Phthalate metabolite levels in serum were measured by isotope diluted online-TurboFlow-LC-MS/MS with preceding enzymatic de-conjugation. Statistical analysis included partial least squares discriminatory analysis (PLS-DA) models and variable importance in the projection (VIP) was used for select those metabolites with a relative importance in the prediction model. In the multivariable regression models, concentrations of each phthalate were natural logtransformed in order to relax the linearity assumption. Models were adjusted for age and sex. The non-targeted analysis in positive mode provided 3735 features, 2917 of which were retained in the statistical analysis which showed 120 features statistically associated with the levels of the sum of phthalates (MxBP), 34 of which were successfully annotated. 216 targeted lipids were significantly associated with the phthalates levels. These preliminary analyses support that exposure to low molecular phthalates may be related to a specific metabolic profile in adipose tissue.

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Section 1 - Poster 31 – S1-P31

Warburg-associated acidification represses lactic fermentation independently of lactate, contribution from real-time NMR on cell-free systems

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Lactate accumulation and acidification in tumours are a cancer hallmark associated with the Warburg effect. Lactic acidosis correlates with cancer malignancy, and the benefit it offers to tumours has been the subject of numerous hypotheses. Strikingly, lactic acidosis enhances cancer cell survival to environmental glucose depletion by repressing high-rate glycolysis and lactic fermentation, and promoting an oxidative metabolism involving reactivated respiration. We used real-time NMR to evaluate how cytosolic lactate accumulation up to 40 mM and acidification up to pH 6.5 individually impact glucose consumption, lactate production and pyruvate evolution in isolated cytosols. We used a reductive cell-free system (CFS) to specifically study cytosolic metabolism independently of other Warburgregulatory mechanisms found in the cell. We assessed the impact of lactate and acidification on the Warburg metabolism of cancer cytosols, and whether this effect extended to different cytosolic phenotypes of lactic fermentation and cancer. We observed that moderate acidification, independently of lactate concentration, drastically reduces the glucose consumption rate and halts lactate production in different lactic fermentation phenotypes. In parallel, for Warburg-type CFS lactate supplementation induces pyruvate accumulation at control pH, and can maintain a higher cytosolic pyruvate pool at low pH. Altogether, we demonstrate that intracellular acidification accounts for the direct repression of lactic fermentation by the Warburg-associated lactic acidosis.

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Section 1 - Poster 32 – S1-P32 (Flash 1)

Ex-vivo study of skin permeability and stability of a topical neurofibromatosis application using a combined LC-MS/MS and MALDI-FTICR imaging worklow

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Neurofibromatosis type 1 (NT1) is a genetic disorder affecting the nervous system leading to the appearance of neurofibromas, which are treated by surgical removal, leading to regrowth and significant surgical risks (1). Recent studies have identified Selumetinib as a promising candidate for clinical trials in NT1-related plexiform neurofibromas (2). To propose a topical formulation of selumetinib, a prerequisite is to characterize its behaviour in the skin, and all the more since it has been shown that selumetinib is amenable to degradation in the presence of sunlight (3). In this study we have investigated the fate of selumetinib when administered to skin explants. The use of MALDI-FTICR analysis showed the permeation of selumetinib and the detection of metabolites from photodegradation processes within the different skin layers. Ultrahigh mass resolution allowed specific distinction of selumetinib from endogenous molecules causing interferences. LC-MS/MS study was carried out on tissue extracts to quantify selumetinib and its degradation product in the different skin layers. The results show limited permeation, often restricted to the epidermis and *via* skin annexes to the dermis. Both techniques conclude that selumetinib is partially photodegradable in the skin, leading to the same compound as identified in solution. This study enabled a better understanding of selumetinib's permeation for topical treatments, and demonstrated the need to obtain a formulation protecting selumetinib and improving its' permeation.

(1) Wilson BN, et al. J Am Acad Dermatol. 2021;84(6):1667-76 ; (2) Gross AM, et al. N Engl J Med. 2020;382(15):1430-42 ; (3) Bouchema TS, et al. Pharmaceutics. 2022;14(12):2651

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Section 1 - Poster 33 – S1-P33 (Flash 2)

Integration of lipidomics and polar metabolomics for a molecular characterization of solvent neurotoxicity

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Glycol ethers such as propylene glycol butyl ether (PGBE) are organic solvents used in many industrial processes and their ubiquitarian presence constitutes a source of human exposure. Associations have been found between occupational solvent exposure and neurogenerative diseases, but neurotoxicity is still not systematically assessed. To investigate neurotoxicity at the molecular level, in vitro human 3D brain spheroids comprising neurons and glial cells were exposed to 5, 10 and 20 mM PGBE and its main metabolite 2-BPA for 2 days and 1 week, respectively. Untargeted chemical profiling was achieved using a double extraction strategy, where the remaining pellet obtained after polar metabolites extraction was re-extracted for lipidomics. Polar metabolomics (zwitterionic Hilic) and lipidomics (RPLC) were acquired in HRMS in data-dependent mode. Polar metabolomics led to 120 manually curated level 1 identifications and 86 relevant level 3/4 annotations. OPLS data analysis showed alterations of the levels of ribonucleotides and carbohydrate phosphates of the glycolysis pathway, suggesting increased energy metabolism after acute exposure (48 h) to the solvents, followed by a strong decrease after prolonged treatment (1 week). Based on the high-quality MS/MS annotation of 713 lipid species, lipidomic analysis revealed that exposures to PGBE and 2-BPA caused significant decreases of phosphatidylethanolamines and hexosylceramides. These lipid alterations may indicate the disruption of the myelin sheath, the multilayered membrane produced by oligodendrocytes which is critical for neuronal signal transmission in axons. These results, complemented by other omics analyses, pave the way for a better understanding of glycol ethers' neurotoxic modes of action.

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Section 1 - Poster 34 – S1-P34 (Flash 3)

Early biomarkers of transition to psychosis detected by NMR IVDr technique : a pilot study

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Introduction : Schizophrenia is a severe mental illness whose onset is frequently preceded by a prodromal phase, with subjects experiencing it being defined as ultra-high risk of psychosis (UHR). Psychotic transition occurs in 25% of cases within a year from the diagnosis, and it has been associated to metabolic dysregulation.

Aim of the study : Detect serum biomarkers predictive of psychosis conversion among UHR patients.

Materials and methods : 14 UHR subjects converting to psychosis (UHR-C) and 21 not converting (UHR-NC) were selected from the project ICAAR (Centre Hospitalier Sainte-Anne, Paris). Serum samples were collected at baseline (M0), after six (M6) and twelvemonths (M12). Serum were analysed using the NMR high-throughput technique IVDr (In Vitro Diagnostic for research) which provided an automated report of 112 lipoprotein parameters and 32 metabolites parameters with absolute quantifications. Statistical analysis were conducted with SIMCA 17.0, MetaboAnalyst 6.0 and R Studio 4.3.0.

Results : Lipoprotein parameters related to high-density lipoprotein subgroup 4 (H4FC, H4A1) and low-density lipoprotein subgroup 4 (L4FC) are reduced in UHR-C compared to UHR-NC at M0. A good discrimination capability at ROC analysis is observed with negative correlations relative to symptoms. Differences in biomarkers is observed due to treatment and according to sex.

Conclusions : This pilot study suggested that NMR could be a useful technique for better understanding the pathophysiology of early psychosis and detecting biomarkers of the psychotic transition. These results need to be validated with a larger cohort, and to find significant biomarkers at follow-up.

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Section 1 - Poster 35 – S1-P35 (Flash 4)

Exploration of the metabolic impact of phenylketonuria by metabolomics on Dried Blood Spot

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Phenylketonuria is a hereditary metabolic disease that causes a variety of damage in patients, including neurological signs and developmental disorders. This disease is detected by the Guthrie test, which determines the blood concentration of phenylalanine on Dried Blood Spot (DBS) collected on the 3rd day of life. The aim of this project is to study the metabolic profiles of patients with phenylketonuria or permanent moderate hyperphenylalaninemia and to compare them with the metabolic profiles of controls. Metabolomics analysis was perfomed by non-targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS). Different protocols were investigated in order to optimize the extraction method and collect a maximum amount of metabolites. The chosen protocol was applied to samples from 30 patients and 30 controls. The metabolic profiles of patients and controls were compared using multivariate statistical approaches. This enabled us to identify significantly different metabolic fingerprints between patients and controls, but also according to disease severity or control by treatment. A number of metabolites and metabolic pathways were shown to be deregulated in patients. This work has validated an extraction method for metabolites on DBS, applicable to other pathologies relevant for newborn screening. It has also highlighted the value of non-targeted metabolomics approaches for studying metabolic disturbances in patients with phenylketonuria and more generally in the context of metabolic diseases.

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Section 1 - Poster 36 – S1-P36 (Flash 5)

New markers for monitoring the elimination of the reactive N-Acetyl-p-benzoquinone imine after paracetamol/acetaminophen hepatotoxicity

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Paracetamol/acetaminophen (N-acetyl-p-aminophenol, APAP) overdose is one of the most important causes of drug-induced liver injury worldwide. Hepatotoxicity induced by APAP is mainly caused by the production of N-acetyl-p-benzoquinone imine (NAPQI), a highly reactive intermediate formed predominantly *via* the cytochrome P450 2E1. Here, we used human studies and in vitro models to demonstrate that NAPQI-glutathione derived thiomethyl metabolites (i.e., S-methyl-3-thioacetaminophen sulfate and S-methyl-3-thioacetaminophen sulphoxide sulfate) identified using high-resolution mass spectrometry could serve to monitor NAPQI detoxification and elimination in patients (after intake at recommended dose or after intoxication). In addition to improve APAP intoxication diagnosis, these biomarkers could also serve to detect inter-individual variability in NAPQI production to assess susceptibility to APAP-induced hepatotoxicity. Using in vitro human models, we showed that these thiomethyl metabolites are directly linked to NAPQI detoxification since they are mainly formed after exposure to glutathione-derived conjugates *via* an overlooked pathway called the thiomethyl shunt. These delayed thiomethyl metabolites have great potential in future clinical studies in order to provide a more reliable history of APAP ingestion in case of acute intoxication or to study underlying causes involved in APAPinduced hepatotoxicity. Further toxicological investigations would also help to understand if the thiomethyl shunt acts only as a pathway for NAPQI detoxification and excretion, or if it contributes to liver or renal toxicity in case of APAP overdose through the generation of the newly identified intermediates with highly reactive sulfur-containing fragment as observed with environmental contaminants.

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Section 1 - Poster 37 – S1-P37

Rôle des polyamines dans la virulence de Pseudomonas aeruginosa au cours de l'infection pulmonaire chronique chez les patients atteints de mucoviscidose

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Objectifs : En utilisant une approche métabolomique non ciblée à haute résolution, nous avons précédemment démontré que les souches de *Pseudomonas aeruginosa* (Pa) provenant de 32 adultes atteints de mucoviscidose pouvaient être classées en 3 métabotypes, les souches de chaque métabotype présentant des profils métaboliques similaires. Parmi les métabolites identifiés, les polyamines notamment la spermidine, représentent un des métabolites discriminants de l'appartenance à un métabotype. Nos travaux ont démontré une corrélation entre l'augmentation de la production bactérienne de ces métabolites et une augmentation de la cytotoxicité des isolats de Pa. Cette étude vise à identifier les mécanismes par lesquels Pa module sa production de polyamines au cours de l'infection chronique chez les patients CF et à déterminer l'impact de cette modulation sur l'expression de ses facteurs de virulence. **Matériels et Méthodes** : À partir de 28 souches sélectionnées parmi une cohorte de 66 isolats provenant de 32 patients, nous avons formé deux groupes de souches : celles produisant un niveau élevé de polyamines (PH) et celles produisant un faible niveau de polyamines (PL). Nous avons ensuite séquencé les génomes des 28 souches et les transcriptomes des 6 souches PH et des 5 souches PL et utilisé une approche de génomique comparative et de transcriptomique pour identifier les mécanismes par lesquels ces souches modulent leur production de polyamines.

Résultats : L'analyse phylogénomique a confirmé l'affiliation des souches provenant d'un même patient (évolution de la même souche au cours de l'infection chez chaque patient). L'analyse de l'expression différentielle des gènes entre les souches PH et PL a confirmé les résultats précédemment obtenus par analyse métabolomique : corrélation entre l'augmentation de l'expression des gènes impliqués dans la biosynthèse des polyamines et l'expression des gènes de virulence chez Pa avec cependant plusieurs mécanismes pouvant conduire aux phénotypes observés.

Discussion et Conclusion : En utilisant une approche combinée de métabolomique, de transcriptomique et de génomique, nous avons identifié comment Pa pouvait moduler sa production de polyamine et l'impact que cela pourrait avoir sur la virulence et la physiopathologie de cette bactérie.

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Section 2 - Poster 1 – S2-P1

Bioinformatics methods for reconstructing a genome-scale metabolic network of a macroalga considering related species

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Ascophyllum nodosum is a brown alga that is abundant along the coast of Brittany. It has industrial applications due, in part, to its biostimulant properties on plants. It is yet undetermined whether these properties come from the alga itself, its microbiota, or the cooperation of both. The reconstruction and analysis of genome-scale metabolic networks (GSMNs) of the alga as well as all associated bacterial and fungal strains provide an approach to address this question. Here we present the reconstruction and the biological knowledge-based curation of the GSMN of the host A. nodosum. Using the in-house bioinformatics tool AuCoMe, we propagated annotations among a corpus of 62 related species to the network. Then, we developed precise manual and automatic curation procedures based on the Meneco gap-filling tool and integrated microbiota information to ensure network functionality. The resulting network contains 3536 metabolites and 3072 biochemical reactions. The network predicts the production of 1023 compounds, starting from a list of 38 metabolites describing the composition of seawater. Each reaction is supported by an enzyme and a corresponding a gene sequence in the genome. Among 1023 compounds, 75 corresponded to those we used to define the algal biomass. The resulting network was used to analyse the metabolic complementarity between the algal host and its microbiota. This microbiota data includes networks created from: 77 bacterial whole-genome sequencing (WGS) genomes, 23 fungal WGS genomes, but also from metabarcoding taxonomic assignments including 316 bacterial taxa and 13 fungal taxa.

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Section 2 - Poster 2 – S2-P2

Chemical investigation of a marine-derived *Penicillium canescens* strain: detection and isolation of halogenated and bioactive compounds

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Fungi are the third most important kingdom regarding the production of specialized metabolites, after plants and animals (1). Although marine-derived strains have been underinvestigated compared to the terrestrial ones, they represent a promising source for the discovery of new bioactive molecules (2). With specific conditions, such as high salinity, the marine environment is conducive to the production of particular chemical entities, including halogenated compounds. Indeed, chlorine and bromine are 60 and 20 times more concentrated in sea water than in terrestrial sediments (1), and 15 to 20% of the described marine natural products contain halogens (2). Many halogenated compounds possess significant bioactivities, and thus represent a valuable class of metabolites for drug discovery (2). According to a previous study, the marine-derived Penicillium canescens strain MMS194, collected from sea water on the Atlantic coast (La Baule, France), was identified as an interesting producer of halogenated compounds (2). This strain was then selected for a comprehensive chemical investigation, in order to isolate new halogenated and bioactive compounds. First, thanks to different automatic detection tools (MeHaloCoA, MZmine and HaloSeeker), halogenated features were specifically highlighted in HPLC-HRMS data of extracts from this fungal strain. Thereafter, a large-scale culture and extraction of the strain were conducted to isolate the targeted compounds. The latter were further identified based on HRMS and NMR data, and their bioactivity will be evaluated.

1. Cochereau et al. Molecules 2022
2. Roullier et al. Anal. Chem. 2016

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Section 2 - Poster 3 – S2-P3

Comparative metabolomic study of a marine algal holobiont: LC/MS-based molecular networking for the exploration of chemical interactions

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Seaweed harbour and interact with associated microbial communities, which contribute to their growth and defence mechanisms. These intricate interactions have led to the conceptualization of seaweed and their associated microbiota as a "supraorganism", collectively referred to as the holobiont. Molecular investigations increasingly point to non-random associations between algae and their microbiota, strongly implying the existence of complex structured and dynamic interactions both within the microbiota itself and with the algal host. Herein, we endeavour to elucidate the chemical interactions occurring between fungi and the algal host, through the determination of the fungal community's metabolome detected in the host, and the understanding of the role of fungal mediators in fungi-host interactions. The brown seaweed Ascophyllum nodosum has been selected regarding its economic interest in agronomy. Indeed, among the commercial products developed to stimulate plant growth and alle*via*te stresses, many contain brown seaweed extracts, among which A. nodosum is widely represented. However, the chemical nature and origin of the bioactive compounds present in A. nodosum extracts have not been fully characterized, even regarding secondary metabolites. Thus, in order to decipher the entire metabolome of A. nodosum holobiont, we isolated and characterized the cultivable microbiota associated with the brown alga. To explore the chemical interactions within this entity, we developed an analytical workflow, based on untargeted metabolomics from LC-MS/MS data and molecular networking with various levels of restrictiveness.

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Section 2 - Poster 4 – S2-P4

Physiological changes during coral larval metamorphosis : Liquid Chromatography – Mass Spectrometry based phenotyping of proteins and metabolites expressed during life cycle transition from swimming planulae to settling larvae.

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Coral larval metamorphosis is a critical life cycle transition from swimming planula to benthic polyp, key for reproductive success and coral populations survival. However, the physiological processes involved during this transition remain largely unknown. Our goal was to identify metamorphosis-related proteins and polar metabolites in settling coral larvae. We conducted a longitudinal study of coral larval proteome and metabolome, *via* label-free quantification proteomics and untargeted metabolomics of swimming planula versus stage 4 settling larvae. Planula larvae emitted from aquarium propagated Pocillopora acuta Lamarck 1816 colonies spontaneously underwent metamorphosis in vitro in filtered seawater. The absence of exogenous microbial biofilm allowed here to focus on the genetically encoded larval metabolic changes. Successive developmental stages were sorted out at onset of metamorphosis according to morphological criteria, and differentially expressed proteins and polar metabolites were analyzed by LC-MS of initial planulae and metamorphosing stage 4 larvae. Metabolomic data were analyzed using an internal workflow to discriminate the two early developmental stages. For the proteomic data, fold change was calculated to identify differentially expressed proteins. Also, statistical procedure like embedding techniques and PLS regression were performed. Finally, the two approaches were integrated using common component analysis to describe the parallel variation of these two data types across the developmental stages, thereby providing a supposed functional link between the metabolome and proteome data of settling larvae.

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Section 2 - Poster 5 – S2-P5

Towards the use of biomarkers of (non)-exposition of mussels and oysters to phycotoxins as an alternative to classical sanitary monitoring programmes.

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Marine micro-algae are at the basis of trophic chains and sustain the growth of exploited filter-feeding bivalves. However, the toxins produced by at least 161 species can be bioaccumulated in shellfish, causing food poisoning in human consumers. As a result, sanitary monitoring programmes have been implemented, based on the quantification of phycotoxins by targeted analyses using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The main drawback of this approach comes from the focusing on a limited number of known molecules (43 for REPHYTOX) while more than 300 phycotoxins exist. The breakthrough of Dervilly-Pinel et al. (2018) (i.e. accreditation for the official control of forbidden compounds in livestock) prompted us to try a metabolomic approach to find biomarkers of exposition of mussels and oysters to phycotoxins, by considering the consequences of a contamination on their global metabolite expression. Samples were selected based on their contamination level to include both "healthy" and "naturally contaminated" organisms and to account for their physiological status. Methanolic extracts of digestive glands were then analyzed by liquid chromatography coupled to high resolution mass spectrometry (Q-TOF at Ifremer and Q-Orbitrap at LABERCA). After data processing on the W4M infrastructure, filtrations and statistical analyses (OPLS-DA or PLS-DA), we obtained 39 and 33/5 (POS/NEG) putative biomarkers for mussels and oysters with the Q-TOF, while 276 and 199 were obtained with the Q-Orbitrap mussel and oyster datasets. The annotation process is still ongoing (using GNPS, SIRIUS, or other databases) but proved highly limited so far (some phospholipids might be present).

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Section 2 - Poster 6 – S2-P6

Validation of chemical and biological methods required for the application of an advanced multi-omics approach to lagoon sediment microbiome profiling.

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The increasing use of chemicals requires a better understanding of their presence and their dynamics in the environment as well as their impact on ecosystems. It is important to set up procedures to answer this key environmental problematic. The aim of this study was to validate the first steps of an innovative multi-omics approach based on the combination of metabolomics and 16S metabarcoding data for future analyses of the fate and impact of contaminants in Mediterranean lagoon environments. The "Canet-St Nazaire" lagoon in France was chosen for this methodological development because previous observations revealed a worrying level of chemical contamination. First, semi targeted analytical procedures for water and sediment matrices were set up in order to evaluate the lagoon contamination status. Forty-six compounds were detected, 28 could be quantified in both water (between 0.09 and 47.4 ng/L) and sediment (between 0.008 and 26.3 ng/g) samples using UHPLC-MS/MS instrument. In addition, metabolomics and metabarcoding approaches were developed on the sediment matrix. Regarding the non-targeted metabolomics study (UHPLC-HRMS), four sample preparation protocols, based on solid/liquid and EDGE™ extractions, were compared in terms of metabolome coverage, efficiency and reproducibility of the extraction methodology. A solid/liquid extraction using acetonitrile/methanol (50/50) solvent was the best protocol. In order to characterize the microbial diversity of the lagoon sediment using metabarcoding, five commercial kits for DNA extraction were evaluated. This study revealed that the DNeasy PowerSoil Pro Qiagen Kit (Promega, USA) was the best compromise for studies assessing microbial diversity in fresh sediment.

