RFMF

Réseau Francophone de Métabolomique et Fluxomique

LIÈGE

23 > 25 MAI 2018

RÉSEAU FRANCOPHONE DE MÉTABOLOMIQUE ET FLUXOMIQUE

11e JOURNÉES SCIENTIFIQUES

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Partenaires Institutionnels des 11èmes Journées Scientifiques *du RFMF*

Center for Interdisciplinary research on Medicines:

http://cirm.uliege.be

Fonds de la Recherche Scientifique :

http://www.fnrs.be

MetaboHUB :

http://www.metabohub.fr

Province de Liège :

http://www.provincedeliege.be

Université de Liège :

https://www.uliege.be

Ville de Liège :

https://www.liege.be/fr

Partenaires Industriels *Platinium* des 11èmes Journées Scientifiques du RFMF

Bruker :

https://www.bruker.com

Sciex :

https://sciex.com

Shimadzu :

https://www.shimadzu.com

Waters :

http://www.waters.com

Partenaires Industriels *Gold* des 11èmes Journées Scientifiques du RFMF

Chenomx :

https://www.chenomx.com

Research Institute for Chromatography :

http://www.richrom.com

Proteigene :

http://proteigene.com

Proteomic solutions :

http://proteomicsolutions.fr

Thermo Fisher Scientific :

https://www.thermofisher.com

Partenaires Industriels *Silver* des 11èmes Journées Scientifiques du RFMF

Eurisotop :

Eurisotop

http://www.eurisotop.com

Partenaires Industriels *Bronze* des 11èmes Journées Scientifiques du RFMF

Cortecnet :

cortecnet
INNOVATION ACCELERATOR IN LIFE SCIENCE

http://www.cortecnet.com

Brasserie de la Lienne :

BRASSERIE DE LA LIENNE

http://www.brasseriedelalienne.be

Le Comité d'Organisation

Comité local d'organisation

- Laure Bindels, Professeur, Université Catholique de Louvain, LDRI
- Jean-Marie Colet, Professeur, Université de Mons, Toxico
- Cédric Delporte, PhD, Université Libre de Bruxelles, Analytical Platform
- Pascal de Tullio, Directeur de Recherches FNRS, Université de Liège, CIRM - METABO-SANTÉ
- Marianne Fillet, Professeur, Université de Liège, CIRM
- Michel Frédérich, Professeur, Université de Liège, CIRM
- Justine Leenders, doctorante, Université de Liège, CIRM - METABO-SANTÉ
- Manon Martin, doctorante, Université Catholique de Louvain, ISBA
- Matthieu Schoumacher, doctorant, Université de Liège, CIRM

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- Floriant Bellvert (IE, CNRS) : Plateforme MetaToul-MetaboHUB
- Frédérique Courant (MCU, Université Montpellier) : UMR Hydrosciences
- Patrick Giraudeau (Professeur, Université de Nantes) : CEISAM
- Yann Guitton (IR, Oniris) : Laboratoire d'Etude des Résidus et Contaminants dans les Aliments
- Anne-Emmanuelle Hay-de Bettignies (MCU, Université Lyon 1, ISPB) : UMR Ecologie Microbienne, CESN
- Fabien Jourdan (DR, INRA toulouse) : UMR Toxalim, MetaboHUB

Le Comité Scientifique

Le Comité Scientifique des 11èmes JS du RFMF est constitué des membres du Conseil d'Administration du RFMF et des membres des universités et instituts belges locaux :

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- Floriant Bellvert (IE, CNRS) : Plateforme MetaToul-MetaboHUB, Toulouse, France
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- Frédérique Courant (MCU, Université Montpellier) : UMR Hydrosciences, Montpellier, France
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- Patrick Giraudeau (Professeur, Université de Nantes) : CEISAM, Nantes, France

- Yann Guitton (IR, Oniris) : Laboratoire d'Etude des Résidus et Contaminants dans les Aliments, Nantes, France
- Anne-Emmanuelle Hay-de Bettignies (MCU, Université Lyon 1) : UMR Ecologie Microbienne, CESN, Lyon, France
- Fabien Jourdan (DR, INRA Toulouse) : UMR Toxalim, MetaboHUB Toulouse, France
- Jean-Charles Martin (DR, INRA) : UMR NORT, plateformes BiomeT et CRIBIOM, Marseille, France
- Etienne Thévenot (Chercheur CEA, Saclay) : CEA LIST, MetaboHUB, Saclay, France

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- Jean-Marie Colet, Professeur, Université de Mons: Toxico, Mons, Belgique
- Pascal de Tullio, Directeur de Recherches FNRS, Université de Liège : CIRM - METABO-SANTÉ, Liège, Belgique
- Marianne Fillet Professeur, Université de Liège, Laboratoire d'Analyse des Médicaments : CIRM, Liège, Belgique
- Michel Frédérich, Professeur, Université de Liège : CIRM, Liège, Belgique
- Bernadette Govaerts, Professeur, Université catholique de Louvain: ISBA, Louvain, Belgique
- Florence Souard, Maître de Conférence, Université Grenoble Alpes : DPM , Grenoble, France
-