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Section 2 - Poster 7 – S2-P7 (Flash 13)

Dealing with non-model organisms: Annotation of micro-algal metabolites using high-resolution mass spectrometry and advanced dereplication tools

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Over the millennia, the particularly harsh conditions prevailing in the oceans have forced marine micro-organisms to produce a wide variety of bioactive molecules in order to survive. These marine natural products (MNPs) represent a reservoir of bioactive compounds with promising pharmaceutical prospects and economic potential. The development of highthroughput metabolite profiling and bioinformatic tools to process large datasets is now accelerating the discovery of new MNPs. The current challenge lies in the annotation of new natural products in complex extracts. The project CEVAMAP focuses on the research of high-value-added metabolites produced by a selection of micro-algae and cyanobacteria strains isolated from French Polynesian lagoons. The 99 micro-algae strains were cultivated over a 6-month period to ensure sufficient biomass for metabolite profiling and bioactivity testing. Extraction conditions (number of cycles, solvent composition, etc.) and purification methods (elimination of salts and polar primary metabolites) were optimized to obtain comprehensive extracts enriched in secondary metabolites. Untargeted analyses were then performed using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS, Obitrap Exploris 120). The resulting dataset was processed with MzMine 3 and annotated through molecular networking (GNPS, cytoscape). The annotation coverage was then improved using SIRIUS and Tima-r tools in addition to in-house database obtained from purified compounds. Antiviral, antibacterial and anticancer activity assays were also carried out on a selection of strains, followed by a micro-fractionation approach to study the families of molecules responsible for these activities and their presence in the dataset.

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Section 2 - Poster 8 – S2-P8 (Flash 15)

Metabolomics and functional genomics of halogenation mechanisms in brown algae

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Some brown algae are known to concentrate halogens such as bromine and iodine to produce halogenated metabolites. Volatile or not, they are still poorly described. In addition the processes and function of halogenation remain uncertain in these marine organisms. Those compounds may have important roles in signalling and/or defense during biotic interactions and physiological responses to environnemental changes. My thesis work under HALOGENE project, aims to explore the production of halogenated metabolites and the role of halogen metabolism in brown algae through functional genomics and metabolomics studies. One of the putative halogenating key enzymes, vanadium-dependent haloperoxidase (vHPO), was inactivated using the CRISPR-Cas9 method in the model brown alga Ectocarpus siliculosus. The extinction of vHPO activity has been validated for 3 independent knock-out mutants. Chemical extractions using different polar and non-polar solvents for metabolomic analysis were carried out. Several analytical optimisations led to the detection of nearly 1,000 features in the wild-type strains under standard culture conditions. A workflow, based on LC-HRMS data and utilizing analytical tools like MZmine3, Sirius, HaloSeeker, and molecular networking, will enable the comparison and description of entire metabolomes between strains, as well as the specific characterization of the halometabolome. The current study will provide new knowledge about the chemical diversity of halogenated metabolites and their biosynthesis in brown algae.

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Section 3 - Poster 1 – S3-P1

A computational solution to match polyphenol-peptides adducts in HRMS-data ?

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Legume cops constitute a promising alternative to reduce meat proteins in human diet. One of the locks to their use is the presence of polyphenols. Condensed tannins (polyphenol polymers) are contained in testa and during the processing steps (i.e alkalinization) some can be processed with the kernel. High pH triggered polyphenols autoxidation that led to quinone formation and thus to nucleophilic attacks (Michael addition or imine formation[1]) on proteins. Despite considerable advances in metabolomics annotation, the metabolite identification remains challenging[2]. If numerous of natural compounds are now well known, the annotation of adducts and derivatives needs to the in-depth comprehension of natural compound reactivity and transformations[3]. Here we propose a computational solution to identify polyphenol-peptides adducts in HRMS data. We first compute possible peptides formation based on amino acids molecular weight (MW), we then calculate the addition of polyphenols through Michael addition and/or imine formation. While the addition reaction is a parameter which has to be set by the user, the polyphenol list remains under his control. At the end, the user will compare his experimental data to the in silico database. The tolerated mass de*via*tion is user-selectable (default 5ppm) and results are finally presented as a table. It gathers the putative adduct annotation, its MW, the feature identity (RT & m/z) that match with and δ ppm and score. After validation, the aim is to release our solution in CRAN repository and to offer a visual interface using RShiny.

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Section 3 - Poster 2 – S3-P2

Application of Liquid Chromatography-High Resolution-Mass Spectrometry to follow-up stilbenes metabolization by the action of laccases

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Stilbenes belong to the large family of polyphenols and are abundant in grapevine woody parts (e.g. canes, trunk, roots), which are common underused by-products. The interest in using stilbenes as biopesticides has emerged due to their promising antimicrobial activities, which could respond to significant agronomic challenges such as gray mold (Botrytis cinerea) in viticulture. However, the use of stilbenes extracts in vineyards has a number of limitations, including their degradation/metabolization by B. cinerea laccases, leading to a loss in their antimicrobial activities. Finding inhibitors to counteract this phenomenon and determining the nature and formation of these metabolites is essential to optimize the efficacy of grapevine cane stilbene enriched extracts as alternative phytosanitary treatments. In our work we analyzed by UHPLC-HRMS/MS the oligomerization of a cane extract rich in stilbenes under different conditions at different times (30, 60 minutes). A targeted quantification was established using calibration curves with 16 stilbene standards previously isolated in our laboratory. Moreover, we also acquired MS/MS data dependent fragmentation throughout the chromatographic run to obtain further information about new metabolites. Our works demonstrate that in the cane extract, most of stilbenes are susceptible to metabolization by laccases. However, we could establish new original combinations of cane extracts with other by-products containing laccase inhibitors. As instance, for all the stilbenes susceptible to degradation, the addition of condensed tannins permitted to reduce their metabolization.

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Section 3 - Poster 3 – S3-P3

Approche de marquage isotopique pour suivre la cinétique de formation de composés phénoliques

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Les suspensions cellulaires de *Vitis sp*. sont un matériel adapté pour la production de métabolites d'intérêt tels que les polyphénols. Un de leur avantage est de pouvoir appliquer de façon contrôlée des stress biotiques et abiotiques (lumière, température, nutrition, élicitation, ...) afin de mieux comprendre les effets que les différents stress peuvent avoir sur la synthèse de ces composés. L'incorporation du marquage au C13, par ajout de précurseurs des polyphénols dans le milieu, est un bon outil pour étudier la formation et la régulation de ces composés. Nous avons étudié l'incorporation du marquage sous différentes conditions de cultures : des cellules soumises à des régulateurs hormonaux (Methyl jasmonate) et des agents de piégeage de polyphénols (Cyclodextrines). Nous avons ensuite prélevé des échantillons à plusieurs temps après application de ces agents. Les échantillons ont été ensuite analysés par une approche de métabolomique ciblée en HRMS utilisant une instrumentation QExactive avec fragmentation MS/MS (data dependent analysis). Ensuite, nous avons exploré pour les polyphénols principaux, à savoir des familles des stilbènes et des flavonoïdes, la présence des différents isotopologues au cours du temps. Ces études nous ont montré la vitesse d'incorporation du marquage sous différentes conditions. Ainsi cela révèle les potentiels intermédiaires de leur formation. Des études ultérieures chercheront à incorporer le profilage non ciblé et à développer des expériences plus poussées en fluxomique.

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Section 3 - Poster 4 – S3-P4

Caractérisation de la pulpe de différentes variétés d'igname pour leurs profils en saponines en lien avec la qualité alimentaire

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L'igname, appartenant à la famille Dioscoreaceae et au genre Dioscorea, est un tubercule comestible réputé pour être l'une des principales sources de saponines stéroïdiennes dans le règne végétal. Ces saponines sont parmi les composés fonctionnels les plus importants des ignames et suscitent un intérêt croissant en raison de leurs nombreuses propriétés bioactives. Parmi ces propriétés, chez les rongeurs, la consommation d'igname ou d'extraits de dioscine a un effet sur la réduction de la prise alimentaire et de l'obésité et sur le contrôle du diabète. Dans un contexte d'épidémie mondiale d'obésité qui affecte lourdement les pays tropicaux, l'igname pourrait jouer un rôle important pour limiter les maladies métaboliques. Le profil en saponines a été établi à partir de poudre d'igname lyophilisée provenant de six cultivars appartenant à quatre espèces différentes cultivées en Guadeloupe : Caribinra et Goana (D. Alata), Grande Savane et Jano (D. Rotundata), Pas possible IRAT (D. Esculenta) et Adon (D. Bulbifera). Le choix a été réalisé sur la base de plusieurs critères (résistance à l'anthracnose, amertume et données bibliographiques relatant la présence de saponines). Un procédé d'extraction a été optimisé, suivi d'une méthode d'analyse par UHPLC-MS de type trappe ionique utilisée en mode négatif. L'identification des composés s'est appuyée sur la comparaison avec des standards commerciaux (temps de rétention et spectres de masse), ainsi que sur des données scientifiques publiées. Une méthode de quantification est en cours de développement et sera appliquée à l'ensemble des échantillons.

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Section 3 - Poster 5 – S3-P5

Caractérisation des lipides cuticulaires des raisins en spectrométrie de masse à haute résolution

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Parmi les nouvelles variétés de vignes résistantes au mildiou (*Plasmopora viticola*) et à l'oïdium (*Erysiphe necator*), certaines sont sensibles au champignon *Botrytis cinerea*, responsable de la pourriture grise qui peut générer des pertes importantes au vignoble et affecter la qualité des vins. Le projet WiVitis, cofinancé par l'Union Européenne, a pour objectif de mettre en évidence des différences entre les variétés sensibles et tolérantes à *B. cinerea*, à travers l'étude de la composition lipidique de la cuticule des baies, afin de déterminer si certaines molécules peuvent expliquer la différence de sensibilité au Botrytis entre les différentes variétés. Une méthode d'extraction des lipides cuticulaires des raisins a été mise au point, ainsi qu'une méthode de séparation et d'identification par chromatographie liquide couplée à de la spectrométrie de masse haute résolution, *via* l'utilisation de deux types de sources d'ionisation : l'APCI (atmospheric pressure chemical ionization) et l'ESI (electrospray ionization). L'utilisation combinée de ces deux sources a permis l'étude de plusieurs familles de composés lipidiques comme les acides gras, les mono-, di- et triglycérides, ainsi que leurs formes glycosylées, les phosphatidylcholines, les phosphatidyléthanolamines et les triterpènes. Combinées à des données de phénotypage de la sensibilité au Botrytis, ces analyses permettront d'évaluer l'impact potentiel de la composition de la cuticule sur la sensibilité à la pourriture grise.

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Section 3 - Poster 6 – S3-P6

Characterisation of beneficial and antinutritional compounds diversity and distribution in *Camelina* seed fractions and extracts

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Camelina is an oilseed crop whose seeds are characterized by high-quality oil. Nevertheless, limited knowledge is available regarding specialized metabolites (SMs) and lipids composition in seed mucilage and other seed fractions. To address this gap, we metabolically characterized seeds from six camelina (Camelina sativa L.) genotypes. An aqueous integrated process was developed to obtain an aqueous phase, an emulsion and a pellet fraction from degummed seeds. Untargeted metabolomics and lipidomics (LC-MS/MS), and fatty acid (GC) analyses were performed on whole seeds, degummed seeds, mucilage, and on three obtained fractions (6 genotypes x 3 replicates x 6 fractions/extracts x 3 analytical techniques). Untargeted metabolomic analyses allowed the detection on > 4500 SM or lipid features. Among the major SMs classes, there were several beneficial (e.g. flavonols and cinnamic acids) or antinutritional (e.g. glucosinolates and alkaloids) metabolites. Untargeted lipidomic analyses allowed the detection of mono- diand tri- acylglycerols (MAG, DAG, TAG), galactolipids, sterols, phosphorylated lipids, and ceramides. The distribution of these compounds was then investigated in whole seeds, mucilage, and the three seed fractions, revealing that extraction methods allowed the concentration of several antinutritional compounds, such as glucosinolates, in specific fractions, thus reducing their level in the oil fraction. In addition, valuable compounds such as Octadecanoids (lipids), dipeptides and some phenylpropanoid classes were highly accumulated in the mucilage extract. Water based extraction and coproduct characterisation using metabolomic techniques allowed the identification of camelina seed fractions with great potential for the agricultural, food, pharmaceutical and industrial sectors.

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Section 3 - Poster 7 – S3-P7

Contribution à la caractérisation du mode d'action de biostimulants microbiens dans les cultures de tomates hydroponiques

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Les données actuelles, montrent que nous devons trouver des solutions alternatives aux systèmes conventionnelles afin d'améliorer la résilience de la production agricole en vue de faire face aux changements climatiques et limiter notre pollution. Dans ce contexte, l'utilisation de produits naturelles tels que les biostimulants offres de nouvelles perspectives pour une agriculture plus résiliente et durable. L'utilisation d'approches analytiques spécialisés tel que la métabolomique peux nous aider à caractériser leurs effets sur les cultures en vue d'améliorer leurs applications. Une étude à été menée au sein du Living Lab Agrolab Biomed en partenariat avec les paysans de Rougeline et Greencell biotechnologies, dans l'objectif d'évaluer l'impact de biostimulants microbiens sur la culture de tomate hors-sol. Les traitements comprennent 3 biostimulants microbiens formulés à partir des souches B25 de Bacillus methylotrophicus, B97 de Trichoderma Harzianum et un cocktail des deux souches. Le design expérimental comprend, 40 pieds de tomates répartis sur 20 blocs de substrat o`u les biostimulants ont été appliqués à différentes doses sous forme de solution liquide. L'analyse phytochimique est dirigé sur les feuilles et sur les fruits. La stratégie analytique repose sur la complémentarité d'une analyse des feuilles par HPLC-HRMS et des fruits par RMN et un traitement des données métabolomiques multiplateforme pour caractériser l'effet des traitements sur la physiologie des plants de tomates. Les premiers résultats obtenus révèlent des profiles métabolique significativement différents entres les plants témoins et traités. A l'aide des banques de données spectral et de logiciels spécialisés (SIRIUS/MS-DIAL) nous avons annoté des molécules d'intérêt.

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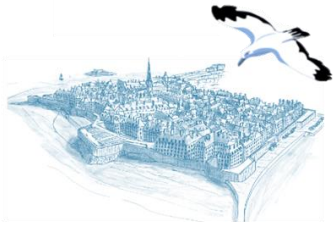

Section 3 - Poster 8 – S3-P8

Development of a non-targeted approach combining High Resolution Mass Spectrometry (LC-HRMS) and High Resolution Tandem Mass Spectrometry (LC-HRMS/MS) approach for assessing the different quality dimensions of the sponge cake and their evolution during food processing

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Characterizing the chemical fingerprint of a processed food is a major challenge to advance food quality. New untargeted approaches based on LC-HRMS analysis can unlock the potential to increase our understanding on how chemical components linked to different food quality dimensions react and evolve during the manufacturing processes and thus contribute to food quality design. The aim of our project is to develop such new approach on a range of baked products (sponge cakes) generated under controlled formulation and processing parameters (time and temperature of cooking) to generate a diversity of chemical profiles. Our approach is based on a method combining LC-HRMS and LC-ddA-HRMS/MS analyses to evaluate at the end whether the use of molecular networks can assist us in better understanding the reactional links between compounds. We report here the different steps followed to build it. We first developed an MS analytical method with a data treatment workflow operated on W4M. PCA treatment successfully discriminated our sponge cakes samples according to their preparation conditions. Several statistical tests (PLS-DA and Volcano plots) were then performed to identify the most significant discriminating features, which would be included later on in the MS/MS method for fragmentation. Surprisingly notable differences were noted and different lists were obtained from the various statistical tests. We proceeded with LC-dda-HRMS/MS acquisition method, using each inclusion list separately. We will explore whether the choice of LC-HRMS selection method has an impact on the construction of molecular networks following LC-HRMS/MS, and what potential interpretations could arise from this.

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Section 3 - Poster 9 – S3-P9

Elicitation du métabolome des graines de tomate par des solutions de biocontrôle pour la protection des semences

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Le projet SeedBioProtect aborde la problématique du mode d'action et de l'efficacité des solutions de biocontrôle pour protéger les semences contre les bioagresseurs. Ces travaux, visent à décortiquer la réponse des graines de Solanum lycopersicum (tomate) issues de plantes-mères traitées par ces solutions pendant le développement/maturation et la germination aux échelles moléculaires (RNA-Seq) et métaboliques (LC-MS/MS) et ainsi comprendre les voies métaboliques stimulés par la graine pour lutter contre des bio-agresseurs, tout en gardant un haut potentiel de germination et de croissance. Des analyses métabolomiques non-ciblées par U-HPLC-ESI+/–Tof nous ont permis de détecter 1017 signaux (ou features) métaboliques " unique " pour les graines de tomate traitées selon 4 modalités : 1 contrôle (H_20), 1 stimulateur de défense des plantes, 1 fongicide naturel de biocontrôle, 1 micro-organisme de biocontrôle. Parmi les catégories de métabolites annotées il y a des composés de la voie des phénylpropanoïdes, alcaloïdes et terpénoïdes connus pour avoir un rôle dans la réponse aux stress et/ou antioxydante. Les tests de statistiques multivari és (clustering hiérarchique, ACP, ANOVA) ont permis d'identifier un grand nombre des métabolites (56% du total) modulés par les traitements utilisés. Des données transcriptomiques (RNA-Seq) et du microbiote (metabarcoding) des graines traitées avec ces stimulateurs des plantes et ces produits de biocontrôle seront intégrées aux résultats des analyses métabolomiques. Cette étude permettra d'identifier des voies métaboliques qui jouent un rôle dans la réponse des graines aux bioagresseurs et de développer des outils d'évaluation robustes sur l'efficacité des solutions de biocontrôle pour la protection des semences.

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Section 3 - Poster 10 – S3-P10

Elucidating the role of glucosinolate modifications regulated by warm temperatures in seeds

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Glucosinolates (GSLs) constitute major antinutritional and defensive compounds in Brassicaceae species such as the model plant Arabidopsis thaliana. While most studies have focused on GSL role in biotic stress responses, the potential roles of GSL in abiotic stress responses has been neglected. This is particularly true in seeds, for which few information are available. In this study, untargeted metabolomic analysis was conducted on A. thaliana seeds developed under warm temperature or control conditions. A large number of detected specialized metabolites (SMs) was affected by warm temperature stress (WTS), revealing a significant stimulation of decorated GSLs, cinnamic acids and flavonols in seeds. Transcriptomic analyses allowed the identification of many differentially expressed genes involved in SM modifications, e.g. acyltransferases and hydroxylases, which are modulated by warm temperatures. Among them, the 2-oxoglutarate-dependent dioxygenase AOP3, involved in aliphatic-GSL side chain hydroxylation, was found to be highly induced by WTS. Seeds of A. thaliana aop3 knock-out mutants display altered seed germination, antioxidant capacity and transcriptomes compared to the wild-type. Moroever, while wild-type seeds showed a strong accumulation of acylated GSL, especially upon WTS, aop3 mutant did not accumulated high levels of these metabolites, suggesting a role of GSL acylation in seed WTS response. To further characterize the role of decorated GSL in seed WTS responses, knock-out mutants for the genes SCPL17 and BZO1, involved in GSL acylation, are being metabolically and functionally characterized. Our results suggested that AOP3 and SCPL17 genes and their associated acylated GSL are involved in A. thaliana seeds response to WTS.

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Section 3 - Poster 11 – S3-P11

Etude de l'efficacité en biocontrôle de l'enrobage des graines de lin par Streptomyces

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Le Lin ou *Linum usitatissimum* est une plante présentant une importance agronomique, valorisée pour ses fibres et son huile riche en oméga 3. Le rendement de cette culture est souvent diminué par l'attaque de différents agents tels que les pathogènes fongiques : *Fusarium oxysporum*, *Verticillium dahliae, Septoria linicola*. Le traitement classique de ces maladies repose sur l'utilisation de produits phytosanitaires ayant des effets néfastes sur l'environnement et l'homme. Dans un contexte de développement durable, l'utilisation de produits phytosanitaires doit être limitée, et l'utilisation de moyens alternatifs doit être développée. Une technique innovante est l'enrobage des semences par des microorganismes pour une application en biocontrôle. Les travaux ici présentés visent à évaluer l'efficacité en biocontrôle/biostimulation d'un modèle par enrobage à faibles coûts d'application, et exempt de toxicité. Trois souches de Streptomyces ont été choisies : *Streptomyces bikiniensis, Streptomyces globisporus, Streptomyces griseorubiginosus*. Un test in-vitro de l'activité directe de ces souches sur les trois agents pathogènes a montré qu'elles limitaient leur développement. Un protocole d'enrobage a été mis en place et appliqué à des graines de lin cultivées ensuite en hydroponie afin d'assurer un contrôle optimal des conditions de culture. L'effet de ce traitement sur la croissance des plantes a été évalué. Une étude de la variation métabolique induite par les bactéries sur les plantes a montré une variation du métabolome des racines entre les plantes traitées par les bactéries et les plantes en condition contrôle.

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Section 3 - Poster 12 – S3-P12

Etude métabolomique d'un kéfir de fruit

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Le kéfir de fruit est une boisson issue de la fermentation d'une solution de saccharose à l'aide d'un ferment appelé " grain de kéfir " constitué d'un consortium de levures, de bactéries acétiques et lactiques niché dans une matrice d'exopolysaccharide. La fermentation du kéfir s'accompagne de dynamiques microbiologiques et métaboliques, qui sont à ce jour peu explor ées. Les métabolites majoritaires présents dans la boisson correspondent principalement aux substrats et produits de la fermentation – sucres, acides, alcools... - et sont identifiables et quantifiables par de méthodes de dosage ciblées. L'objectif de cette étude est de mieux comprendre les interactions métaboliques au sein du consortium microbien au cours de la fermentation du kéfir, *via* une caractérisation longitudinale non ciblée de l'exo métabolome d'un kéfir de fruit. Dans cette optique, une approche métabolomique par résonance magnétique nucléaire (RMN) et chromatographie liquide couplée à la spectrométrie de masse (LC-MS) a été déployée pour suivre la fermentation du kéfir. L'analyse des données a mis en évidence les variations longitudinales du métabolome, partant d'une composition très simple (principalement du saccharose) à une complexification montrant des profils chimiques distincts à 16h, 24h et 48h. Les données LC-MS/MS ont été utilisées pour construire un réseau moléculaire en vue d'identifier les métabolites produits au cours de la fermentation. Au-delà de mettre en évidence les interactions écologiques au sein du consortium microbien, le projet pourrait permettre, à plus long terme, de caractériser dans la boisson, au niveau moléculaire, des fonctionnalités de nature pré, probiotique ou organoleptique.

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Section 3 - Poster 13 – S3-P13

Etude métabolomique par SPME-GC-MS et annotation de biomarqueurs de la Vanille Mahoraise

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La vanille est une épice de plusieurs espèces du genre Vanilla, appartenant à la famille des Orchidaceae. Outre la vanilline, la vanille renferme également d'autres composés aromatiques tels que les acétates, les alcools et les aldéhydes, contribuant à sa complexité sensorielle. En se penchant sur la vanille de Mayotte, couronnée de la médaille d'argent à plusieurs reprises, nous explorons un terroir spécifique propice à la culture de Vanilla planifolia, espèce de vanille la plus couramment cultivée. Le premier objectif de cette étude est de démontrer la présence d'une spécificité de la vanille de Mayotte par rapport à 3 autres grands producteurs qui sont les Comores, Madagascar et la Réunion. Les analyses en SPME-GC-MS sont réalisées sur 84 extraits de vanille provenant des 4 îles. Les résultats sont exploités de manière indépendante avec une manière "traditionnels" consistant à réaliser une matrice manuelle. Parallèlement, une seconde matrice issue des mêmes données est réalisée avec le logiciel MzMine. Les matrices sont exploitées statistiquement avec le logiciel R dans le but de déterminer des biomarqueurs pertinents appartenant à la vanille de Mayotte. Ces derniers sont par la suite annotés en combinant indice de Kovats et comparaison à la banque spectrale NIST. Les résultats permettent de mettre en avant la spécificité de la vanille de Mayotte par rapport à celle des Comores, de Madagascar et de la Réunion. Mais aussi, ils amorcent une seconde étape cruciale pour démontrer son authenticité avec la présence potentielle d'un effet terroir spécifique à Mayotte.

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Section 3 - Poster 14 – S3-P14

Evaluation de l'impact des pratiques culturales sur la vigne *via* le développement d'une approche basée sur la métabolomique

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Le modèle cultural de la vigne est en France un modèle de culture intensif qui a besoin d'un usage important d'intrants de synthèse. De la bouillie bordelaise, qui a pour principe actif le sulfate de cuivre, est notamment utilisée pour lutter contre le mildiou et l'oïdium de la vigne. L'utilisation du sulfate de cuivre a contaminé le sol des vignes en cuivre. Ce dernier peut être délétère pour les plantes puisqu'il peut affecter leur croissance et réduire les rendements. Des enjeux environnementaux et sociétaux ont poussé les agriculteurs à faire évoluer leurs modèles vers des modèles plus respectueux de l'environnement. Pour réduire l'utilisation des intrants de synthèse, les agriculteurs utilisent de plus en plus de bio-intrants. Ce projet a pour objectif d'évaluer l'impact d'un stimulateur de défense des plantes (COSOGA) sur le métabolome de la vigne (Syrah). Pour cela, une étude cinétique a été réalisée sur 49 jours avec 14 pas de temps et 5 réplicats pour chaque modalité de temps et de traitement. Cette cinétique a été effectuée sur une parcelle expérimentale mise à notre disposition. Une approche basée sur la métabolomique non ciblée (LC-MS) a été développée pour analyser les échantillons. A notre connaissance, c'est le premier projet qui étudie le COS-OGA sur Syrah par métabolomique dans le Languedoc- Roussillon. Des analyses statistiques multivariées ont permis d'observer une séparation des modalités non traités et COS-OGA. Des variables discriminantes, qui sont potentiellement des métabolites du COS-OGA et de réponse de la plante, ont pu être mise en évidence.

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Section 3 - Poster 15 – S3-P15

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

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Pea (*Pisum sativum L.*) is a legume species widely cultivated due to its high seed protein content for feed and food applications. While many seed specialized metabolites (SM) play essential physiological functions by protecting seeds against stresses, the presence of certain SMs in pea seeds can alter the organoleptic or functional properties of protein fractions. However, SM diversity and plasticity in response to the environment remain largely underexplored. In addition, few information is available about genes and enzymes involved in the biosynthesis and modification of SM in seeds. In the LETSPROSEED project, we aim at exploring SM in pea seeds to elucidate the impact of domestication and/or evolution on seed SM diversity, and to investigate the extent to which SM composition influences seed quality. As a first step to achieve these goals, seed cotyledons and coats from 204 pea genotypes were subjected to untargeted metabolomic analyses (LC-MS/MS). The collection includes wild and domesticated Pisum species and subspecies, together with widely cultivated varieties in France. Our first results revealed significant differential accumulation of many SM categories among species and/or subspecies, suggesting a major impact of the evolution and/or domestication on the accumulation of SM with potential roles in seed defence and quality. To identify the genetic determinants with a role in SM accumulation, a metabolomic-GWAS approach is foreseen during the project. These preliminary 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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Section 3 - Poster 16 – S3-P16

Exploring the Impact of Homocysteine Supplementation on A*via*n Embryonic Metabolism Through Mass Spectrometry-Based Metabolomics Analysis

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Embryonic malformations are often attributed to environmental factors, including folate deficiency. A*via*n models can recapitulate human folate deficiencies by adding homocysteine into the egg. However, how this treatment impacts the metabolite levels during embryonic development remains poorly understood. This project aims to fill this gap by studying how the supplementation of homocysteine affects the metabolite composition across various matrices. We used our expertise in mass spectrometry-based metabolomics to develop a method for sample preparation and analysis on the different parts of the egg : yolk, white, and embryo, in order to study the impact of homocysteine supplementation on metabolite profiles during embryonic development. Samples were analyzed using HILIC technology on a Vanquish- QExactive+ coupling (Thermo) in ESI-NEG and ESI-POS, full scan mode and targeted reprocessing on 150 metabolites. Our preliminary results show satisfactory metabolic coverage, with 103 metabolites detected across the 3 matrices studied. The next quantitative study of 60 samples will show the impact of homocysteine supplementation at different doses, on the metabolism of these 3 matrices at different stages of embryonic development. It will allow us to determine the dynamics of metabolite levels in homocysteine-supplemented eggs across developmental time.

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Section 3 - Poster 17 – S3-P17

Impact de bactériocines sur le microbiote colique a*via*ire : étude *in vitro*

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Dans cette étude, nous avons utilisé le modèle de fermentation continue in vitro PolyFerm S pour simuler le microbiote colique de poulet. L'étude a évalué l'impact de trois bactériocines (microcine J25, nisine Z, pédiocine PA1) et de l'antibiotique bacitracine sur ce microbiote. La stabilité et l'efficacité des bactériocines ont été mesurées par un test de diffusion en gélose pendant 48 heures. La microcine J25 a maintenu une forte activité inhibitrice contre Salmonella enterica sérotype Newport même après 24 heures, contrairement à la pédiocine PA1 qui a perdu son activité après 8 heures, et la nisine Z qui n'a montré aucun effet inhibiteur. Les résultats ont été appuyés par une analyse LC-MS/MS, identifiant les bactériocines et leurs produits de dégradation. L'analyse des acides gras à chaîne courte a montré une réduction significative de l'acide butyrique avec la nisine Z après 24 heures, et de l'acide propionique avec la bacitracine après 48 heures. L'acide acétique n'a présenté aucune variation notable. L'étude de la composition du microbiote par metabarcoding 16S a révélé un impact marquant de la nisine Z, tandis que la microcine J25 n'avait pas d'effet significatif, et la pédiocine PA1 un effet modéré. Une analyse métabolomique par LC-MS a confirmé un impact plus marqué de la nisine Z. Ces observations suggèrent que la microcine J25 est la plus stable, avec le moins d'impact sur la composition et le métabolisme du microbiote colique a*via*ire.

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Section 3 - Poster 18 – S3-P18

Impact of global climate change on barley secondary metabolism

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One of the aims of the European Biodiversa SuppressSOIL project (Germany, France, witzerland) is to generate knowledge that will help define management strategies to improve crop health in soils facing the challenge of global change. Within this framework, a barley field cropping approach was carried out, mimicking (i) two agricultural management systems (conventional versus organic farming) and (ii) two different climatic conditions (current and future - heat and water stress). Untargeted metabolomics in UHPLC-DAD-QTOF, on aerial and root parts of barley, at two different stages of development, were used to classify the relative effects of climatic conditions (current versus simulated future conditions of global change) and farm management (organic versus conventional farming) on plant growth and health. Multivariate analysis conducted by separating the data by farming system (conventional versus organic) showed a significant effect of climate on the metabolic profile of both barley roots and leaves, whatever the stage of development. We were thus able to annotate biomarkers of metabolic pathways involved in adaptation to climate change.

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Section 3 - Poster 19 – S3-P19

Influence des caractéristiques des LOts de semences de maïs sur la TOlérance de la plante aux attaques de taupins (LOTO)

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Les taupins, ravageurs du maïs, causent des pertes importantes de production en France, atteignant 7,8% pour le grain et 3,5% pour le fourrage. Le taux de plantes de maïs attaquées au niveau du collet par les taupins varie selon les lots de semences, suggérant des différences d'attractivité ou de résistance. L'objectif du projet LOTO vise à identifier les mécanismes favorisant les interactions entre la plantule de maïs et les larves de taupins, en particulier les métabolites impliqués. La stratégie a consisté à réaliser un phénotypage sur la sensibilité de plantules aux attaques de taupins, afin de sélectionner des lots de semences présentant des réponses extrêmes de sensibilité. Sur ces lots, les métabolites différentiellement exprimés au niveau du collet, à un stade de développement précoce des plantules, ont été recherchés. Trois approches chromatographiques orthogonales (C18, Hilic, Lipidomique) couplées à des analyses par spectrométrie de masse haute résolution (HRMS) ont été réalisées sur les métabolites extraits des collets de plantule collectés en champ. Ces approches multiplexées ont permis d'obtenir une cartographie métabolique complète réalisée à l'aide d'un outil en cours de développement (MS-Net : Multi-similarity based network annotation). Des statistiques multiblocs ont permis de lister les métabolites différentiellement produits par les plantules en lien avec leurs phénotypes de sensibilité aux taupins. L'objectif final sera de caractériser des familles de composés ou des voies métaboliques impliquées dans la sensibilité des plantules puis de valider les résultats par des essais au champ.

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Section 3 - Poster 20 – S3-P20

Intégration multiblocs de quatre jeux de données métabolomiques acquises en LC-HRMS pour l'amélioration du dépistage de l'administration de promoteur de croissance chez les animaux de production.

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Afin de garantir la sécurité sanitaire des denrées d'origine animale, dépister l'administration de promoteurs de croissance chez les bovins est une priorité des pouvoirs publics. En ce sens, de nombreuses études ont montré le potentiel de la métabolomique pour la découverte de nouveaux biomarqueurs d'effet. Cependant, elles n'abordaient qu'une classe de composés, et ce, avec une couverture limitée du métabolome. La présente étude s'est donc intéressée à mettre en place un modèle de classification global d'échantillons, s'appuyant sur une évaluation plus exhaustive du métabolome. Pour cela, 502 prélèvements urinaires issus de six expérimentations impliquant l'administration de cinq promoteurs de croissance ont été analysés par LC-HRMS en RPLC-ESI+, RPLCESI-, HILIC-ESI+ et HILIC-ESI-. Face à l'importante variabilité inter-individuelle liée aux six expérimentations, une normalisation adaptée du z-score a été réalisée, avant que les quatre jeux de données soient intégrés au moyen d'une analyse multiblocs Consensus-OPLS-DA. Le modèle obtenu a montré une bonne classification des échantillons (R2Y=0.678, Q2Y=0.662, R2X=0.02) et une amélioration du pouvoir prédictif comparé aux modèles spécifiques des différentes analyses LC-HRMS. De plus, l'intégration multiblocs révèle une contribution comparable des quatre jeux de données, illustrant la complémentarité des analyses. Enfin, les analyses univariées et l'annotation des variables ont permis d'identifier les potentielles voies métaboliques impactées par l'administration des promoteurs de croissance. La combinaison de six expérimentations et quatre analyses LC-HRMS, illustre la complémentarité des approches en termes de couverture métabolique et leur intégration multiblocs a permis l'implémentation d'un modèle de classification global capable de prédire l'administration de promoteur de croissance.

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Section 3 - Poster 21 – S3-P21

LC-MS and NMR multiplex metabolomics approach for classifying monovarietal wines

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Wine is among the most consumed beverages worldwide, renowned for its complex composition and significant economic value. The value of wine largely derives from its type, vintage, grape variety, and production region, all of which contribute to its identity. Given its high value, wine is particularly susceptible to fraud and counterfeiting. Ensuring traceability is therefore crucial for both the production chain and consumers. To address this issue, metabolomics-specifically, wineomics-plays a pivotal role by employing techniques such as MS and NMR. This study aims to enhance the coverage of the metabolome of interest by applying multiplex analysis through NMR and MS. The goal is to distinguish 26 monovarietal wines-specifically Cabernet Sauvignon, Pinot, and Merlot-from the Languedoc region in France using untargeted approaches. NMR data was collected using a Bruker 500 MHz AVANCE III, utilizing zgpr and noesygpps1d pulse sequences, and processed with NMRProcFlow software, yielding 331 features. MS/MS data dependent analysis was obtained *via* a UHPLC-MS/MS system (Orbitrap Q-Exactive™), and processed with MSDial 4.94, producing 8,500 features. The data underwent multivariate analysis using SIMCA 17 software. The unsupervised chemometric models from the untargeted analysis indicated consistent clustering behavior between NMR and MS PCAs, with monovarietal Pinot wines showing significant variations compared to Merlot and Cabernet Sauvignon. Supervised modeling *via* PLS demonstrated good clustering ; but showed limitations for group classification ($Q2 \leq 0.6$ (PRESS crossvalidation)). Future work will explore a NMR/MS data fusion approach in pursuit of improved wines classification.

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Section 3 - Poster 22 – S3-P22

Metabolome modeling of agroecological practices.

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Agroecological metabolomics is a specialized branch of ecological metabolomics that aims to investigate the relation between agroecological practices and plant metabolism. In the context of stagnating crop yields, escalating concerns about climate change, and a growing shift towards ecological agriculture, it is important to shed light on the metabolic dynamics of plants within agricultural ecosystems. By employing metabolomics and machine learning techniques, it is possible to comprehend the relationship between agroecological practices, plant metabolism, and ecosystemic services. This study focuses on two primary objectives : Firstly, to gain insights into the influence of diverse farming practices on plant metabolism. This involves understanding how different agricultural systems, such as conventional, biological, and zero pesticide residue systems, impact plants' metabolomes, and identify metabolic indicators of plant performance under agroecological practices and predict ecosystemic services. Secondly, explore generic mechanisms towards multispecies adaptation. By analyzing and comparing metabolic responses across agroecological practices, identifying commonalities that can be extrapolated to different plant species. This can be done through a comprehensive metabolomic analysis using (LCMS) techniques, analyzing the metabolite composition of fruits, roots, leaves, and soil samples collected from each farming system. The results are expected to present potential variations in the metabolomic profiles of carrots and tomatoes grown under different agricultural systems. In addition to identify metabolic signatures associated with each agricultural practice, with specific metabolites showing differential abundance levels across the studied conditions. Furthermore, highlighting common metabolic responses shared between the two plant species, suggesting generic mechanisms underlying plant metabolic adaptation to agroecological practices.

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Section 3 - Poster 23 – S3-P23

Metabolomic Analysis of Camellia: Unlocking Phenotypic Insights and Industrial Applications

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The Camellia genus, renowned in cosmetics for its polyphenols, triterpenoids, saponins, and oils, and in horticulture for its flowers' beauty, is the subject of innovations aimed at creating new varieties. These efforts seek to enhance its components and aesthetic traits. The main challenge is the long 3 to 7-year wait for the Camellia to blossom, crucial for validating hybrids' phenotypic attributes. Integrating innovative techniques is imperative to accelerate the characterisation of floral phenotypes, representing a significant opportunity for the agronomics and cosmetics sectors. In this context, an original predictive metabolomics study of 200 samples from 49 distinct Camellia taxa has provided invaluable insights. Untargeted metabolomic analysis was performed on ethanolic leaf extracts using reverse-phase liquid chromatography coupled to an Elite-Orbitrap high resolution mass spectrometer with electrospray ionisation interface (UHPLC-(ESI)-HRMS), in negative ion mode. Following this analysis, advanced prediction was performed on the metabolomic variables obtained, by applying Ridge, Lasso and Elastic- Net regression methods. Metabolomics uncovered more than 5,000 metabolomic signatures, of which 1,600 showed statistically significant responses to flower shape and colour. Through predictive modelling including all features and based on 100 generalised linear models, excellent prediction accuracies exceeding 95% were obtained for the two phenotypic parameters studied. In addition, this approach shed light on specific families of molecules accountable for each trait. Beyond its primary objective of expediting phenotypic characterisation, this innovative approach holds promise in facilitating future investigations into the biological effects of Camellia extracts on the skin.

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Section 3 - Poster 24 – S3-P24

Metabolomics approach to study the impact of industrial processes on yeast metabolism

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Better understanding of yeast metabolism under different fermentation conditions in industry is crucial to better adapt and evolve industrial processes in order to remain competitive. In this context, metabolomics is one of the approaches making it possible to answer the various questions associated with these industrial processes. Beforehand, a study comparing the different protocols for quenching and extraction of the yeast endometabolome (*S. cerevisiae*) was invastigated to select the most appropriate method. A metabolomic study was then carried out on several samples from different industrial conditions to validate the protocol and to highlight the first insights of correlation between composition and industrial processes. Other approaches were also used as part of this study, notably transcriptomics, proteomics and lipidomics. All these results should be used to develop multi-omics in the future.

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Section 3 - Poster 25 – S3-P25

MS profiling: a tool to identify antioxidant compounds in complex matrices and investigate synergic antioxidant activities in model solutions.

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Phenolic compounds are a large family of secondary metabolites specific to plants. They are known for their many bio-activities, including their ability to oxidise, which helps protect other elements from oxidation. In the food industry, this antioxidant capacity can be used to protect macro- and micro- nutrients from oxidation, thereby improving food preservation. By replacing synthetic preservatives, polyphenols, sourced from plant co-products (in this case apple pomace and buckwheat hulls), appear to be a more virtuous solution that respects both consumers and the environment. The aim of this study is to identify the molecular determinants of the antioxidant action of mixed polyphenols and to understand the mechanisms involved, in particular by looking at the concepts of synergy, additivity and antagonism. In a 1st step, LC-UV-MS was used to profile and identify the main polyphenols in apple pomace and buckwheat hulls. This enabled the identification of 5'-O-caffeoylquinic acid (hydroxycinnamic acid), phloridzin (dihydrochalcone), (-)-epicatechin and condensed tannins (flavan-3-ols) in apple pomace. In buckwheat hulls, (iso-)vitexin and (iso-)orientin (flavones), rutin and hyperoside (flavonols), and condensed tannins were in the majority. In a 2nd step, the antioxidant potential of the matrices was determined using DPPH and FRAP assays. The standards for some of the main polyphenols identified were worked up in model solutions, separately and in mixtures of different concentrations.

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Section 3 - Poster 26 – S3-P26

Polyphenolic profiles of faba beans and enriched fraction interactions with legume albumins?

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As an alternative to meat, legumes represent a promising alternative to fulfil both agrienvironmental issues and feeding the planet. Despite faba bean protein richness, several locks remain to its consumption. The main issue is its polyphenolic content that alter its organoleptic and physico-chemical products properties. Flavan-3-ols monomers (catechin, epicatechin and galloyl derivatives) and oligomers (proanthocyanidins) were detected in faba bean methanolic extract[1] in genotypes dependant manner[2]. Tannin depolymerization gave more information about their structure and mean degree of polymerization3. During transformation, seeds undergo alkalinization (pH8) to precipitate proteins and obtain proteins isolates. Such condition leads to polyphenol autoxidation that forms quinones and reactive intermediates able to interact with proteins. If it is well known that the non-covalent and covalent bounding4 depended on multiple factors (temperature, pH and conformation/type), the relevance of the polyphenolic pool oxidative status on the interaction determinism needed to be investigated. Here, we performed a metabolomics profiling of 12 faba beans genotypes. The study relied on both direct UHPLC-DAD-LTQ analysis or after a depolymerization step. Varieties were separated by chemometrics tools through their growth seasonality. We then used centrifugal partition chromatography and preparative HPLC to obtain a tannin-rich fraction that we set at pH8 for night in order to oxidize the polyphenolic pool. This fraction was then put into contact with a fraction of faba bean albumin enriched fraction and interactions were monitored. Fluorescence quenching was revealed and high molecular weight bands on gel electrophoresis were observed that proved that oxidized polyphenols covalently bounded to proteins.

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Section 3 - Poster 27 – S3-P27

Targeted and untargeted UHPLC-MS metabolomic approaches for the study of monovarietal wines: preliminary tests

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The viticulture and oenology sectors are currently facing several new challenges and trends: wine traceability and identity, global warming effects on wine components, reduction in the use of synthetic pesticides, organic farming, innovation on new resistant grape varieties and/or grape varieties better adapted to global warming and drought, etc. Metabolomics approaches are highly suitable for studying these new topics since they can provide a detailed chemical characterisation of wines and grapes and assist in the finding of relationships between composition and wine quality traits. Our global project aims to establish a comparison of French and Italian wines from different cultivars (autochthonous and international), terroirs and different conditions (organic/ conventional wines, etc) and develop interring targeted and non-targeted analysis methods based on the use of NMR and MS, coupled with HPLC and GC. An initial targeted and non-targeted profiling of 38 commercial wines using UHPLC-HRMS/MS has already been carried out. The targeted analysis was conducted using a calibration curve of over 45 polyphenols, while the untargeted approach was based on a full scan HRMS acquisition with data-dependent fragmentation. The initial results showed a clear discrimination between wines based on the grape variety and the storage conditions of the samples. Concretely, the storage condition at 4◦C showed a clear effect on some polyphenols while others remained unchanged independently of the wine cultivar, which is a significant result for developing analytical protocols on wine sample analyses by metabolomics. Further research will be carried out to determine new biomarkers specifically involved in these discriminations.