Comité scientifique Junior

- Djawed Bennouna (Doctorant, Faculté de Médecine, Aix-Marseille Université) : UMR NORT (nutrition, obésité, risque thrombotique), Marseille, France
- Antoine Buonomo (Ingénieur chimiste) : Rovaltain Research Company, Alixan, France
- Sylvain Dechaumet (Doctorant, UMR INRA 1349) : Institut de Génétique, Environnement et Protection des Plantes, Le Rheu, France
- Grégory Genta-Jouve (MCU, Université Paris Descartes) : C-TAC, UMR 8638 COMETE, Paris, France
- Maximilien Gonçalves-Martins (IE), Novi Sad, Serbie
- Corentine Goossens (PhD RMN Métabolomique), Montréal, Canada
- Justine Leenders (PhD, Université de Liège) : CIRM - METABO-SANTÉ , Liège, Belgique
- Guillaume Meiffren (IE, Université Lyon 1) : UMR Ecologie Microbienne, CESN, Lyon, France
- Sandrine Aros (PhD, biochimie) : MedDay Pharmaceuticals, CEA Saclay, France

Ateliers des 11émes Journées Scientifiques du Réseau Francophone de Métabolomique et Fluxomique

22 mai 2018
Université de Liège, Place du 20 août

Mardi 22 mai 2018

12:30 - 15:00 : Atelier Présentation et application de PepsNMR, un package R pour le prétraitement de spectres ^{1}H-NMR. (Manon Martin et Bernadette Govaerts)
Salle Philo 2
(sur inscription préalable validée)

12:30 - 15:00: Atelier Outils d'annotation de données MS/MS : applications pratique (Etienne Thevenot, David Touboul, Alexis Delabrière, Yann Guitton, Emilien Jamin et Grégory Genta-Jouve)
Salle Lumière
(sur inscription préalable validée)

15:15 – 17:45 : Atelier La quantification en métabolomique
(Justine Bertrand-Michel, Marlène Lacroix et Cécile Canlet)
Salle Philo 2
(sur inscription préalable validée)

15:15 – 17:45 : Atelier Méta-métabolomique, discussion autour des méthodes d'analyse des données. Focus sur les aspects multiblock, multitableaux. (Cyril Jousse, Jean-Charles Martin, Binta Dieme, Gabriel Markov et Julien Boccard)
Salle Lumière
(sur inscription préalable validée)

18:00 - 20:00 : Table ronde Junior : Interventions et discussions autour des problématiques propres aux juniors dans les domaines de la métabolomique et de la fluxomique
Salle Lumière
(sur inscription préalable validée)

Programme des 11émes Journées Scientifiques du Réseau Francophone de Métabolomique et Fluxomique

23-25 mai 2018
Université de Liège, Théâtre de Liège

Mercredi 23 mai 2018

8h30-9h00 Accueil des participants

9h00-9h20 Allocution de Bienvenue

Ouverture des 11émes JS du RFMF par le Prof. Rudy Cloots, Vice-recteur à la Recherche et le Prof. Vincent d'Orio, Doyen de la Faculté de Médecine, de l'Université de Liège.

9h20-9h30 Introduction aux 11émes journées Scientifiques du RFMF

Fabien Jourdan, Président du RFMF et Pascal de Tullio, Coordinateur du Comité Local d'Organisation

9h30-10h15 Conférencier invité

Chairman: Michel Frédérich, Liège, Belgique

Opening new fields of natural products research by metabolomics tools

Prof. Young Hae Choi, Natural Products Laboratory, University of Leiden, The Netherlands OP1

10h15-12h15 Session Environnement et microbiologie 1

Chairmen: Frédérique Courant, Montpellier, France et Sylvain Dechaumet, Rennes, France

10h15-10h35 **Study of the environmental impact of Bti *vs.* chemical insecticides by metabolic foot-printing approach.**
Patil Chandrashekhar, CRIOBE USR3278, Université de Perpignan, France O1

10h35-10h55 **Etude par métabolomique et protéomique de la réponse de la bactérie marine *Pseudoalteromonas lipolytica* TC8 à un stress cuprique.**

Laurie Favre, The New Zealand Institute for Plant Food Research Limited, New-Zealand O2

10h55-11h25 Pause-Echanges informels-Visite des stands

11h25-12h15 Session Environnement et microbiologie 2

Chairmen : Cyril Jousse, Clermont-Ferrand, France et Léa Roch, Bordeaux, France

11h25-11h45 **Métabolomique des interactions plantes/ bactéries dans la rhizosphère: activité modulatrice de la plante hôte sur le métabolisme secondaire bactérien**

Laura Rieusset, Laboratoire d'écologie microbienne, Université Claude Bernard Lyon1, France O3

11h45-12h00 **High-resolution mass spectrometry based non-targeted metabolomic analyses for the study of the toasting/ tannin potential interaction in oak wood.**

Nolwenn Wirgot, [1]UMR A 02.102 PAM laboratoire PAPC AgroSup Dijon,Université de Bourgogne, France OJ1

12h00-12h15 **Utilisation en routine et applications de la nouvelle microLC Nexera Mikros en couplage avec l'Ultra Fast MS LCMS-8060**

Thierry Legoupil, SHIMADZU, Marne la Vallée, France OS1

12h15-13h15 Déjeuner

13h15-14h00 Session poster 1

14h00-14h45 Conférencier invité

Chairman: Fabien Jourdan, Toulouse, France

Metabolomics at 5000m and other stories: Applications of metabolomics to understanding human physiology.