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Section 3 - Poster 28 – S3-P28

Unravelling protein turn-over and allocation of fixed carbon in metabolism in response to changes in photosynthetic rate in sunflower leaves

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Photosynthesis is the cornerstone of plant carbon primary metabolism, providing source carbon for many metabolic pathways, including nucleic acid and protein synthesis. Photosynthesis is prone to variations through several factors like CO_2 mole fraction, with reciprocal changes in photorespiration. It is currently unclear whether CO_2-driven changes in photosynthesis impact on other metabolic pathways in the short term. It has been recently shown that in addition to changes in many pathways like base synthesis, protein production increases when photosynthetic activity increased when CO_2 mole fraction increases or O_2 fraction decreases, *via* the regulation of translation initiation. Nevertheless, it is unlikely that all proteins are up-regulated similarly when photosynthesis increases. It raises the question of specific proteins that are concerned by higher translation activity and also, how metabolism adjusts to match amino acid requirement. To address this question, sunflower leaves have been subjected to different CO_2 mole fractions for few hours and labelled with $13CO_2$. The incorporation of 13C in proteins has been studied by shotgun proteomics. Our first results showed a relatively slow protein turn-over in sunflower mature leaves regardless of photosynthetic activity and thus isotopic patterns are hardly detectable. To gain more sensitivity in 13C analysis of peptides, the experiment has been repeated with 13C-lysine labelling. Then, the partitioning of 13C in metabolism has been studied by 13C-NMR, GCMS and EA-IRMS. Our results indicate a strong labelling in metabolites like sugars. Also, we apply non-targeted 13C-NMR analysis to look at changes in 13C allocation when CO_2 mole fraction varies.

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Section 3 - Poster 29 – S3-P29

Unveiling the Impact of Biostimulant Application on Tomato Metabolism through a metabolomics strategy

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The framework of our research work is the GPR Bordeaux Plant Sciences (WP PHYTOSTIM). Our focus lies in investigating plant stimulation using biostimulants, to tackle environmental stresses and investigate underlying physiological and metabolic tradeoffs. Tomato (*Solanum lycopersicum* L.) is an important horticultural crop, typically cultivated during the summer months, aiming for a commercial maturity from July, in Europe. However, with the escalating frequency and intensity of high temperatures due to climate change, greenhouse tomato culture faces the challenge of navigating through heatwave periods, particularly during the reproductive stage. Our study aims to evaluate the impact of a biostimulant application on tomato metabolism, with or without thermic stress, at the beginning of the summer. Over two cultivation periods (2021 and 2022), employing a biostimulant derived from seaweed extract (*Ascophyllum nodosum*, L., Stim Pure liquid, Van Iparen), we gathered phenotypic observations and tomato samples from three organ types (Apex Leaf, Fully Expanded Leaf, and pericarp). Additionally, we performed targeted and non-targeted high-throughput analytical strategies by enzymatic measurements, and Liquid Chromatography coupled to tandem Mass Spectrometry on the leaf and fruit samples. Fruit yield is the variable that reflects crop performance. The results showed the effect of the condition of application (ferti-irrigation or rootball soaking at potting time). The fruit and leaf metabolome allowed discriminating between different biostimulant application methods and the control (treatment with water). The perspective of this work is to predict the performance of tomato crop and to gain highlight on molecular, metabolic, cellular and physiological mechanisms, underlying biostimulant efficacy.

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Section 3 - Poster 30 – S3-P30

Deciphering Prunus Responses to PPV Infection: A Way toward the Use of Metabolomics Approach for the Diagnostic of Sharka Disease

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Plum pox virus (PPV) causes the incurable sharka disease, affecting peach. Today, no preventive treatment or resistant cultivar are available. To prevent the spread of PPV, prophylactic methods are implemented. Early detection and removal of infected trees are crucial to managing sharka outbreaks. To date, orchard monitoring is based on visual detection of symptoms or serological and molecular analysis. However, these methods can lead to false negatives at early stages of infection due to heterogeneous distribution of virus and symptoms. Thus, these methods are not suitable for large-scale early detection. These limitations prompted the development of an innovative approach based on metabolic responses of plants to PPV-infection.

Two monitored experimentations on peach cultivars (PPV-infected vs. control) were conducted and studied using a non-targeted metabolomics approach (UHPLC-HRMS ESI+/-). The first study revealed an early metabolic response, before the onset of PPV-symptoms, despite negative RT-qPCR results. Meanwhile, the second study revealed biomarkers revealing PPV-infection in both symptomatic and asymptomatic leaves, suggesting a potential systemic metabolic response. Furthermore, some biomarkers appear to be specific to PPV-infection when compared to ACLSV-infection. These results highlight the potential of metabolomics for detection of infected trees even before the onset of symptoms and negative RT-qPCR results. Nevertheless, correlating results obtained under monitored conditions with field conditions remains a challenge.

To deal with this problematic the identification of affected pathways by PPV-infection could permit to have a robust diagnostic of infected trees. Therefore, putative annotation of biomarkers and affected pathways is currently underway using different platforms, notably GLOMICAVE.

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Section 3 - Poster 31 – S3-P31

Using ^{13}C metabolic probes to assess carbon allocation processes within central metabolism of *Brassica napus* source leaves

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Plant central metabolism comprises several essential metabolic pathways acting together to support plant growth and yield establishment. The deployment of 13C-tracing dynamic approaches in the model brassica specie Arabidopsis allowed to improve our understanding of this complex metabolic network by identifying major leaf carbon allocation processes. However, some questions remained open for Brassica napus leaves, an economically relevant oleaginous crop experiencing sequential senescence during its vegetative growth. Notably, the functioning of the tricarboxylic acid (TCA) cycle in the light in relation to glycolysis, Calvin cycle, photorespiration and branched-chain amino acid catabolism could be substantially different in source leaves in order to support senescence-associated remobilization processes. Here, we investigated these carbon fluxes by performing short-term incorporations of different fully 13C-labelled metabolic probes into Brassica napus leaf discs. Indeed, this experimental setup showed relatively similar photosynthetic and respiratory capacities compared to attached leaves. Evaluation of 13C-enrichments at molecular and isotopologue levels by GCMS identified light/dark regulation of key carbon fluxes and separated competing metabolic contributions to metabolite biosynthesis. Notably, the results supported: i) the occurrence of both cyclic and non-cyclic flux modes of TCA cycle in the light; ii) the contribution of glycolysis rather than stored citrate to TCA cycle functioning in the light; iii) the reallocation of TCA cycle decarboxylations back to the TCA cycle through photorespiration and glycolysis; iv) the multiple contributions of branched-chain amino acid catabolism to TCA cycle in the light. Overall, our results improved our understanding of central metabolism in source leaves of Brassica napus.

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Section 3 - Poster 32 – S3-P32 (Flash 9)

Unraveling *Brassica napus* leaf metabolic diversity: leveraging machine learning for agronomic traits prediction

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Rapeseed (*Brassica napus*) emerged through interspecific hybridization between Brassica rapa and Brassica oleracea. Subsequent genetic breeding efforts focused on reducing grain erucic acid and glucosinolates due to their toxicity, reflecting modern accessions. Creating crops with agroecological relevant metabolic profiles requires a chemical diversity characterization. Despite recent reports on vegetative parts involving glucosinolates and phenolics, intraspecific phytochemical diversity in leaves is understudied. Moreover, leveraging vegetative metabolomics to predict agronomic traits holds breeding potential. This study aimed to analyze the leaf metabolome across 304 brassica accessions and associate metabolome with agronomic traits using machine learning-based predictive approaches. LC-MS/MS metabolomics was performed on ethanolic extracts. MS-signals were processed by MSDIAL, resulting in 16,192 features, and annotations were performed using an in-house database. Predictive metabolomics based on LASSO, Ridge, and Elastic-Net models were employed to predict qualitative and quantitative traits using 4,919 curated features. Classification analyses achieved high accuracies (> 95%, p< 2e-16), with perfect accuracy (100%) for species prediction, revealing 261 predictive biomarkers, including some glucosinolates, and pointing out the modernity of accessions. Regression results showed r2-values above 0.75 for phenolic, glucosinolates, and erucic leaf content, and r2-values under 0.6 for protein and lipid leaf content, and leaf surface and area. Future predictions will incorporate root metabolome to associate with these agronomic traits, and a smaller panel of spring accessions (170) to predict grain yield under different conditions. Overall, predictive modeling facilitates understanding metabolic-phenotypic associations, aiding genotype selection for plant performance, including yield and flowering. This metabolomics approach holds promise for future breeding endeavors.

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Section 3 - Poster 33 – S3-P33 (Flash 10)

Diversité phytochimique de collections génétiques de Brassicacées pour la recherche de caractères d'intérêt agronomiques et agroécologiques

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Le métabolisme spécialisé représente une dimension essentielle des interactions entre les plantes et leurs bioagresseurs. Sa caractérisation au sein des espèces cultivées est donc un enjeu majeur, en particulier dans des programmes de sélection variétale. Néanmoins, chez les Brassicaceae et plus particulièrement chez le colza (Brassica napus), ce métabolisme est encore trop partiellement connu. Notre objectif est de caractériser la diversité phytochimique qui existe au sein de collections génétiques chez des espèces du genre Brassica et d'identifier les déterminismes génétiques qui contrôlent ce métabolisme spécialisé par le biais d'approches combinées de génétique, de génomique et de métabolomique. Les projets de recherche menés ces dernières années nous ont permis de (1) mettre en évidence des liens entre la résistance à un agent pathogène racinaire et des variations alléliques sur plusieurs loci, affectant les teneurs en certains métabolites spécialisés, (2) démontrer que l'architecture génétique globale du métabolisme spécialisé est organisée chez le colza sous forme de petits réseaux de QTL métaboliques qui contrôlent indépendamment des sous catégories de composés et (3) montrer qu'il était possible d'enrichir le répertoire métabolique du colza et d'élucider des voies de biosynthèse encore inexplorées, en s'appuyant sur des stratégies innovantes de croisement et d'introgression. Ces travaux ouvrent la voie vers une meilleure compréhension du métabolisme spécialisé chez les plantes de la famille des Brassicaceae. Ils ont également permis de générer des ressources destinées à soutenir les efforts de recherche pour l'étude des interactions entre les plantes du genre Brassica avec différents bioagresseurs et avec le microbiote racinaire.

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Section 3 - Poster 34 – S3-P34 (Flash 11)

Metabolomics approaches of seed-borne fungal endophytes for enhancing tomato seed performance in challenging environments

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Seed germination is drastically decreased by biotic and abiotic stresses. The improvement of seed germination efficiency is, hence, a strategic priority to increase food production. In order to substitute chemical treatments currently used, there is a need to develop environmentally-friendly solutions. A way to enhance seed performance is to exploit the beneficial impact of endophytes on plant fitness. The current view is that such phenomenon relies on chemical mediations using the large variety of molecules produced by endophytes. Tomato is one of the most important crops worldwide undergoing important periodic losses due to abiotic and biotic stresses. Tomato seed germination and seedling establishment are particularly sensitive to salt and water stress as well as fungal pathogens. Recent studies support the potential of endophytes to improve tomato tolerance to salt stress or pathogens. Here, we explore the potential of metabolites from tomato seed-borne fungal endophytes to improve tomato seed germination under stress. We thus isolated and identified 63 cultivable fungal strains from a core collection of tomato seeds. We have initiated bioactivity screening to evaluate their efficacy in combating pathogens and enhancing seed germination under salt/drought stress conditions. Furthermore, the metabolome of the most promising strains is being investigated using LC-MS/MS and molecular networking techniques to dereplicate extracts and identify the most active compounds. The next step will be to isolate the most active compounds in order to generate new high-value molecules capable of providing alternative and environmentally friendly solutions to the currently used pesticides.

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Section 3 - Poster 35 – S3-P35 (Flash 12)

The UNTWIST project : Unraveling Stress Response Mechanisms in *Camelina sativa* for Enhanced Crop Resilience in European Agriculture

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Climate change, particularly variability, challenges European agriculture, causing drought and high-temperature stress, reducing productivity and yield. Camelina sativa, a native European oilseed crop, has regained attention for its adaptability, yield stability, and high performance in variable environments. The UNTWIST project aims to understand the stress response mechanisms of Camelina sativa using a multidisciplinary approach, creating an unprecedented dataset from a core collection of 54 Camelina lines grown under various stresses and locations across Europe.
Employing top-down modeling, we predict phenotypic field traits from metabolic data using machine learning. A predictive model was developed by combining phenotypic field data with targeted and LCMS based untargeted metabolomics data from early-stage leaves of Camelina lines grown under control, water, or thermal stress conditions. This model enables the prediction of Thousand Kernel Weight (TKW) and fatty acid content under various stresses. Current efforts involve comparing these results with those of genomic prediction based on the same data.

The bottom-up approach focuses on the growth and development of Camelina fruit by reconstructing four compartmentalized genome-scale metabolic networks of diverse stress responsive lines. Metabolic data is being utilized to calculate fluxes for each model, constraining networks to provide insights into drought and heat tolerance mechanisms. These networks will be further refined with omics from the focus lines.
The UNTWIST project's comprehensive analysis of Camelina sativa's stress response mechanisms will advance our understanding of crop resilience, ultimately contributing to the development of more sustainable and climate-resilient farming practices.

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Section 4 - Poster 1 – S4-P1

Analyse métabolomique des sols dans la forêt de la Massane

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La Réserve Naturelle Nationale de la forêt de la Massane (Pyrénées-Orientales) abrite une vieille forêt, en libre évolution depuis 150 ans, caractérisée par des peuplements de hêtres et des faciès plus diversifiés. Nous proposons de cartographier la diversité chimique des sols au regard de la diversité biologique locale afin de comprendre l'influence des différences de peuplements forestiers sur les métabolites du sol, et in fine de mettre en lumière les facteurs permettant de rendre compte des éventuelles évolutions des profils chimiques (saison, conditions climatiques, etc.). Onze sites distincts sont étudiés, caractérisés par la présence de hêtres, de mélanges hêtres/chênes, et de peuplements mixtes d'origine sylvopastorale. Les échantillons de sol sans litière (triplicata) sont lyophilisés, tamisés et extraits à l'aide d'un mélange méthanol / isopropanol / eau (3:3:2 v/v/v). Après centrifugation, les surnageant sont prélevés et analysés en UHPLCHRMS/ MS en mode d'ionisation positif. Les données obtenues ont ensuite été explorées à l'aide de l'outil FBMN sur la plateforme GNPS. Les composés clés ont été annotés manuellement, éventuellement avec l'aide du logiciel Sirius. L'analyse du réseau moléculaire révèle que chaque type de peuplement forestier induit un profil chimique distinct, avec des familles moléculaires (céramides, prénols) associées spécifiquement aux hêtres, ainsi que des dérivés de stéroïdes, des triterpénoïdes et des lipopeptides typiques des peuplements hêtres/chênes. Ces résultats montrent la possibilité de détecter la variabilité spatiale du métabolome des échantillons, pour une meilleure compréhension du lien entre la diversité chimique des sols et les caractéristiques écologiques et historiques des sites.

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Section 4 - Poster 2 – S4-P2

Caractérisation métabolique et modélisation des flux de la microalgue verte Coelastrella en réponse au stress induit par l'uranium.

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L'uranium est un métal lourd naturel pouvant s'accumuler avec les éruptions volcaniques, l'exploitation minière, les déchets nucléaires et la fertilisation agricole (Duhan et al., 2023). En excès, il induit des effets néfastes sur les organismes vivants et l'environnement à cause de sa toxicité et ses rayonnements ionisants. Cette pollution risque de s'amplifier par l'augmentation des besoins en minéraux, en électricité (AIE, 2021) et en nourriture d'une population mondiale grandissante. Il est donc primordial de développer des stratégies de dépollution comme des processus de bioremédiation. Dans cette optique, le projet DemoniaCO souhaite comprendre les mécanismes de tolérance et d'accumulation d'une microalgue verte hyper-tolérante de genre Coelastrella en réponse au stress induit par l'uranium (Beaulier, 2023 et 2024). Ainsi, l'algue a été cultivée en milieu synthétique avec ou sans uranium et des échantillons ont été prélevés à différents temps de culture. L'un des objectifs de ce projet est de déterminer les flux métaboliques de l'algue pour identifier les réactions et les voies impliquées dans la tolérance à l'uranium. Pour cela, les concentrations en métabolites intracellulaires et extracellulaires ont été mesurées pour obtenir le profil métabolique de l'algue. Celui-ci sera intégré dans un modèle stoechiométrique de Chlamydomonas reinhardtii, le plus proche parent de Coelastrella référencé dans la littérature, pour calculer les flux métaboliques avec la méthode de modélisation sous-contrainte. A ce stade, nous avons constaté des différences significatives du profil métabolique selon les différentes conditions. Ainsi, des modifications sont attendues dans les flux et les voies métaboliques de Coelastrella en présence d'uranium.

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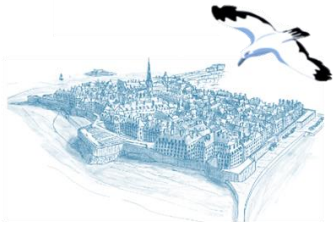

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Section 4 - Poster 3 – S4-P3

Characterization of the metabolomic response of freshwater periphytic biofilm exposed to different concentrations of glyphosate

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Glyphosate is a widely used herbicide detected ubiquitously in European freshwaters. However, there still is a lack of knowledge about its impact on aquatic ecosystems. To fill this gap, periphytic community playing a pivotal role in ecosystem functions, is a relevant model. Also, untargeted meta-metabolomics is an approach through its ability to sensitively characterize the molecular/biochemical community phenotype. In this context, this study aims to characterize the meta-metabolome response of periphytic biofilm exposed during 14 days to different concentrations of glyphosate (0.1-150 µg.L-1). The meta-metabolome was characterized by an untargeted approach on a UPLC-HRMS-TOF system. Acquired data were processed in W4M and further analyzed with chemometric methods. In particular, DROMICS was used to characterize trend (U, Bell, Increase, Decrease) and sensitivity (BMD1sd) of each metabolomic features as well as their aggregated response. Finally, the signals of interest were annotated by combining MS-DIAL and SIRIUS5. Chemometrics revealed that 7276 features reacted to glyphosate. Only 2288 could be annotated. Among them alkaloids (n=662) and amino-acids and peptides (n=952) classes were the more reactive. Surprisingly, low effect of glyphosate was noted on the shikimates while this herbicide targets their biosynthesis. DROMICS showed no discrepancy in the proportion of each trend and the sensitivity to glyphosate between the classes. However, the strengh of the response (i.e. fold change) slightly change between the classes, as terpenoids and amino-acids were th most down-regulated classes. Altogether, our results higlighted a complex metabolic response of periphyton to glyphosate that questions potential impairment of ecological functions of these communities.

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Section 4 - Poster 4 – S4-P4

Développement d'un protocole de co-extractions transcriptomiques et métabolomiques pour la caractérisation des photogranules oxygéniques

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Le projet ANR PANORAMICS a pour but de mieux caractériser les photogranules oxygéniques (agrégats microbiens photosynthétiques compacts et sphériques) dans l'objectif de substituer à terme le procédé à boues activées pour la dépollution des eaux usées. Afin d'accroˆıtre les connaissances sur les photogranules, il est nécessaire de développer de nouvelles méthodologies de couplage des analyses multi-omiques dans des séries longitudinales. Les travaux présentés visent à proposer un protocole de co-extractions réalisées à partir d'un homogénat commun adapté à la fois aux analyses métabolomique et transcriptomique. Des lots de 50 photogranules de diamètre moyen de 800 µm ont été soumis à des cycles de lumière et d'obscurité, en présence ou non de substrats carbonés. En fonction des conditions appliquées, les activités photosynthétiques et/ou hétérotrophes étaient stimulées. Des essais ont été réalisés dans le but d'optimiser la technique d'extraction des photogranules en minimisant le nombre de photogranules à utiliser pour obtenir des spectres RMN et des extraits d'ARN de bonne qualité. Le protocole optimisé a été utilisé pour l'extraction de 200 échantillons et a permis de combiner métabolomique et métatranscriptomique à partir d'un même échantillon de départ d'environ 200 mg. La qualité des spectres RMN obtenus et la qualité des ARNs ainsi que leur concentration démontrent que ce protocole a fonctionné. L'utilisation d'un protocole de co-extraction permet de s'affranchir de quelques biais notamment des variabilités pouvant provenir de l'échantillonnage. Cette recherche a été financée par l'Agence Nationale de la Recherche (ANR-21-CE45-0036- 01), et a bénéficié des équipements de la plateforme Bio2E (doi.org/10.15454/1.557234103446854E12).

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Section 4 - Poster 5 – S4-P5

Développement d'une approche multimodale pour l'étude d'interactions plantes-microorganismes par imagerie par spectrométrie de masse, imagerie Raman et métabolomique non ciblée.

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Il y a 450 millions d'années, la symbiose mycorhizienne arbusculaire (AMS) est survenue entre les plantes et les champignons Gloméromycètes. Cette étape a facilité la colonisation du sol par les premières plantes terrestres et, dès lors, a joué un rôle central dans les écosystèmes naturels et agricoles. L'établissement de l'AMS débute par un dialogue moléculaire entre deux partenaires localisés indépendamment dans le sol. Ce dialogue est suivi par la formation de structures fongiques spécialisées dans les racines : les arbuscules, principaux sites d'échange de nutriments entre les deux organismes. Accéder au dialogue métabolique entre une plante et sa mycorhize représente, à ce jour, un enjeu important pour mieux décrire la cascade de voies symbiotiques communes impliquées. La multimodalité offerte par la métabolomique globale par LC-HRMS, l'imagerie par spectrométrie de masse MALDI-ToF et la microscopie Raman stimulée, permet d'y répondre en localisant des métabolites sur des coupes de la matrice étudiée tout en s'affranchissant de colorations ou de marquages possiblement invasifs. Ces approches aux vastes spectres de résolution spatiale et aux capacit és spécifiques, sont complémentaires pour identifier, cartographier finement et quantifier les métabolites d'intérêts au sein d'une AMS selon l'état de mycorhization. L'originalité du couplage à la microscopie Raman permet de compléter et d'améliorer le champ de vision du paysage métabolique de ce mutualisme, à l'échelle de la cellule mycorhizée. Les premiers résultats de cette étude originale sont présentés chez *Marchantia paleacea* mycorhizée par *Rhizophagus irregularis*.

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Section 4 - Poster 6 – S4-P6

Elaboration d'un workflow pour l'identification des voies métaboliques des plantes impactées par l'utilisation de biostimulants

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La transition agricole vers des pratiques plus responsables et basées sur la gestion intégrée des cultures met en avant les biostimulants comme des solutions prometteuses. Ces produits, issus de sources naturelles, agissent en stimulant les processus de nutrition des plantes pour atténuer le stress abiotique, indépendamment de leur contenu nutritif. Cependant, les biostimulants se heurtent souvent à des niveaux d'efficacité partiels, principalement en raison d'une compréhension limitée de leur mode d'action. L'utilisation de la métabolomique apparaˆıt comme un outil puissant pour explorer les interactions complexes entre les biostimulants et les plantes. L'objectif de cette étude est donc d'analyser l'impact d'un biostimulant combinant le champignon *Trichoderma harzianum* et la bactérie *Bacillus methylotrophicus* sur les voies métaboliques des plants de tomates cultivés hors sol. Les méthodes utilisées comprennent des analyses métabolomiques non ciblées par UPLC-HRMS et RMN et dans un second temps des comparaisons avec les profils métaboliques de plants traités avec des biostimulants dont le mode d'action est connu. Ce travail s'intègre au projet Microsos, qui explore l'utilisation de microorganismes pour aider les plantes à s'adapter au changement climatique. Ce projet vise à utiliser une combinaison des données métabolomiques et transcriptomiques pour identifier les voies métaboliques affectées, contribuant ainsi à une meilleure compréhension du mode d'action des biostimulants.

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Section 4 - Poster 7 – S4-P7

Etude de la dissipation et de l'impact d'un biofongicide d'origine végétale sur le sol par une approche métabolomique non ciblée

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Dans le cadre du développement d'une agriculture durable, les produits de biocontrôle (PBs) représentent une alternative pour l'environnement. Cependant, leur devenir et leur impact environnemental doivent être évalués dans un cadre réglementaire. Le suivi des PBs est un défi majeur en raison du mélange complexe qui compose l'extrait végétal auquel est ajouté des formulants. Ainsi, une approche innovante basée sur la métabolomique, l'Environmental Metabolic Footprinting (EMF), été développée pour évaluer le temps de résilience qui correspond au temps nécessaire à la dissipation du biofongicide, de ses produits de dégradation et de leurs effets sur la matrice. Un nouveau paramètre a été défini, le temps de dissipation, qui évalue uniquement la dissipation du biofongicide appliqué sur le sol. Ces deux paramètres sont obtenus grâce au profilage des méta-métabolomes du sol d'échantillons traités et contrôles au cours d'une cinétique par UHPLC-HRMS. D'abord, quatre solvants d'extraction ont été testés : un mélange Acétonitrile/Méthanol/H2O acidifié ou non et un mélange Isopropanol/Acétonitrile/H2O acidifié ou non. Suite au traitement des données (ACP, Heatmap, CV), le mélange Isopropanol/Acétonitrile/H2O non acidifié a été choisi. Une cinétique est en cours pour estimer ces 2 paramètres et ils permettront de distinguer la dissipation et l'impact du biofongicide sur le sol.

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Section 4 - Poster 8 – S4-P8

How molecular networks can add value to the whole plant

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Each part of the plant has an important function in ecosystem. Roots provide nutrition for the plant by drawing water and minerals necessary for its development. Stems enable support for the plant, the transport of saps allowing its nutrition and growth. Leaves are an essential component for photosynthesis, breathing and transpiration. Flowers are necessary for reproduction and seeds for the birth of a new plant. Each organ, undergoing different stress depending on its role will produce different secondary metabolites. These metabolites could be interesting in various fields through their diverse activities, however, their accessibility may vary according to certain constraints. Here, an ultrasonic extraction step using a hydroalcoholic solvent was used to obtain a molecular richness with a wide range of polarity on 3 different organs of a plant from Theaceae, roots, leaves and flowers. All the extracts have been analyzed by UHPLC-HRMS2-QTOF in negative or positive mode. A pre-processing with MzMine was carried out on the data of each sample. GNPS was used to perform molecular networking and to aid in the extract comparison and identification of compounds. This tools have made it possible to visualize different clusters specific of the plant of interest such as polyphenols and saponins, which were also highlighted in all organs. Interestingly a specific condensed tannins family (catechin monomers) in high concentration was identified in roots. Additionally, biological activity tests were carried out on extracts, such as collagenase, superoxide dismutase or cyclooxygenase-2. These results were fed into the network to identify possible structure-activity relationships.

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Section 4 - Poster 9 – S4-P9

Influence de l'altitude et du type de sol sur la composition en métabolites des feuilles de *Laurus nobilis* du Liban

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Le laurier (*Laurus nobilis*), arbre répandu dans tout le bassin méditerranéen (Mansou et al. 2018) a été l'objet d'études approfondies sur ses composants antioxydants (Özcan et Chalchat 2005). Dans cette étude, une analyse de la teneur en métabolites du laurier du Liban a été menée dans différentes conditions pédoclimatiques. La zone d'étude, située dans le sud du Liban, a été choisie en raison de l'abondance de cet arbre dans la région, ainsi que de la diversité des altitudes et des types de sols. Les altitudes varient de 0 à 1000 mètres et deux types de sols dominent : le sol rouge (limoneux riche en éléments nutritifs) et le sol blanc (sableux calcaire). Deux types d'extraits de feuilles ont été étudiés : les huiles essentielles et les extraits méthanoliques. Les techniques d'analyse utilisées sont : GC-MS, RMN et LCMS. Les résultats ont montré que le type de sol et l'altitude contribuent à la variation observée mais l'altitude était le principal facteur influençant le contenu en métabolites secondaires des feuilles de laurier, surtout les composés volatils. Les taux de sesquiterpènes et de monoterpènes augmentent avec l'altitude pour les échantillons sur sol rouge, tandis qu'ils diminuent avec l'altitude pour les échantillons sur sol blanc. Pour les autres métabolites secondaires, la plupart sont plus abondants à des altitudes plus élevées. Les métabolites primaires ont été faiblement influencés. L'interaction entre le type de sol et l'altitude a également montré une influence significative sur le contenu en métabolites secondaires du laurier.

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Section 4 - Poster 10 – S4-P10

La métabolomique au secours de la détermination pour deux genres alpins botaniquement complexes

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Le projet de thèse vise à appliquer des approches combinées de métabolomique, taxonomie et écologie chimique à une sélection d'espèces végétales alpines appartenant aux genres Rubus et Alchemilla et adaptées à des situations environnementales contrastées. La particularité conférée par l'apomixie et le polymorphisme inter-espèce de ces deux modèles, en ont fait un tel défi pour les botanistes que leurs études ont été pendant longtemps délaissées. La conception d'une nouvelle méthode de détermination et de classification est donc un réel enjeu. D'autre part, l'étude vise à étudier le profil métabolomique des deux modèles en fonction de l'environnement alpin, et enfin à terme, d'étudier plus précisément la composition phytochimique et l'activité biologique des extraits en vue d'une valorisation en cosmétique. Un échantillonnage à grande échelle sur 21 points de récoltes le long d'un gradient altitudinal d'étendant de 856 à 1856m a été réalisé sur des plantes sauvages des Alpes du Nord (Massif de la Tournette, 74). Au total, 107 Rubus et 354 Alchemilla ont été prélevés, et les identifications morphologiques associées ont révélé la présence de 5 et 15 espèces différentes chez Rubus et Alchemilla respectivement. Les extraits éthanoliques des feuilles ont été analysés en LC-MS. Les premières analyses multivariées (ACP, PLS-DA) ont déjà montré un " effet espèce " net qui tend à séparer *R. idaeus* (framboisier) des autres espèces de ronces. La confrontation des résultats des analyses métabolomiques avec les identifications morphologiques en cours sera complétée par la définition des paramètres stationnels les plus pertinents pour les différentes espèces.

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Section 4 - Poster 11 – S4-P11

Mass Spectrometry-Based Metabolomic Assessment of Environmental Platinoids and Heavy Metals-Induced Disruptions in Forest Mosses

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The transport sector is among the main contributors to atmospheric contamination. Since 1990s, many countries have made catalytic converters compulsory for petrol or diesel engines to limit emissions of pollutants (CO, NOx, unburnt hydrocarbons) (1, 2). However, this technology generates emergent pollution problems linked to platinum group elements (PGE) at the origin of toxicological problems. The objective of the study is to get significant links between metabolomic fingerprints obtained by FIA-ESI-MS (-MS/MS) using bb-CID activation, in positive and negative ionization modes from direct injection of extracts prepared from dry powder of terrestrial mosses (two species collected on 405 geographical sites) and PGEs or heavy metals data established by ICP-MS. Few Principal Components (5 and 4 PCs in positive and negative modes, respectively) were sufficient to concentrate more than 90% of the total metabolomic information. In positive mode, a clear "Species" effect is detected from PC2 with a partial differentiation of Pseudoscleropodium purum extracts from Hypnum cupressiforme ones. Different chemometric approaches including Common Component Analysis are used to get significant canonical links between some candidate biomarkers selected from MS or MS/MS data obtained in both ionization modes and considering the complementary exposure metadata in heavy metals or PGEs.

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Section 4 - Poster 12 – S4-P12

Metabolite Profiling and Molecular Network Shows Kinkeloids as promoting of collagen synthesis from *Combretum micranthum*

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This presentation will investigate the molecular composition and a new cosmetically relevant biological activity of Combretum micranthum extract and enriched fractions to begin to establish a structure-activity relationship. Combretum micranthum, a plant native to Africa, has a well-documented traditional use in the treatment of various ailments such as fever, diabetes and malaria (Yoro, Tine et al., 2024). Its pharmaceutical benefits include nephroprotective, anti-inflammatory, antioxidant and antimicrobial properties have been demonstrated (Olajide, Olumayokun et al., 2003). In addition, its potential for cosmetic applications is being explored due to its depigmenting, anti-inflammatory and UV damage repairing properties. First, an extract of Combretum micranthum was prepared and selected for its overall biological response, then fractionated to obtain simplified molecular fractions. One fraction was particularly enriched in kinkeloids, a family of compounds specific to this species. All fractions and the crude extract were then tested on biological targets to evaluate and compare their cosmetic activities. Molecular networks were constructed from the UHPLC-MS/HRMS data to better characterize the extract and fractions and to highlight structure-activity relationships. This study highlights the metabolic profiling of a butylene glycol extract of Combretum micranthum, showing its main chemical families and revealing that the kinkeloids identified by HRMS and NMR promote an increase in collagen I synthesis, an interesting cosmetic activity not previously described for these compounds or for any Combretum micranthum extract.

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Section 4 - Poster 13 – S4-P13

Metabolomic insight in species sensitivity differences within periphytic communities

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Facing global change, periphyton is a relevant model to evaluate the impairment of ecosystem functions in aquatic systems. However, there is still paucity knowledge about the differences in species sensitivity to chemical stress in these communities, which is critical to their structure and functioning. In this context, this study aims to fill this gap at the biochemical level through the comparison of the molecular phenotype of a cyanobacteria, a green algea, a diatom and their co-culture facing a chemical stress. To this end, the metabolome of the three species and their co-culture were characterized using UPLC-HRMS-based untargeted metabolomics, prior and following a seven days exposure to atrazine, S-metolachlor and their mixture at three concentrations (10, 100 and 1000 µg/L). The comparison of the metabolic fingerprints highlighted that prior to exposure, the metabolisms of the diatom and green algae were more similar than that of the cyanobacteria, while the co-culture was closer to the diatom metabolism. Both herbicides caused a shift in the metabolic fingerprint of the three species and the consortia, but the modulated metabolic features and/or the intensity of the modulations differed between the species. Moreover, atrazine and S-metolachlor as well as their mixture modulated the metabolome differently for each species, suggesting a potential discrepancy in the toxicity pathways. Further investigations are ongoing to annotate the metabolites and pathways involved in the discrepancies of the molecular phenotypes before and after the exposure. This study will provide new knowledge to better understand species sensitivity differences to chemical stress in periphyton.

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Section 4 - Poster 14 – S4-P14

Metabolomics and lipidomics investigation of organ-specific perturbations induced by Pravastatine and Cadmium exposure on the freshwater sentinel crustaceans Gammarus fossarum by high resolution mass spectrometry.

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Many chemicals found in the environment have been proved to cause endocrine perturbation and induce metabolites and lipids homeostasis disruption. Sentinel species such as the crustacean Gammarus fossarum play an indispensable role in monitoring environmental pollution in freshwater ecosystems. Here, we investigated chronic effects on the metabolic and lipidomic assets in ovaries and hepatopancreas of female G. fossarum organisms exposed to pravastatin or cadmium. Reproductive toxicity assays were performed according to a standardized procedure and a multi-omics extraction protocol used for the concomitant extraction of metabolites and lipids in pooled organs. Samples were submitted to data dependent acquisition analysis (DDA-MS) either under collision induced dissociation (CID) and electron induced dissociation (EAD) on a high-resolution ZenoTOF 7600 instrument (Sciex). Multivariate data analysis including Principal Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA) enabled to highlight organ-specific metabolomic and lipidomic changes and identify statistically significant features responsible for group segregation. Molecular networking constructed either under CID or EAD dissociation conditions enabled to reach confidents annotations for the proposed metabolomics and lipidomics signatures connected to endocrine disruption.

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Section 4 - Poster 15 – S4-P15

Prediction of oak species by UHPLC/HRMS and chemometrics tools

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The oak forests spread through the Centre-Val de Loire region and are worldwide known for their high quality to produce barrels. Two species are dominant : Pedunculate and Sessile oak. The first one, rather rich in tannins and low in aromatic compounds is mainly used in brandies aging. The second one is often richer in aromatic compounds and lower in tannins, which enables wine aging. If the morphological characteristics of trees (acorns, leaves) make it possible to distinguish pure species in forest, it is not true for hybrids or oaks logs used by coopers. Until now, DNA analysis has been the only reliable method for species differentiation using fresh tissue. However, recent studies of oak extracts using different analytical methods such as UHPLC/HRMS/MS and NMR have highlighted specific markers of oak species[1-2]. This is why the objective of this study was to set up a prediction model for the two species using UHPLC/HRMS based on our previous non-targeted metabolomic method[1]. Thus data from 80 samples of species genetically identified, injected at 3 different times were used to build and evaluate 5 different prediction models (decision trees, random forest, naive Bayes, support-vector machine, neural networks). After studding the impact of the origin of the sample (forest, stavework) and the number of variables, the developed models allow the species prediction of the forest samples. Three of them are more efficient, particularly the random forest model which has a better performance rate of 99.15%, with less than 2% error per species.

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[2] DOI:10.1021/acs.jafc.5b05056

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Section 4 - Poster 16 – S4-P16

Toxicokinetic study of inhaled Cis- and Trans-permethrin in rats using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

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Permethrin is one of the most widely used pyrethroids (pesticide). It is present in commercial formulations as a mixture of *cis*- and *trans*- isoforms. Data on the toxicokinetics and metabolism of inhaled permethrin are scarce. We therefore conducted a toxicokinetics study in the rat and developed a dedicated absolute LC-MS/MS quantification method to explore the absorption, distribution, metabolism and elimination parameters of *cis*- and *trans*-permethrin and its three derived metabolites. Several key tissues were thus analyzed : plasma, blood, adipose tissue, brain, liver and lung. For the in vivo experiment, rats (n = 3) were exposed by inhalation to nebulized *cis*- and *trans*-permethrin at 18.4 mg/kg (subtoxic dose level) for 4 hours. Quantifications were performed after 2 and 4 hours following initiation of inhalation and at 0.5, 1 and 3 hours after completion of the 4h of inhalation. An optimized sample preparation and an analytical method using UPLC-ESI-MS/MS were developed for the separation and absolute quantification of *cis*- and *trans*-permethrin and their respective metabolites (*cis*- and *trans*-DCCA and 3-phenoxybenzoic acid (3-PBA). *Cis*- and *trans*-permethrin, 3-PBA and *trans*-DCCA were detected and quantified in all the samples and tissues. However, *Cis*-DCCA was only quantified in adipose tissue and liver. These data allowed the development of a physiologically based kinetic (PBK) model to assess inhaled permethrin exposure in rats. The model accurately predicted concentrations, suggesting its potential for other pyrethroids and metabolites. It can also be adapted to human populations, reducing the need for animal testing.

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Section 4 - Poster 17 – S4-P17 (Flash 14)

Insight on the relationship between the meta-metabolome, photosynthesis sensitivities and their natural fluctuation in freshwater microbial communities exposed to a model herbicide

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Facing aquatic chemical pollution, the study of microbial communities improves the ecological dimension of ecotoxicology. Despite growing knowledge on the effects of contaminants on biofilms, there is still a paucity of information about the natural fluctuation of the sensitivity of these communities to chemical stress. This is particularly the case of periphyton's photosynthesis sensitivity, for which the molecular/biochemical processes underlying its response remains partly described. To tackle this issue, untargeted meta-metabolomics can provide a comprehensive picture of the molecular/biochemical response prior physiological/functional impairment. In this context, the present study aims to characterize the monthly changes of periphyton's sensitivity over a year at the physiological and molecular levels by measuring the photosynthetic yield and the meta-metabolome. Periphyton were monthly-colonized in-situ and acutely exposed in controlled conditions to terbuthylazine. Sensitivity fluctuation was assessed by determination of benchmark-dose-1SD for the photosynthetic yield and the whole meta-metabolome. The results showed the strong sensitivity shift of the metametabolome vs the lesser one of the photosynthetic yield. Moreover, this study confirmed the higher sensitivity of the meta-metabolome, as most of signals responded prior to the photosynthesis. Further annotation of metabolites underlined the response of various classes including alkaloids and fatty acids. Among them, oxylipins were identified as part of the response to the oxidative stress enhanced by terbuthylazine exposure. Overall, this study highlighted the need to take into account of the natural fluctuation of microbial sensitivity in order to get better mechanistic understanding of the periphyton's meta-metabolome response to chemical stress.

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Section 5 - Poster 1 – S5-P1

Analyse d'échantillons sanguins : Pourquoi choisir entre métabolomique et lipidomique ? La quête d'une extraction tout-en-un, rapide et monophasique

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L'essor de la métabolomique entraine une demande croissante de caractérisation de métabolites à grande échelle dans diverses matrices. MetaboHUB, en tant qu'infrastructure nationale, s'efforce de répondre à ces besoins en fournissant des outils et méthodes analytiques de pointe.
Pour une analyse globale d'échantillons sanguins, deux protocoles distincts sont souvent utilisés : l'un dédié aux métabolites polaires, l'autre aux lipides apolaires. Pour l'étude de grandes cohortes, un protocole tout-en-un de préparation des échantillons capable d'extraire à la fois les métabolites et les lipides d'intérêt semble d'intérêt majeur.
Le choix du solvant d'extraction est un élément clé de l'optimisation des protocoles et de leur automatisation : le méthanol est couramment utilisé en métabolomique, tandis que l'isopropanol est préféré en lipidomique. Nous avons comparé l'extraction de plasma et du sérum humain par ces deux solvants purs ou en mélanges, respectivement à 3:1 et 2:2 (isopropanol : méthanol, v:v), , utilisés à un ratio commun de 1:4 (matrice:solvant, v:v). Une approche métabolomique non-ciblée a été réalisée : acquisition des données par LC-QToF, puis retraitement sur W4M. Les ions extraits ont été filtrés selon plusieurs critères qualités. L'efficacité d'extraction des solvants a été évaluée en termes de profils globaux et nombres d'annotations (banque interne, HMDB, Lipidmaps).
Les premiers résultats métabolomiques montrent un effet de la méthode de préparation sur la répétabilité des analyses et sur le nombre de métabolites et lipides extraits. Le mélange 2:2 semblerait offrir le meilleur compromis. Ce choix sera complété par une analyse avec un protocole MS optimisé pour la lipidomique.

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Section 5 - Poster 2 – S5-P2

Automation of metabolite extraction from human plasma for high-throughput metabolomics

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Metabolomics refers to the large-scale detection, quantification, and analysis of the whole set of metabolites present in biological media. Metabolomics has already demonstrated its relevance for clinical studies, with an ever increasing size of clinical cohorts frequently composed of thousands of individuals. Sample preparation is often the most limiting step for the high-throughput analysis of such large-scale cohorts.
In that context, we have transferred our well-established manual metabolite extraction protocol onto an automated liquid handling platform that processes samples in 96-well plates and performs protein precipitation, sample centrifugation and supernatant withdrawal. Here, we present the different steps and difficulties encountered during this protocol implementation.

We first optimized methanol-assisted extraction yield and accuracy through the monitoring of 295 human plasma metabolites by LC-HRMS. Then, we comparatively evaluated metabolomics fingerprints from 37 plasma samples prepared manually and with the automated protocol for validation purposes, evaluating presence, peak quality, and intensity of the targeted metabolites. Automated extraction yielded equivalent results to manual. The automated protocol has been used on a cohort of 300 samples, and will be applied to larger cohorts of 1,000 and 6,000 samples.
On-going efforts are made towards implementing other extraction protocols for the metabolomics analysis of various matrices (stools, urines and tissues) and to total lipid extraction to achieve high-throughput metabolomics and lipidomics in the field of human health and large cohort analysis.

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Section 5 - Poster 3 – S5-P3

Caractérisation par LC-HR-MS/MS de polluants émergents issus de la dégradation de composés organiques en milieux aqueux : Apport de la mobilité ionique

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La pollution des eaux superficielles et souterraines par des composés organiques est une préoccupation pour la préservation de l'environnement. Les pesticides et leurs composés de dégradation suscitent une attention croissante du fait de leurs effets néfastes sur la santé humaine et sur les écosystèmes (1). De nombreuses études analytiques et éco-toxicologiques examinent leur impact sur les populations aquatiques et terrestres mais elles portent le plus souvent sur les pesticides. Ces molécules peuvent évoluer dans l'environnement, réagir en milieu aqueux sous l'action d'agents biologiques, ou chimiques. Les produits de transformation tendent à remplacer le pesticide parent dans l'environnement. Parmi les produits de dégradation caractérisés, des isomères (2-3) peuvent être formés qui ne sont pas toujours différenciables par HR-MS/MS. Comme ces composés peuvent avoir des propriétés différentes, nous tentons d'évaluer l'apport de la mobilité ionique dans la différenciation de ces structures. La mesure de la mobilité ionique réduite des ions moléculaires, permet d'accéder à une nouvelle donnée la section efficace de collision (CCS Cross section collision) qui apporte un degré de caract érisation supplémentaire. Les CCS seront aussi évaluées à l'aide de calculs de chimie quantique pour quelques molécules de référence et comparées aux valeurs de CCS obtenues par les softwares en accès libre.

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Section 5 - Poster 4 – S5-P4

Carbon-13-Isotopomics and Metabolomics of Fatty Acids from Triacylglycerols

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The 13C isotopic analysis of free fatty acids (FFAs) derived from triacylglycerols (TAGs) is crucial for understanding lipid metabolism and examining fraud actions, especially in food products. However, the analysis of fatty acid methyl esters (FAMEs) using conventional analytical methods, notably gas chromatography coupled with isotope ratio mass spectrometry (GC-C-IRMS), face inherent challenges in accurately characterizing short and medium chain FAs (C-4 to C-10) due to their volatility generating losses during the derivatization and thus inducing isotope fractionation. These limitations require innovative approaches to overcome analytical constraints and provide insights into FA metabolism and dietary sources. A new protocol was designed1by analyzing the individual FAs instead of FAMEs as usually performed in the literature2. For short chain FAs, the addition of the methyl group requires a correction of the δ13C, increasing an uncertainty on the measured values. Therefore, the advantage of this method is that it allows for the observation of volatile short chain FA without making a correction for the contribution of the methyl. The same experiment also provides the FA profile, the relative percentage of each FA in the TAG hydrolysate in the studied matrix. The method exhibited high precision, as evidenced by the repeatability and within-lab reproducibility of results when tested on TAGs from both animal and vegetal origins. Commercial samples of cow milk, butter, cheese, and coconut oil were used for analysis and comparison. Overall, we hope that this innovative methodology will offer a powerful tool for elucidating the origins of specific FAs within biological lipids.

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Section 5 - Poster 5 – S5-P5

Challenge autour d'un développement d'une méthode de quantification large échelle

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L'enjeu aujourd'hui en métabolomique non ciblée, dans les domaines de la santé et nutrition, est de découvrir de potentiels biomarqueurs de pathologie ou état nutritionnel. Afin de les valider et comparer les études menées entre elles, une grande importance est donnée à la quantification absolue de ces métabolites. Une méthode de quantification pour chaque biomarqueur peut être limitée par la disponibilité, le prix ou l'utilisation à grande échelle des standards commerciaux. Une combinaison de biomarqueurs peut donner une plus grande robustesse et spécificité qu'un biomarqueur unique, aussi le challenge actuel est de développer une méthode de quantification large échelle de plusieurs dizaines/centaines de composés d'intérêt.

La méthode a été développée sur biofluides humains contenant des centaines de métabolites aux propriétés physico chimiques et concentrations variées. L'enjeu est de pouvoir proposer la plus grande couverture analytique avec cette nouvelle méthode. Les données ont été acquises sur LC-QTRAP®, puis retraitées à partir des logiciels constructeur et Skyline en vue de partager les méthodes de retraitement des données à la communauté.

Les premiers résultats ont été obtenus sur l'analyse des standards en solvant après optimisation en infusion puis en couplage LCMS. L'étape suivante a été de travailler en matrice pour se rapprocher des conditions réelles et évaluer les effets observés pour les corriger. L'objectif de cette présentation est de mettre en évidence tous les points de vigilance qui ont été soulevés afin de proposer cette nouvelle méthode de quantification large échelle.

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Section 5 - Poster 6 – S5-P6

Classification of ricin samples according to purification levels using untargeted 1D-1H NMR and supervised multivariate analysis

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Ricin is a highly toxic protein listed as a scheduled 1 controlled substance under the Chemical Weapons Convention, present at high concentrations in the seeds of the plant Ricinus Communis. It is widespread both as an ornamental plant and to produce castor oil. Moreover, ricin extraction and purification are relatively simple. Therefore, it has been frequently involved in intoxication attempts over the last fifty years.

The elucidation of the provenance of a ricin sample is a complex task in which chemical forensic approaches can bring relevant information. One of the questions that can be asked to the analytical chemist is to evaluate to what extent an unknown ricin sample has been purified.

In this preliminary study, crude and purified samples from nine cultivars were analyzed with 1D-1H NMR technique. Two sample preparation methods were used for both approach optimization and ruggedness assessment. Data were then bucketed using NMRProcFlow. OPLS-DA was used to build a supervised classification model and external test samples were projected into the model for validation. Samples were well classified according to purification level, even when small experimental variations such as primary seed extraction method and sample treatment before analysis were introduced in the data. Discriminating variables (mainly carbohydrates) were found to be relevant regarding the chemical changes expected during sample purification.

Future work will involve the combination of multiple analytical techniques (such as NMR and LC-HRMS) to evaluate the possibility to extract additional forensic information from samples (i.e. cultivar information, preparation method and storage conditions among others).

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Section 5 - Poster 7 – S5-P7

Comparative Evaluation of Metabolomic Information, Lipidomic Information and Quantitative Coverage of Dried Blood Spot

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DBS (Dried Blood Spot) are used for the screening of neonatal diseases and monitoring adults suffering from certain diseases in clinical context. DBS is a self-sampling device which is less invasive and requires less sample than a blood test. They can be sent by mail to the hospital allowing everyone to get access easily to biological analysis even in remote area or for elderly patients with mobility problems. Their use in new contexts has been widespread: carrying out anti-doping tests, the research of biomarkers of galactosemia or detection of cancer. In this work, we propose to compare quantitative data and exploratory metabolomic data between DSB and serum. For quantitative data, we quantified 6 short-chain fatty acids (SCFA), 20 bile acids, 20 tryptophan intermediates and 8 organic acids from TCA cycle. Two trends emerge : the first one is that the majority of the serum information is found in DBS. The second one is that DBS brings complementary information not found in serum. Indeed, an overlay of the metabolic cards of serum and DBS highlights a wider metabolic coverage for DBS. These results make it possible to envisage the use of DBS in both quantitative and exploratory metabolomic analyses. However, building up a cohort can last several months or years, so it will be necessary to clearly define the impact of storage conditions (temperature, hygrometry and light exposure) as well as its lasting.

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Section 5 - Poster 8 – S5-P8

Comparison of different pooled QC samples to correct interbatch-effect in untargeted UHPLC-HRMS analysis on two different MS platforms

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Quality control (QC) samples are commonly used in metabolomics approaches for three main reasons (Maertens et al. 2023, Analytical Chemistry) : (i) the initial conditioning of the column ; (ii) the evaluation of measurement precision ; and (iii) the correction of analytical drift especially between batches. In practice there are several ways to prepare and conserve QC samples. The most common in untargeted metabolomics is to pool samples after or before extraction, in order to obtain pooled QC samples accounting respectively for analytical variance or for both analytical and sample preparation variances (Broeckling et al. 2023, Metabolites). In this study, focusing on untargeted analysis of tea leaves, we compared three ways of preparing pooled QC samples and their efficiency to perform interbatch correction on datasets obtained using two mass spectrometry (MS) platforms (Orbitrap and time of flight (Tof)). Batch correction usually includes two steps : analytical drift correction and quality metrics selection (< 30% of variation in the QC samples). Here we investigated each step and its effect on the structure of the datasets. Interestingly, datasets were impacted differently depending on the platform used for acquisition (datasets from the Orbitrap contained more features with low intensity than datasets from the Tof). Generally, our results show that data acquired on the Tof platform were more stable and less prone to an interbatch-effect as compared to the Orbitrap platform.

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Section 5 - Poster 9 – S5-P9

DESI-TQ-MS imaging for ex vivo brain biodistribution evaluation of radiotracer : example of LBT-999 a ligand of the dopamine transporter

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Rationale : Radiolabeling is a challenging step of developing radiotracers, especially for the brain. Mass spectrometry (MS) is an alternative to investigate the ex-vivo characteristics of unlabeled candidates, but most of MS studies are confounded by the pharmacological doses of unlabeled tracer injected, and the dissection of regions to study the tracer biodistribution.

Methods : We used triple quadrupole analyzer (TQ LC-MS/MS) implemented with desorption electrospray ionization (DESI) to quantify an unlabeled validated radiotracer targeting the dopamine transporter (LBT-999) on dissected regions, and to study its biodistribution on brain sections.

Results : TQ LC-MS/MS quantified injected subtracer doses of LBT-999 in dissected striata, and DESI implemented on the TQ analyzer provided images of LBT-999 biodistribution on sagittal sections in agreement to positron emission tomography, with the additional ability to simultaneously image other metabolites such as dopamine.

Conclusion : This clearly shows the advantage of using LC-TQ MS/MS and DESI imaging to accelerate the development of radiotracers.

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Section 5 - Poster 10 – S5-P10

Development and validation of 1H-NRM metabolomics for wine authenticity: towards official accreditation

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The assessment of wine authenticity relies heavily on understanding its chemical composition, which serves as a distinctive fingerprint influenced by various factors such as grape variety, terroir, and winemaking processes (1). 1H-NMR spectroscopy metabolomics is a powerful tool in characterizing these profiles as non-destructive technique with rapid analysis over minimal sample preparation. If previous studies have demonstrated that 1H-NMR metabolomics can enable the quantification of a few compounds (2,3), we aim to explore a broader range of compounds for targeted analysis of wines. Our study addresses methodological and metrological challenges in sample preparation and spectrum analysis. We refined 1H-NRM metabolomics methodology towards automation. We optimized sample preparation parameters (buffer, quantitation standard, wine and D2O ratio) and discussed challenges in signal treatment using generic spectrum quantification tools for targeted analysis with the aim to finally propose an accredited method for official analyzes.
Successful adjustments in sample preparation were achieved, enabling potential automation on different NMR spectrometers. We emphasized the need for assessing correction factors for quantifying numerous compounds and developing specific tools for wine matrix analysis. This study is a significant step towards establishing standardized methods for accrediting wine authenticity through 1H-NRM metabolomics.

1. Le Mao et al., 2023 : 1H-NMR metabolomics for wine screening and analysis
2. OIV, 2020 : Resolution Oeno 618/2020
3. Godelmann et al., 2016 : Quantitation of compounds in wine using 1H NMR spectroscopy: Description of the method and collaborative study

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Section 5 - Poster 11 – S5-P11

Development of a high-throughput and high-metabolome coverage UHPLC-HRMS method for the accurate profiling of large clinical cohorts (> 1000 samples)

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Untargeted metabolomics offers biomedical researchers a powerful means of assessing and comparing human phenotypes via the comparative measurement of the metabolome from different biological samples. Current non-targeted metabolomics methods based on UHPLC-HRMS are often time-consuming and may lack of sufficient robustness to enable successful large-scale cohort analysis (> 1000 samples). In that context, and to analyze a cohort of 6000 human plasma samples (PEPR ProPsy project), we are developing an untargeted metabolomics method to reduce analytical times.

Our routinely used workflow involves two injections per sample using two complementary methods UHPLC-HRMS platforms, both involving a Q-Orbitrap mass spectrometer : C18 combined with detection in the positive ionization mode over 30 minutes and ZIC-pHILIC with detection in the negative mode over 42 minutes. This proves impractical for a 6000- sample cohort, compromising the robustness and reproducibility of the analyses. The BEH amide column was selected based on an extensive literature search. Mobile phase composition, gradient conditions, and source parameters were thoroughly optimized. Currently, the method is undergoing validation, and robustness tests conducted on 300 plasma injections showing excellent coefficients of variation (0.7% for retention times and 12% for peak areas). By performing 2 injections on the same column, in both positive and negative ionization modes, this method achieves a detection coverage of 90% of our library of 1200-compounds in 38 minutes per sample. Moreover, we will use a dual HPLC system allowing parallel sample analysis, thereby theoretically reducing the time to ˜24 minutes per sample, i.e. a third of the time currently required.

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Section 5 - Poster 12 – S5-P12

Development of Headspace GC-MS Method for VOC Analysis in Biological Material

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Platform for Translationnal Oncometabolomics Headspace coupled with gas chromatographymass spectrometry (GC-MS) offers an attractive approach for comprehensive metabolic profiling of diverse volatile organic compounds (VOCs) from complex matrices with minimal sample preparation. Certain headspace techniques also provide solutions for concentrating the volatile phase, notably using SPME (solid-phase microextraction) fibers. In the present study our objective was to compare standard (static) headspace with SPME headspace analysis of solid and fluid biological samples. We further show an untargeted approach that allows for the profiling of various biological conditions in a hypothesis-independent manner. As a proof of concept, we compared fecal samples from immune-depleted mice transplanted with subcutaneous tumors and treated with or without a KRAS inhibitor, using both static and SPME headspace methods. In conclusion, headspace proved as a reliable and informative technique for analysis of biological samples. Future studies should aim to couple this sampling methodology with targeted MS analysis, a combination that could further increase the sensitivity and enable more robust quantification.

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Section 5 - Poster 13 – S5-P13

Fast and Quantitative 2D NMR for metabolomic applications within MetaboHUB

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Conventional 1D 1H NMR is hampered by overlapping signals which are typically encountered in biological samples. Over the years, it has been shown that advanced fast and quantitative 2D NMR methods can overcome this issue while staying compatible with highthroughput and quantitative needs of metabolomics (1, 2, 3). However, these methods are yet to be widely used, thus highlighting the need to build a bridge between NMR developments and metabolomics applications. Here, we will show the work of optimization and harmonization that was performed in order to extend the field of applications of fast and quantitative 2D NMR within MetaboHUB. This infrastructure offers a unique collaborative framework throughout France, involving 7 NMR metabolomic platforms scattered across 5 cities : Bordeaux, Clermont-Ferrand, Nantes, Toulouse and Tours (4).

Fast 2D NMR was successfully implemented within the involved NMR platforms and applied to various biological matrices, such as liver extract, wine or serum. Such work paves the way toward new biological discoveries in the respective fields of all the involved platforms as well as harmonized uses within MetaboHUB, thus building shared foundation for interlaboratory studies.

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Section 5 - Poster 14 – S5-P14

Impact du mode de prélèvement de la sueur sur le profil métabolique

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L'un des atouts majeurs de la métabolomique est le fait de pouvoir étudier les profils métaboliques à partir de différents tissus et biofluides. Parmi ces derniers, le plasma et le sérum font partie des matrices les plus communément analysées. Cependant, leur recueil est invasif, contrairement à la sueur qui, ces dernières années, a été utilisée dans le cadre d'études sur la fibrose ou certaines dermatites par exemple. Toutefois, le recueil de la sueur n'est pas aisé et le mode de prélèvement diffère selon les études. Ainsi, dans le cadre d'un projet étudiant l'effet de la dette de sommeil sur la réponse neurophysiologique à la chaleur, nous avons comparé les profils métaboliques d'un recueil de sueur fraîche vs un recueil sur un dispositif de micro-prélèvement calibré. Une hyperthermie (38,3 ± 0,2◦C) a été induite passivement chez 12 sujets par exposition à la chaleur (45◦C) après une nuit normale et une nuit de restriction de sommeil. Une poche a été placée sur leur peau afin de recueillir la sueur dite " fraiche " et le micro-prélèvement (30 µL) a été réalisé directement à partir de gouttes de sueur sur la peau. Tous les échantillons ont été analysés sur un appareil UHPLC-QToF en phase inverse et HILIC ainsi qu'en ionisation positive et négative. Les données ont été extraites sur Galaxy-Workflow4metabolomics. Malgré une forte variabilité interindividuelle, des profils métaboliques différents ont été observés selon le mode de prélèvement montrant l'importance d'avoir une réflexion quant aux conditions de recueil.

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Section 5 - Poster 15 – S5-P15

Implementation of an Automated Sample Preparation Workflow for Comprehensive Serum Metabolomics

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Metabolomics plays a pivotal role in clinical research, uncovering variations in the human metabolome and its correlation with health-related outcomes. However, the analysis of large-scale studies involving thousands of individuals poses a significant challenge for many untargeted analytical methods. Consequently, the automation of sample processing and analysis has emerged as a crucial advancement to meet the demanding experimental throughput. In this study, we established and evaluated a high-throughput metabolomics workflow for processing commercial human serum samples. Samples underwent extraction for metabolomics analyses using a Janus G3 (Revvity) liquid handling robot, employing two different extraction methods suitable for two separate and complementary chromatographic methods (C18 and HILIC). Samples were then analyzed using ultra-performance liquid chromatography coupled with HRMS (Vanquish Duo system coupled to a Thermo Scientific Orbitrap Exploris 240 mass spectrometer) in positive and negative ionization modes on the Integrative-Metabolome AnalytiCs for Translational and Precision Medicine (IMPACT-PM) platform in Lille. For both the total number of detected and annotated features, their coefficients of variation (CVs) across quality control samples (QCs) were assessed, along with intensity plots across the injection sequence. Additionally, technical internal standards (tISs) were employed to monitor data quality, all of which exhibited coefficients of variation (CVs) < 30% in the QCs. This workflow provides expanded coverage of serum metabolites, rendering the platform suitable for conducting serum untargeted metabolomic analysis, and it will prove beneficial for applications in larger cohort studies.

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Section 5 - Poster 16 – S5-P16

Insight into the metabolome by NMR: photo-CIDNP developments for metabolomics

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The versatility of NMR for analyzing complex biological mixtures has been described in recent years, 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 major 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) method is cost-effective and easy to implement on any NMR instrument. It is based on a radical-pair mechanism between a metabolite and an excited dye, induced by light irradiation.

Photo-CIDNP has not been employed to date in metabolomics studies, involving the sequential analysis of a large number of samples. Since it is successful when the target metabolite is highly conjugated, here we present the first developments for complex mixtures where aromatic compounds are not directly accessible by standard NMR due to their low concentration.

Preliminary results will be shown for the sensitivity enhancement of over 20-fold of flavonoid derivatives at the micromolar range, as well as an application for tea extracts obtained at different conditions. 1H photo-CIDNP data are acquired with a 1-scan 1D standard sequence, in a fast and efficient manner, which paves the way for a unique metabolomics protocol dedicated to aromatic or highly conjugated compounds.

1) Nagana Gowda, G. A. et al Anal. Chem. 2017, 89, 490–510
2) Eills, J. et al Chem. Rev. 2023, 123, 1417–1551.
3) Okuno, Y. eMagRes 2017, 6, 283-314.

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Section 5 - Poster 17 – S5-P17

Metafollow: one-year longitudinal follow-up to assess metabolites' variations in healthy subjects

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In healthcare, almost all metabolomics' studies focus on pathologies by studying interindividual variation of metabolites. But if we want to apply metabolomics to personalized medicine, we must first understand normal intra-individual variations of metabolites before preventively detect a pathological deviation. Our knowledge of the intra-individual "normal" variations of the metabolome is currently very poor and to expand our expertise, we have first to explore healthy people's metabolome over a certain period.

For this purpose, we selected 30 healthy volunteers that we followed for one year. According to gold standards of Clinical Chemistry, blood, urine and saliva samples were first collected each week for ten weeks and then each month for ten months. This work first focused on the blood NMR analysis. For the ten weeks' period, results showed that metabolites could be classified according to their variation, from the less to the most variable ones. The same approach has been applied to the monthly samples and have also highlighted a classification in the metabolites' variabilities very similar to the weekly ones. Metabolites were also linked to their metabolic pathways to identify the most variable networks. The results obtained in this preliminary work make it possible to stratify blood metabolites according to their short and long-term variations. The subsequent urine and saliva analyses will complete our data and are expected to give a more complete overview of the normal human metabolome's variation, which is very important for the application of metabolomics in the context of personalized medicine.

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Section 5 - Poster 18 – S5-P18

New method to characterize epidermal Ceramides by Supercritical Fluid Chromatography (SFC) coupled to High Resolution Mass (Q-Tof) : application to various samples with skin barrier default

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Lipids are the main constituent of skin and epidermis, and are mainly constituted by ceramides with prevalent very long acyl chains, free fatty acids, and cholesterol. Epidermal ceramides, specificaly ω-O-Acylceramide, will have a crucial role for skin barrier function so their full characterization are essential. It is a large and complex family of 12 sub-classes and their structural diversity, their wide range of concentration and the few standards available make this analysis very challenging.
The aim of this work was to develop an original profiling of the epidermal ceramides using Supercritical Fluid Chromatography (SFC) (UPC2 Waters) coupled to a Q-TOF (Xevo, Waters). The profiling was developed on a Torus DIOL column with a gradient of a mixture of isopropanol, methanol and acetonitrile (0.1% formic acid) in CO2 under pressure with ionisation in negative mode in presence of methanol. In studies involving the skin we can find many samples: it can be whole skin biopsies, cigarette paper impregnated with sebum, strip but also reconstructed human epidermis (RHE) for in vitro skin studies. Sample preparation needs to be adapted to sampling. We will present the development and the validation of this new method from the sample preparation to the data treatment to the chromatographic separation and structural characterisation.
Mutation in patatin-like phospholipase domain-containing 1 (PNPLA1) causes autosomal recessive congenital ichthyosis and conducts to lethal phenotype with major defects in the epidermal barrier. We will show the first application of the developed ceramide profiling on mutated Pnlpa1 mice and RHE samples.

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Section 5 - Poster 19 – S5-P19

Non-Targeted profiling of the plant volatolome: New perspectives enabled by VASE (Vacuum Assisted Sorbent Extraction) technology

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Plant volatolome is a very diverse chemical universe composed of secondary metabolites belonging to Volatiles Organic Compounds (VOCs). The structures, properties, emission rate of these VOCs by plant highly depends on the emitting organ, the growth stage, and obviously species and environmental biotic and abiotic conditions. For example, Brassica plants are well known to emit sulfur compounds like sulfides or thiols. Theses VOCs allow plants to communicate with other organisms for different attractive or repulsive purposes. Thus, they represent a source of interest in chemical ecology and putative reliable targets for plant performance protection.
Diverse techniques exist to trap these plant VOCs. The most common way of doing it is by a Dynamic Headspace method with a trapping on an adsorbent. However, such methods may present a lack of efficiency to capture the whole plant volatolome. To solve this problem, a new technology is tested from intact plant tissues called Vacuum Assisted Sorbent Extraction (VASE). This technique has a lot of advantages such as a simple workflow, no solvents, and the strength of vacuum allows the extraction of VOCS in quantity and in quality that wouldn't be accessible to other techniques. Feasibility test made on young oilseed rape plants showed convincing results in term of quantity and diversity of VOCs extracted compared to a parallel and conventional Dynamic Headspace method. The poster illustrates preliminary, although convincing evidences about the VASE technology performance on plant volatolome.

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Section 5 - Poster 20 – S5-P20

Optimisation de l'étape pré-analytique pour l'analyse métabolomique non-ciblée et le dosage ciblé de l'atorvastatine : une approche automatisée pour les grandes cohortes de patients

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Avec le développement des approches métabolomiques, nous observons une augmentation significative de la taille des cohortes, complexifiant l'étape d'extraction des échantillons. L'étape pré-analytique est une étape critique pour ces analyses très sensibles à toutes modifications apportées sur l'échantillon. Nombreux biais et erreurs peuvent être introduit lors de cette étape rendant difficile l'interprétation des phénomènes biologiques étudiés. Usuellement au laboratoire, les extractions des échantillons en métabolomiques non ciblées sont réalisées par précipitation des protéines par de l'acétonitrile froid. Toutefois, l'extraction manuelle de grandes cohortes est difficile à mettre en oeuvre en métabolomique non-ciblée. Le manque de robustesse et/ou l'introduction d'un effet batch sont des cas de figures souvent rencontrées en dépit des précautions prises, comme l'ajout de Pool QC et d'un matériel certifié.

Dans cette étude, nous avons eu recours à l'automate Extrahera LV200 de Biotage avec un double objectif : (i) comparer les performances de l'extraction usuelle manuelle à l'extraction via des plaques d'extraction type PPT, (ii) créer une méthode d'extraction, à partir d'un même volume d'échantillon de plasma précieux, pouvant réaliser une analyse métabolomique non ciblée et une analyse ciblée dosant l'atorvastatines et ses produits de métabolisation. Pour cela, différentes plaques et paramètres ont été optimisé afin de répondre à ce cahier des charges pour permettre l'extraction de 1800 échantillons plasmatiques ainsi qu'une perspective d'application sur l'extraction de fèces de manière automatisée. Ce développement s'inscrit dans l'ANR Strat Mi-UP o`u l'objectif est d'observer l'effet des statines sur le microbiote au sein d'une population atteint d'hypercholestérolémie.

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Section 5 - Poster 21 – S5-P21

Production in vitro de métabolites de xénobiotiques et enrichissement de la base de données PeakForest afin d'améliorer leurs détections dans des matrices complexes

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L'une des principales difficultés rencontrées dans les analyses métabolomiques non ciblées réside dans la présence fréquente de métabolites de contaminants, entravant ainsi l'identification précise de biomarqueurs d'effets significatifs. De plus, la rareté des composés commerciaux complique la confirmation de l'identité structurale de ces métabolites par spectrométrie de masse. L'objectif principal consiste donc à biosynthétiser des standards de xénobiotiques, tels que des biocides domestiques, des désinfectants et des tensioactifs, à partir de fractions subcellulaires S9 hépatiques humaines. Cette approche vise à produire des métabolites de contaminants afin d'obtenir des données de référence avec un niveau 2 d'identification. Six xénobiotiques, notamment des biocides comme le thiaclopride, la picaridine, le napropamide, le tépraloxydime, ainsi que les désinfectants 4-nonylphénol et 4-octylphénol, ont été soumis à une incubation, suivie de l'identification des métabolites candidats par LC-HRMS et MS/MS. Les analyses ont été réalisées à l'aide d'un LTQ-Orbitrap couplé à un système UHPLCWaters ACQUITY. Les métabolites ont été détectés grâce au peak picking et au logiciel MetaSense (ACD Labs), puis confirmés par analyse MS/MS. Les temps de rétention et les spectres MS/MS de ces métabolites ont ensuite été intégrés dans une base de données interne et implémentés dans PeakForest pour faciliter la caractérisation future de ces composés.

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Section 5 - Poster 22 – S5-P22 (Flash 7)

LC-HRMS-based metabolomics as a tool to develop analytical methods : How to choose the best extraction protocol when it comes to untargeted analysis ?

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Among lots of exigencies, two major requirements are necessary to assure the best performance of untargeted metabolomics-based approaches : (1) covering the maximum number of compounds present in the studied samples, and (2) determining the "optimal" analytical conditions to do so.
The present work suggests an approach to address these exigencies. This approach is based on LC-HRMS untargeted analyses and metabolomics computational tools. It will be developed and applied to assess the optimal extraction protocol dedicated to analyze a wide range of pesticides formulates and microbial metabolites at once, all hidden in a complex environmental matrix : agricultural soil.

Therefore, to compare five different extraction protocols applied on two types of soil and two formulated herbicides, four criteria were selected : (1) the coverage of compounds in term of polarity, (2) the quantitative performance, (3) the repeatability, and (4) the capacity to discriminate between contaminated and non-contaminated soils. To assess each criterion, different data analysis and visualization tools were used (e.g. hierarchically-clustered and polarity-segmented Heatmaps, van Krevelen diagrams, Euclidean Distances, RSD density plots, and OPLS-DA). They will be presented, explained and discussed in order to show their advantages, limitations and complementarities, as well as giving the best practices and tricks to render their exploration optimal.
These tools finally allowed for the selection of the best extraction protocol. They are thus suggested to assess the performance of analytical methods when it comes to untargeted metabolomics analyses.

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Section 5 - Poster 23 – S5-P23 (Flash 8)

Discovery of the first archaeal terpene synthases: metabolic engineering meets untargeted metabolomics

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Our lab developed a machine learning model that detects terpene synthases. Our model predicted terpene synthase activity for 7,000 ”dark matter” protein sequences from UniRef50 with no InterProScan signatures (even ”domain of unknown function”).
We expressed 17 ”dark matter” proteins in Saccharomyces cerevisiae JWY501, genetically modified to overproduce sesquiterpenes (C15H24) and diterpenes (C20H32). To discover their terpene synthase activity, we had to integrate untargeted metabolomics into the metabolic engineering workflow.

Metabolic engineering for terpene synthesis discovery relies on GC-EI-MS as a detector. In most cases, the product is known or guessed based on literature. Therefore, the major drawback of electronic impact (low or no signal from molecular ion) is circumvented by searching diagnostic fragments and NIST-EI library queries. This strategy only confirmed 3 enzymes as terpene synthases.

Electrospray is not commonly used for terpenes, but we found it can ionize terpene scaffolds surprisingly well. Thus, we took advantage of untargeted metabolomics strategies (MetaCorrelate module in MZmine 3, introduced for the Ion Identity Networking workflow) to search for possible chemical variations of terpene scaffolds and discovered that 4 additional enzymes were producing terpenes. We went back to GC-EI-MS data confirmed that 2 of the enzymes discovered using LC-HRMS were missed in our first analysis. Out of 7 enzymes we confirmed activity, 3 were detected by GC-MS and LC-HRMS, 2 by GC-MS and 2 by LC-HRMS. Among the 7 confirmed terpene synthases, 3 are of archaeal origin. This work constitutes the first experimental evidence of active terpene biosynthesis in the Archaea kingdom.

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Section 5 - Poster 24 – S5-P24

The Chenomx NMR Suite all-new COMPLETE Autofit Tool

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The mathematical challenges associated with automatic reconstruction and fitting of high-resolution proton NMR spectra has been the bottleneck of high-throughput analysis of NMR metabolomics data since its infancy. Quantitative or targeted profiling offers an alternative route to spectral reduction techniques such as binning, where experimental spectra are reconstituted at their full resolution from a sum of their underlying components using a reference compound library. In this approach, compounds are identified and quantified prior to performing any kind of multivariate statistical analyses. The challenge in quantitative metabolomics lies in the time and effort needed to identify and quantify compounds in biofluid mixtures. Different approaches have been formulated over the years, with more or less success, or limited applicability, due to the sensitive nature of the NMR signal with experimental conditions (solvent, magnet strength, pulse sequence and parameters) and the mathematical complexity of spectral deconvolution, whereby the parameter space to explore is enormous and hundreds if not thousands of variables need to be optimized simultaneously. Here we present a new, fully automated fitting algorithm based on mathematical methods derived from artificial intelligence that improve peak positioning and concentration fitting.

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Section 5 - Poster 25 – S5-P25

Développement d'une approche " hybride " pour l'analyse hautement multiplexée en métabolomique

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La métabolomique vise à étudier l'ensemble des molécules de bas poids moléculaire contenues dans des cellules, tissus, organismes ou fluides biologiques afin d'avoir une vision intégrée du métabolisme proche du phénotype et de proposer de nouveaux biomarqueurs. Pour cela, différentes stratégies d'analyses globales ou/et ciblées sont généralement définies selon la question biologique posées et impliquent des méthodologies analytiques différentes de production et de traitement de données. L'approche non ciblée se concentre sur l'analyse de tous les métabolites alors que l'approche ciblée s'adresse à des biomarqueurs potentiels prédéfinis ou présélectionnés à l'issue des analyses non ciblées. Cette dernière approche ciblée nécessitant un nombre important de standards et des efforts considérables pour être implémentée, nous proposons une approche dite hybride combinant une analyse en haute résolution puis plusieurs analyses ciblées pour rapidement évaluer et qualifier des biomarqueurs. Elle repose (i) sur l'utilisation d'un mode de balayage ciblé hautement multiplexé : Scout MRM développé par notre Laboratoire et (ii), le développement d'un algorithme " HRMS vers Scout-MRM ". Cet algorithme permet de retraiter les données de hautes résolutions et de générer très rapidement des méthodes Scout-MRM fortement multiplexées.

Pour démontrer l'intérêt de notre méthode, un jeu d'échantillon biologique de foies de porc ayant subi une ischémie reperfusion a été analysés avec l'approche classique non ciblée en mode d'acquisition indépendant de la donnée (DIA) et avec l'approche hybride.

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Section 5 - Poster 26 – S5-P26

Développement et mise en œuvre d'une approche innovante de criblage à grande échelle pour une caractérisation étendue de l'exposition humaine aux pesticides

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L'exposition chimique aux pesticides est un sujet de préoccupation majeure en santé. Actuellement, l'analyse de cette exposition chimique est effectuée de manière plus ou moins directe, par exemple via des questionnaires ou en quantifiant un nombre restreint de substances dans des matrices biologiques, sang ou urine (méthodes ciblées de biosurveillance). Ces approches sont insuffisantes pour prendre en compte la complexité et la totalité des expositions auxquelles les individus sont exposés. L'objectif de ce projet est de développer un nouveau workflow analytique multiplexe en associant trois techniques analytiques complémentaires (HILIC-LC-HRMS ; LC-ESI-HRMS ; GC-HRMS) et des outils bio-informatiques innovants permettant de caractériser à large échelle, l'exposition interne de l'homme aux pesticides. L'approche utilisée est celle du « profilage de suspects » en utilisant des données acquises en spectrométrie de masse haute résolution afin de cribler plusieurs milliers de marqueurs provenant de produits phytosanitaires, leurs métabolites (phase I et II) ou produits de dégradation. Une librairie de marqueurs de plus de 200 pesticides et de leurs métabolites (i.e., via Biotransformer) a été élaborée pour couvrir une large gamme de propriétés physicochimiques (logKow : (-4.71;8.38) ; MM/g.mol (72;720). Les données LC-ESI-HRMS présentées ont été pré-traitées par Galaxy (XCMS) et le profilage de suspect grâce à l'outil Scannotation et MS-DIAL4. Cette approche multiple permettra de caractériser et de complémenter les données d'exposition liées à l'usage de pesticides et de tester leurs relations avec des paramètres de santé afin d'identifier des substances délétères pour la santé humaine.

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Section 5 - Poster 27 – S5-P27

Plan d'expérience pour le développement d'une méthode HILIC LC-ESI-HRMS non-ciblée pour la caractérisation de l'exposome chimique

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Le concept d'exposome chimique a pour ambition de capturer la diversité des expositions humaines au cours de la vie et d'étudier leur impact sur la santé. La chromatographie liquide couplée à la spectrométrie de masse haute résolution (LC-HRMS) est une approche prometteuse pour caractériser l'exposome chimique interne de manière globale sur des échantillons biologiques. Cependant, les composés d'intérêt sont souvent très peu abondants et présentent une large variabilité de propriétés physico-chimiques. Bien que moins utilisées en exposomique, les phases HILIC sont pertinentes pour la séparation des composés excrétés sous forme plus polaires, en particulier dans l'urine.

Nous présentons ici le développement d'une méthode HILIC LC-HRMS non-ciblée par plan d'expérience visant à caractériser l'exposome chimique via l'analyse d'échantillon urinaire. Le développement se base sur l'utilisation d'un mélange de 164 standards (molécules endogènes et exogènes) en solvant et dopés dans l'urine. 5 facteurs ont été sélectionnés (conditions initiales, débit, pH, concentration en sel et température) et 5 réponses ont été mesurées (% de pics ayant une rétention inférieure à 1, efficacité, capacité de pic, résolution). L'étape de criblage des paramètres importants a été réalisée grâce à une matrice factorielle fractionnaire. Les paramètres clés ont ensuite été optimisés par la méthode de modélisation des surfaces de réponse.

Les résultats montrent que l'approche par plan d'expérience est efficace pour le développement analytique de méthodes d'analyse non-ciblée, car elle permet d'étudier l'influence de chaque facteur expérimental simultanément à différent niveau avec un nombre prédéfini d'expériences et en prenant en compte toutes les interactions possibles.

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Section 5 - Poster 28 – S5-P28

Toward modular quantifications methods for amino acids families, tryptophan derivatives, organics acids and bile acids

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Targeted quantitation of endogenous metabolites as a complete solution is increasingly in demand for clinical purpose. Consequently, it is important to adapt demand by developing a simple, fast, flexible and low-cost LC-MS/MS analysis method for quantifying specific lists of constantly evolving compounds.

Here we have adapted an accurate method for amino acids, tryptophan derivatives, organic acids and bile acids. Plasma, or urine, samples are prepared with 96 deep-well plates including two distinct derivatizations: one for secondary amines using phenylisothiocyanate and the other with 3-nitrophenylhydrazine for carboxylic acids. Plates are then analyzed in LC-MRM using a Sciex QTRAP 6500+ operating in positive ionization for amino acids and tryptophan derivatives and in negative ionization for organic acids and bile acids. A dedicated data processing involving Skyline, for peak integration, and a homemade R shiny app for calibration curves and analyte concentration calculations, have also been developed for this method.

A total of about one hundred metabolites from four different molecular families are able to be quantified in plasma and urine. From sample preparation to data processing, concentrations for about 78 samples can be obtained in a short period of time. Other endogenous compounds from several families of interest, such as steroids, biogenic amines, sugars, lipids, acylcarnitines, etc ..., will soon be developed and optimized for implementation into this method.

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Section 5 - Poster 29 – S5-P29

CINDERELLA: A semi-automatic user-friendly tool for curation of mass spectrometry metabolomics data

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In non-targeted metabolomics, the automatic pre-processing of mass spectrometry data, coupled with liquid or gas chromatography, is of great importance to highlight true biomarkers of interest while limiting false positives or false negatives. Many software offer more or less robust algorithms for the pre-processing stages (peak detection, alignment and integration), which generate hundreds or even thousands of features, but do not necessarily integrate pre-treatment (or cleaning) methods. In fact, a significant proportion of the signals extracted at the end of data pre-processing still need to be corrected or eliminated before the data can be statistically processed. Existing automated tools are often too strict and lack in visualisation steps. Thus, we are developing CINDERELLA (Comprehensive Integrated Data Editing and Refinement for metabolomics by ELiminating Low-quality signals and Artifacts), an R tool with a user-friendly interface for semi-automatic and supervised artifact elimination. Based on pre-processed data and sample metadata, CINDERELLA can, at the user's request, (i) remove features and/or samples containing too many missing values and replace the remaining missing values so as not to impact statistical tests, (ii) perform sample normalisation with the internal standard values and/or the amount of biological samples, (iii) correct batch effects, (iv) filter out artifacts and biologically irrelevant signals and (v) assess the quality of the remaining features. Each step is accompanied by interactive decision-support tables and graphs, which are compiled in an automatic output report for monitoring the data treatment process.

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Section 5 - Poster 30 – S5-P30

Conversion from metabolomics raw data to open format: ensuring MS and MS/MS data quality and software compatibility

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In a context of open-science, extracting information from high-throughput MS/MS metabolomics experiments has led to the necessity of converting raw MS data into open formats capable of handling MS/MS. However, several formats and conversion software exist, involving heterogeneous FAIR adherence in terms of reproducibility, retrocompatibility and interoperability. Format quality and software compatibility were studied within and across datasets using netCDF, mzML and mzXML open formats. Those were obtained from raw data using different versions of ProteoWizard's MSConvert and constructor-specific software. Inter-software and version evaluations were performed using in-house datasets acquired with high-resolution Bruker Impact HDII UHR-QTOF (Bruker Daltonics, Wissembourg, France) in 2018 and 2022, as well as an inter-laboratory study using constructor-provided datasets issued from the analysis of the same biological samples with various MS/MS analytical conditions. Data extraction differences were then evaluated using Galaxy's XCMS and MSPurity. Our results showed that altough open formats are capable of storing data obtained from various technologies, in some cases, conversion solutions may have difficulties to retrieve all the information without alteration. Consequently extraction results were affected. Worse still, interoperability and software compatibility were shown not guaranteed, with a generation of mzML and mzXML not compatible with some software yet designed to support these formats. Software version and data acquisition date also affect reproducibility. In conclusion, open data quality is highly sensitive to data age, instrument used, conversion software including version and output format. This emphasized the need for rigorous hardware and software monitoring and the criticality of metadata reporting in publications.

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Section 5 - Poster 31 – S5-P31

Development of an untargeted workflow for the identification of new lipids by mass spectrometry analysis

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Mass spectrometry (MS) has shown significant potential in measuring metabolites at very low concentrations over a wide dynamic range with targeted or untargeted approaches. Lipids, constituting the hydrophobic fraction of small biological molecules, play a crucial role in cellular physiology and are implicated in various diseases such as Alzheimer's, diabetes, or cancer.
The project aims to develop an untargeted workflow for the profiling of lipids by supercritical fluid (SFC) or liquid (LC) chromatography coupled to high resolution mass spectrometry (HRMS) with the final objective to identify new discriminated lipids. Currently, although several software packages exist for the treatment of MS lipidomic data for targeted and semi-targeted studies, the untargeted workflow for lipid identification remains poorly understood. Our objective is to compare two analytical techniques, SFC-QTOF (Waters) and LC-Orbitrap (Thermo), using different software to select the optimal process for untargeted lipid analyses, and subsequently develop a bioinformatics pipeline based on benchmarking of various software.

Raw data will be converted to mzXML format for analysis using software or workflows such as W4M, MZmine, MSDial, and SIRIUS. Benchmarking will be conducted to select the most efficient software, and a combination of functionalities from different software packages will be utilized to achieve optimal detection. In conclusion, the implementation of this untargeted tool will streamline lipid identification via MS, and additional statistical analysis functionalities will be added to investigate differentially expressed lipids under various conditions.

16 èmes Journées Scientifiques du RFMF
4-6 juin 2024, Saint-Malo

Section 5 - Poster 32 – S5-P32

Intelligent modeling and classification of NMR spectroscopic data

Fabien Torralba[1]

[1] Evear Extraction – Université Lyon 1 – France

This poster presents the research project of my Cifre thesis at Evear extraction in partnership with University of Lyon 1, on the modeling and intelligent classification of NMR spectroscopic data, utilizing a 400MHz NMR spectrometer and AI models trained on 4 Nvidia L4 GPUs. The main goal is to develop a metric for comparing two NMR spectra, enabling rapid and precise detection of similarities. This approach facilitates the identification of components in complex samples which would associate each peak in the NMR spectrum with a specific molecule. Particular attention is given to the classification of plants from their NMR spectra, aiming to specifically identify the studied plant 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. Early results on a machine learning workflow based on a random forest algorithm will be presented. This project promises to significantly improve the speed and accuracy of chemical compound identification, 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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Section 5 - Poster 33 – S5-P33

Mapping metabolomics data: complexity and issues

Mathieu Umec[1], Ghina Hajjar[1], Franck Giacomoni[1], Blandine Comte[1], and Estelle Pujos-Guillot[1]

[1] INRAE – Université Clermont Auvergne, INRAE, UNH, Plateforme

To optimize the translation of large-scale metabolomics by defining meaningful results, data contextualization is mandatory. Although a number of tools and methods have been developed, there is still no standardization of practices.
In this context, the objective of the work was to evaluate pathway analysis to biologically contextualize metabolomics data, identify bottlenecks and optimize workflows to provide reproducible information able to guide biological interpretation. To fulfil this objective, a published dataset including a list of identified metabolites modulated with metabolic syndrome in elderly men was used (Comte et al. 2021).

A large number of tools using neither the same methods nor the same databases were first evaluated. Then, four alternative mapping tools (i.e. ConsensusPathDB, MetaboAnalyst, MetExplore and RaMP) enabling to cover a wide range of methods, from the use of a single metabolic network to the use of multiple databases with different identifiers were deeper investigated.
Our work showed that, before taking an interest in mapping methods, it is essential to produce complete lists of adequate identifiers regarding the used databases or network, which often results in loss of information. Using multiple pathway databases was found to be a good strategy to derive a consensus pathway signature and increase the metabolome coverage. For fully identified metabolites, the use of metabolic network and subnetwork extraction appeared to be more pertinent to go deeper into metabolic exploration. In each case, the level of knowledge about the annotated metabolites, as well as the contextualization objective should guide the design of optimal workflows.

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Section 5 - Poster 34 – S5-P34

Multi-Platform Analysis for Quantitative Metabolomics

Jérémy Monteiro[1], Camille Dupuy[1], Lefèvre Antoine[1], Adeline Oury[1], Fréderic Montigny[1], Gabrielle Chicheri[1], Laurent Galineau[1], Hélène Blasco[1,2], Patrick Emond[1,3], and Lydie Nadal-Desbarats[1]

[1] Université de Tours, INSERM, Imaging Brain Neuropsychiatry iBraiN U1253, MetaboHub Tours – Université de Tours, Institut National de la Santé et de la Recherche Médicale - INSERM – France
[2] Service de Biochimie et Biologie Moléculaire – CHRU Tours – France
[3] CHRU de Tours, Service de Médecine Nucléaire In Vitro – France

Metabolomics, the science of identifying and quantifying cellular metabolites, employs advanced analytical techniques such as mass spectrometry (MS) or nuclear magnetic resonance (NMR), yet no single method comprehensively covers the entire metabolome. NMR offers advantages such as the ease of sample preparation, reproducibility, and non-destructive analysis, which facilitates rapid profiling for disease identification. Conversely, mass spectrometry provides high sensitivity and specificity, allowing analysis across a wide range of metabolites. Integrating multiple analytical platforms enhances the coverage and quantitative data, addressing challenges such as misidentification and database limitations. Innovative approaches like CoNaM, a workflow developed to increase the identification reliability by statistical correlation of NMR and LC-MS spectra, can facilitate the identification of individual metabolites in the metabolomics mixtures and enable efficient metabolite identification and data interpretation. In this work, we propose to characterise a serum QC sample, pooled from 10 samples, by NMR and LC-MS in order to reintegrated it in an exploratory LC-MS analysis to be able to propose semi-quantitative screening. First, the screening powers of NMR and LC-MS will be evaluated. Then, the QC will be quantified by both technics. For NMR, it will be quantified by 2 methods, calibration curve and estimation with reference, through several software. For LC-MS analysis, the quantified process is based on the calibration curve and the internal standard. The objective of this work is to simplify and extend quantification processes in further untargeted studies and to optimise metabolomics methodologies to improve our understanding of biological systems.

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Section 5 - Poster 35 – S5-P35

Multiblock Omics data fusion using the Consensus OPLS R package

Céline Bougel[1], Van Du Tran[2], **Julien Boccard**[3], **Marie Tremblay-Franco**[1], and **Florence Mehl**[2]

[1] INRAE Toxalim Axiom platform Metatoul Metabohub – Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) : UMR1331 – France
[2] SIB Swiss Institute of Bioinformatics – Suisse
[3] Biomedical and Metabolomics Analysis, School of Pharmaceutical Sciences, University of Geneva – Suisse

Omics approaches have proven their value to provide a broad monitoring of biological systems. However, despite the wealth of data generated by modern analytical platforms, the analysis of a single dataset is still limited and insufficient to reveal the full biochemical complexity of biological samples. The fusion of information from several data sources constitutes therefore a relevant approach to assess biochemical events more comprehensively. However, inherent problems encountered when analysing single tables are amplified with the generation of multiblock datasets and finding the relationships between data layers of increasing complexity constitutes a challenging task. For that purpose, a versatile methodology is proposed by combining the strengths of established data analysis strategies, i.e. multiblock approaches and the OPLS-DA framework to offer an efficient tool for the fusion of Omics data obtained from multiple sources.

The method, already available in MATLAB, has been translated into an R package with additional functionalities (e.g. VIPs, prediction of new samples). The package has been validated on a published dataset (transcriptomics, plasma shotgun MS lipidomics, and targeted LC-MS sphingolipids data) from living human pancreatic islet donors (n=51) with various diabetes status. Two analyses were performed: (i) a discriminant analysis to distinguish patients with impaired glucose tolerance, type 2 diabetes, and type 3c diabetes, and (ii) a regression analysis to model the level of HbA1c, a parameter of longer-term glycaemia. OPLS-DA multiblock models enabled the identification of significant biomarkers across various blocks, leading to novel biological insights.

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Section 5 - Poster 36 – S5-P36

Semi-automatic generation of a spectral library for metabolomics studies

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[1] European Genomic Institute for Diabetes – EGENODIA, INSERM U1283, CNRS UMR8199, Institut Pasteur de Lille, Lille University Hospital, University of Lille – France
[2] Section of Biomolecular Medicine, Division of Systems Medicine, Department of Metabolism, Digestion and Reproduction, Imperial College London – Royaume-Uni

Introduction

Untargeted UHPLC-MS metabolomics analyses are constrained by the spectral databases used for annotation. Building these databases is challenging since it requires injecting hundreds to several thousands of standards and manually curating the chemical information.

Technological and methodological innovation

A new workflow written in R is proposed for building spectral databases with minimal manual curation work. The workflow consists in the automatic import of the raw files, peak picking, extraction of MS/MS spectra and database compilation. Compared to the commercial alternative mzVault (ThermoFisher), it also includes a step of manual data inspection of the chromatographic profile and the corresponding MS/MS for final curation before compilation into the most widely used formats (.db, .msp). This workflow was validated with the IROA MSMLS chemical library (603 standards) and its performance was compared to that of mzVault.

Results and impact

The workflow presented was proven to be easy-to-use. The inclusion of the manual step of data inspection before compilation permitted the user to assess with confidence the reliability of the built database. In mzVault, which does not have this step, it is recommended to extract the MS/MS from the most common adducts only ((M+H)+, (M-H)-) limiting the number of MS/MS extracted (786 MS/MS spectra from 410 compounds with mzVault, versus 1,908 MS/MS spectra from 466 compounds with the proposed method). In conclusion, this workflow not only facilitates but also allows for the construction of more exhaustive spectral databases for metabolomics.

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Section 5 - Poster 37 – S5-P37

The FORUM Knowledge Graph 2.0: New Features and Future Directions for Advancing Metabolomics Data Understanding

Clément Frainay[1,2], Maxime Delmas3, Meije Mathe[1], Guillaume Laisney[2,4], Christophe Duperier[2,5], Nils Paulhe[2,5], Florence Vinson[1,2], Marie Lefebvre[2,5], Fabien Jourdan[1,2], Olivier Filangi[2,4], and Franck Giacomoni[2,5]

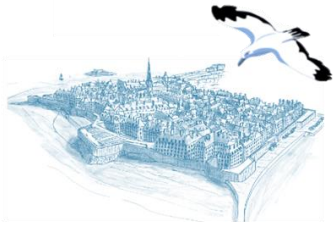
[1] Toxalim (Research Center in Food Toxicology) – INRAE, Universé de Toulouse, école Nationale Vétérinaire de Toulouse - ENVT, INP-Purpan, Université Paul Sabatier-Toulouse III - UPS – France
[2] MetaboHUB – INRAE – France
[3] IDIAP Research Institute – Suisse
[4] INRAE UMR 1349 IGEPP, Université de Rennes, Agrocampus Ouest, Institut national d'enseignement supérieur pour l'agriculture, l'alimentation et l'environnement – France
[5] Plateforme Exploration du Métabolisme – INRAE, Université Clermont Auvergne, INRA, UNH – France

With the rapid growth of metabolomics research, scientists face a significant challenge in navigating and interpreting this wealth of information. The diverse and large dataset of compounds identified in metabolomics, combined with the sheer volume of metabolitephenotype associations documented in scientific literature, creates an information overload that can hinder meaningful insights and discoveries.

To address the complexities of metabolomics data interpretation, the knowledge graph FORUM has recently emerged as a valuable resource, offering a solution to information overload. A knowledge graph is a structured representation of knowledge, comprising interconnected entities (nodes) and their relationships (edges). In the case of FORUM, this graph links metabolites with diseases based on evidence extracted from vast amounts of scientific literature using automated literature mining techniques.

Recently, FORUM underwent a significant update to enhance its capabilities and utility. This update includes the integration of newly published datasets and the incorporation of the human metabolic network into the knowledge graph. By expanding its scope, FORUM now offers a more comprehensive view of metabolite-disease associations, facilitating more nuanced interpretations of metabolomics data by fostering indirect relationships through metabolism. These features aim to streamline access to valuable insights and enable researchers to explore metabolite-disease relationships with greater depth and efficiency. We also present the ongoing developments and planned directions for FORUM, including the enhancement of the infrastructure to support more frequent updates, the integration of additional datasets to further enrich the knowledge graph, and the ongoing revamping of the web portal for an improved user experience.

RFMF
Réseau Francophone de Métabolomique et Fluxomique



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4-6 juin 2024, Saint-Malo

Section 5 - Poster 38 – S5-P38 (Flash 6)

Développement d'un workflow computationnel pour la fluxomique ^{13}C

Loïc Le Grégam[1,2], Yann Guitton[3,4], Floriant Bellvert[2,5], Fabien Jourdan[2,6], Jean-Charles Portais[2,7,8], and Pierre Millard[1,2]

[1] Toulouse Biotechnology Institute, TBI-INSA Institut National des Sciences Appliquées de Toulouse INSA/CNRS 5504-UMR INSA/INRA 798, 5504 Toulouse -– France

[2] MetaboHUB-MetaToul, National Infrastructure of Metabolomics and Fluxomics, Toulouse – Institut National des Sciences Appliquées - Toulouse, Institut National des Sciences Appliquées - Toulouse – France

[3] MetaboHUB-Grand-Ouest – MetaboHUB – France

[4] Laboratoire d'étude des Résidus et Contaminants dans les Aliments – Ecole Nationale Vétérinaire, Agroalimentaire et de l'alimentation Nantes-Atlantique, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, INRAE – France

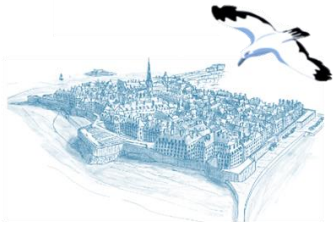
[5] Toulouse Biotechnology Institute – Institut National des Sciences Appliquées - Toulouse, Centre National de la Recherche Scientifique, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement : UMR0792 – France

[6] ToxAlim – Univ. Toulouse III - Paul Sabatier, Ecole Nationale Vétérinaire de Toulouse, Institut National Polytechnique (Toulouse), Ecole d'Ingénieurs de Purpan, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – France

[7] Geroscience and rejuvenation research center – Univ. Toulouse III - Paul Sabatier, EFS, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique – France

[8] Université Toulouse III - Paul Sabatier – Université de Toulouse – France

En biologie des systèmes, l'étude des flux métaboliques est cruciale pour comprendre le phénotype des organismes vivants. Dans les domaines de la santé et de la biotechnologie, elle facilite la découverte des mécanismes régulateurs clés des maladies ou l'optimisation des processus biologiques pour la production de molécules d'intérêt. Une des techniques majeures utilisée est l'Analyse des Flux Métaboliques (MFA), qui consiste à étudier et quantifier les vitesses de réactions biochimiques au sein des réseaux métaboliques grâce à des méthodes de modélisation mathématique basée sur contraintes. En 13C-MFA, l'utilisation d'isotopes stables du carbone dans des expériences de marquage isotopique (ILE) fournis des données expérimentales permettant de contraindre les modèles et ainsi augmenter la précision des flux calculés. Récemment, un nombre croissant d'études démontrent qu'il est possible d'augmenter le débit des ILE et des étapes analytiques. Cependant, le traitement des données et la modélisation des flux reste un goulot d'étranglement en 13C-MFA. Ceci est dû à la complexité qui découle de la multiplication des logiciels disponibles et du manque d'interopérabilité entre ces derniers. Cette partie reste donc majoritairement manuelle et sujette aux erreurs humaines, diminuant la reproductibilité et augmentant les coûts. Pour répondre à ce problème, nous avons intégré et/ou développé plusieurs outils/modules pour effectuer les différentes parties du workflow global (approche en microservices). Disponibles sur la plateformeWorkflow4metabolomics et rendus interopérables, nous les avons implémentés au sein d'un workflow computationnel automatisé, flexible, reproductible, et haut débit pour la 13C-MFA.



16 èmes Journées Scientifiques du RFMF

4-6 juin 2024, Saint-Malo

Détails des visites du Jeudi 6 juin 2024

Des visites sont organisées à la suite du congrès, le **jeudi 6 juin après-midi**, à 14h pour que tous puissent profiter une dernière fois de la cité Corsaire avant le départ.

Nous proposons donc 2 visites commentées par des guides locaux. Les visites sont à votre charge et **à régler le jour-même**. Au moins un organisateur du RFMF participera aux visites et organisera un départ depuis le Palais du Grand Large pour ceux qui le désirent.

Le fort national (Durée : 45 min) – Prix : 4€/pers

Faisant corps avec le rocher, ce bastion avancé, assurant la protection de la cité, a été édifié par Vauban en 1689. C'est aujourd'hui une propriété privée. La vue des remparts est exceptionnelle : de l'estuaire de la Rance aux îles Chausey. Et la visite du cachot, impressionnante !

Le fort ouvre ses portes vers 13h15 pour un début de visite à 14h qui dure environ 45min. Vous avez donc la possibilité de manger sur place avant la visite dans le respect du site. L'accès s'effectue à marée basse depuis la grande plage de Saint-Malo (dite la plage de l'Eventail), face au Château. Vous traverserez la plage depuis la Porte Saint-Thomas ou le parking de la Galère (300 mètres à pied). Une gestion des valises sera mise en place par le comité local d'organisation du RFMF.

https://www.fortnational.com/fort-national.html

La ville de Saint-Malo (Durée : 1h30) – Prix : 6,50€/pers

Loin des présentations touristiques habituelles, la visite insolite de Saint-Malo vous fait découvrir les ruelles anciennes de la cité corsaire. Les origines de la ville au XII°s, ses remparts, le pourpris, les chiens du guet, les attaques anglaises et le commerce interlope… Les anecdotes permettent d'aborder l'Histoire sous un angle très vivant et accessible : le guide saura s'adapter à tout type de population.

Départ de la demeure de Corsaire à 14h. Possibilité de laisser les valises à la demeure de Corsaire le temps de la visite.

Informations pratiques

- Plan d'accès

Lieu du congrès

Palais du Grand Large - Centre des Congrès
1 Quai Duguay-Trouin, 35400 Saint-Malo

Diner de Gala du 5 juin – 20h

La Demeure de Corsaire
5 Rue d'Asfeld, 35400 Saint-Malo

Visites du Jeudi 6 juin – 14h

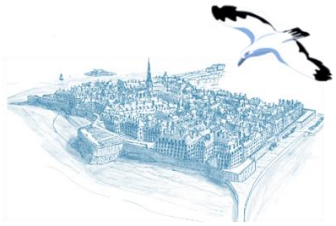
Fort National (45 min) – 4€/pers
60 Chau. du Sillon, 35400 Saint-Malo, France

Visite de Saint-Malo (1h30) – 6,5€/pers
Départ Demeure de Corsaire, 35400 Saint-Malo

- Code WIFI

Identifiant : **RFMF**

Mot de passe : **stmalo2024**



- Liste Ingrédients « Goodies alimentaires »

Caramels tendres au beurre salé

Ingrédients : Lait entier bio, sucre bio, sirop de glucose, crème crue bio, dextrose, beurre salé bio, sel de guérande, bicarbonate de soude, arôme naturel vanille, huile essentielle bio de citron

Galettes fines

Ingrédients : Farine de blé, sucre, beurre demi-sel, œufs entiers plein air, poudre levante (bicarbonate de soude, carbonate d'ammonium), sel, dorure : poudre de lait, œufs entiers, eau

Palets bretons citron gingembre

Ingrédients : farine de blé, beurre demi-sel, sucre, jaunes d'œufs plein air, zeste de citron, poudre levante (bicarbonate de sodium), gingembre. Dorure : lait

Palets bretons au caramel au beurre salé

Ingrédients : farine de blé, beurre demi-sel, sucre, jaunes d'œufs plein air, caramel au beurre salé (sucre, crème fraiche, beurre frais, sirop de glucose, sel de guérande, fleur de sel de guérande), arôme naturel, éclats de caramel au beurre salé, poudre levante (bicarbonate de sodium). Dorure : lait demi-écrémé

Palets bretons au chocolat

Ingrédient : farine de blé, beurre breton demi-sel, sucre, perles de chocolat noir (pâte de cacao, sucre, beurre de cacaco, émulsifiant : lécithine de soja), jaunes d'œufs plein air, poudre levante (bicarbonate de sodium). Dorure : lait demi-écrémé

Palets bretons nature

Ingrédient : farine de blé, beurre demi-sel, sucre, jaunes d'œufs plein air, poudre levante (bicarbonate de sodium). Dorure : lait demi-écrémé

- Les alentours du Palais du Grand Large

Intra-muros, la vieille ville :

Le centre historique de Saint-Malo regorge d'histoire, de ruelles secrètes, et de commerces animés. La cité Corsaire est aussi entourée de remparts desquels on peut apprécier la vue sur les îles aux alentours.

Bars et restaurants :

- Les Terroiristes Associés, bar à vin et tapas
- Le Penjab, restaurant Indien
- La Rose Des Vents, crêperie
- Le Chateaubriand, cuisine française
- La Trinquette, bar
- La Belle Epoque, bar
- L'Alambic, bar
- Le Saint-Patrick, bar
- Le 109, boîte de nuit

Le Sillon

Deux kilomètres de digue longent la Grande plage, la plage du Sillon et la plage de Rochebonne, reliant ainsi Intra-muros au quartier de Paramé. En y marchant, on peut admirer Intra-muros, la pointe de la Varde et les plus belles villas malouines.

Bars et restaurants :

- Les Ambassadeurs, crêperie et terrasse panoramique
- Les Charmettes, bar restaurant

- La Kaziela, restaurant créole
- La Corniche, restaurant
- L'Olivier, pizzeria

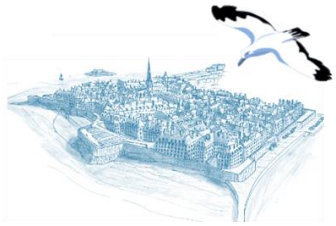
Solidor

A 30 minutes à pied du Palais du Grand Large, au milieu du quartier de Saint-Servan et à l'embouchure de la Rance, trône la tour Solidor, un des monuments emblématiques de Saint-Malo, offrant à ce quartier animé un décor de carte postale.

Bars et restaurants :

- La Cale, restaurant de poissons et de fruits de mer
- Le Cancalais, Bar Tapas
- Le Point Zéro, bar éphémère avec vue panoramique
- Atypic, restaurant
- Le Vomb, bar restaurant

RÉSEAU FRANCOPHONE DE MÉTABOLOMIQUE ET FLUXOMIQUE



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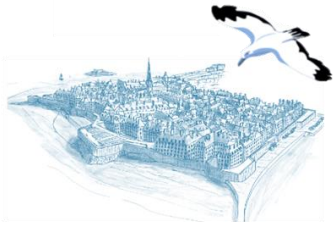
Liste des participants



- **ALAMIL Héléna** - Bruker France - Champs-sur-Marne
- **ANDRUSZKOW Léo** - IGEPP - Rennes - Le Rheu
- **ANTÓNIO Carla** - Plant Metabolomics Portugal - University of Lisbon
- **ARNAUD Luc** - Agilent Technologies Technologies - Les Ulis
- **ASSEMAT Olivier** - Bruker - Wissembourg
- **AV Luce-Flour** - Œnologie - INRAE - Bordeaux
- **AVELLA Maria Teresa** - IPNP - Paris Cité
- **AWADA Fatme** - BIOPI - Université Jules Verne - Amiens
- **BARREDA Lea** - IJPB - INRAE - Versailles
- **BEAUMONT Eric** - Centre INRAE Bretagne Normandie
- **BELLVERT Floriant** - MetaboHUB - Toulouse
- **BERARDOCCO Solenne** - IGEPP - Rennes - Le Rheu
- **BERGES Cécilia** - Metatoul - Toulouse
- **BERTHO Gildas** - Université Paris Cité - Paris
- **BERTON Thierry** - Givaudan Naturals France - Avignon
- **BERTRAND Marylène** - CBM - CNRS - Orléans
- **BERTRAND Cédric** - CRIOBE- AKINAO - Perpignan
- **BERTRAND Samuel** - ISOMer - Nantes Université
- **BERTRAND-MICHEL Justine** - Metatoul - Toulouse
- **BILLET Kevin** - BIA - INRAE - Rennes - Le Rheu
- **BOCCA Cinzia** - MITOVASC - CHU - Université d'Angers
- **BOCCARD Julien** - Université de Genève
- **BOISSIN Louise** - LABERCA - ONIRIS - Nantes
- **BONNARD Isabelle** - CRIOBE - Perpignan
- **BOUCHEREAU Alain** - IGEPP - Rennes - Le Rheu
- **BOUDRY Félix** - CRIOBE - Perpignan
- **BOURAFAI AZIEZ Asma** - EVEAR Extraction - Couture
- **BOURCIER Sophie** - LCM Polytech - Palaiseau
- **BOURGEON Frédéric** - BIO CHENE VERT - Chateaubourg
- **BOURSEAU Coline** - BIA - INRAE - Rennes - Le Rheu
- **BOUTET Stéphanie** - IJPB - INRAE - Versailles
- **BRIAND Olivier** - EHESP - Rennes
- **BROSSE Ludovic** - LNI-Swissgas
- **BRUNET Thomas** - ISA - Université Claude Bernard Lyon
- **BUSONT Océane** - ICOA - Université d'Orléans
- **BUTIN Noémie** - RESTORE - Université de Toulouse
- **CABASSON Cécile** - BFP - INRAE - Villenave d'Ornon
- **CAHIER Karine** - CNRS - SB Roscoff
- **CAHUZAC Robin** - SAS AKINAO - Perpignan
- **CAMPAS Manon** - CIRM - Université de Liège
- **CANLET Cécile** - Metatoul - Toulouse

- **CARADEUC Cédric** - Université Paris Cité
- **CASTRO Inès** - EGENODIA - Université de Lille
- **CAUSSE Julie** - IRBA - Brétigny-sur-Orge
- **CELLIER Benoit** - SCIEX - Villebon-sur-Yvette
- **CHAKER Jade** - EHESP - Rennes
- **CHARTON Sophie** - IGEPP - Rennes - Le Rheu
- **CHÉREAU Sylvain** - IGEPP - Rennes - Le Rheu
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