Prof, Jules Griffin, Department of Biochemistry, University of Cambridge, UK OP2

14h45-16h00 Session Santé 1

Chairmen: Jean-Marie Collet, Mons, Belgique et Sandrine Aros, Gif-sur-Yvette, France

14h45-15h05 **Metabolic reprogramming in IDH1 Mutant Acute Myeloid Leukemia Cells**

Jean-Charles Portais, Metatoul – Metabohub, Université de Toulouse, France O4

15h05-15h25 **Exudative Age-Related Macular Degeneration: from metabolomics to the identification of lactate as a key functional and targetable metabolite for personalized medicine**.

Vincent Lambert, Department of Ophthalmology- Laboratory of Tumor and Development Biology, Université de Liège, Belgique O5

15h25-15h45 **Consequences of blunting the mevalonate pathway in cancer identified by a pluri-omics approach.**

Laurent Corcos, INSERM U1078, Université de Brest, France O6

15h45-16h00 **Gut microbiota and faecal levels of short chain fatty acids differ upon blood pressure levels in man.**

Justine Huart, GIGA, Sciences Cardio-vasculaires Université de Liège, Belgique OJ2

16h00-16h30 Pause-Echanges informels-Visite des stands

16h30-17h55 Session Développement méthodologique 1

Chairmen: Florian Bellvert, Toulouse, France et Grégory Genta-Jouve, Paris, France

16h30-16h50 **Inferring new biochemical reactions and new metabolite structures to cope with metabolic pathway drift in emerging model organisms**

Gabriel Markov, Laboratoire de Biologie Intégrative des Modèles Marins, CNRS : UMR8227, Roscoff, France O7

16h50-17h10 **Getting more from less: optimizing the investment of analytical resources in metabolomic studies by using a scoring algorithm**

Julian Pezzatti, School of pharmaceutical Sciences, University of Geneva, Suisse O8

17h10-17h30 **Volumetric absorptive microsampling for targeted metabolomics of whole blood**

Miranda Kok, Laboratory for the Analysis of Medicines, CIRM, University of Liege, Belgium O9

17h30-17h45 **Benefits of SWATH® Acquisition over Traditional DDA for High Resolution Untargeted Metabolomics Applications**

Henri Nicar, SCIEX, Paris, France OS2

17h45-17h55 Session Flash 1 (3 Présentations)

Modérateurs: Floriant Bellvert et Pascal de Tullio

MetWork: a Web Server for in-silico Metabolization of Natural Products

Yann Beauxis, Chimie Organique, Médicinale et Extractive et Toxicologie Expérimentale, Université Paris Descartes, France F1-P1

Metabolomics as a tool to identify phenolic compounds involved in spatial distribution of flax leaves

Job Tchoumtchoua, Biologie des Plantes et Innovation, Université de Picardie Jules Verne, France F2-P2

Influence of psychosocial stress on salivary metabonomic profile

Gilson Romoaldo, Laboratory of Human Biology and toxicity, Universoté de Mons, Belgique F3-P3

18h00-19h30 Assemblée générale du Réseau Francophone de Métabolomique et de Fluxomique (RFMF)

19h30-21h00 Verres de bienvenue

Jeudi 24 mai 2018

9h00-9h45 Conférencier invite

Chairman: Julien Boccard, Genève, Suisse

From multivariate to multiblock: tackling data structures in Metabolomics

Prof. Serge Rudaz, *School of Pharmaceutical Sciences, University of Geneva*, Suisse OP3

9h45-10h40 Session Développement méthodologique 2

Chairmen : Etienne Thévenot, Paris, France et Maxime Chazalviel, Toulouse, France

9h45-10h05 **Improved NMR methods for 13C NMR based fluxomics**

Guy Lippens, Laboratoire d'ingénierie des Systèmes Biologiques et des Procédés, Toulouse, France O10

10h05-10h25 **From a metabolomics research study to ISO17025 method accreditation**

Anne-Lise Royer, LABERCA, Oniris, INRA, Université Bretagne-Loire, France O11

10h25-10h40 **About the use of surface-enhanced Raman scattering to monitor the cellular release of neurotransmitters.**

Elodie Dumont, CIRM, VibraSanté HUB, Université de Liège, Belgique OJ3

10h40-11h10 Pause-Echanges informels-Visite des stands

11h10-12h20 Session Agro-ressources et Agro-alimentaire

Chairmen: Alain Bouchereau, Rennes, France et Camille Rozier, Lyon, France

11h10-11h30 **Message in a bottle: metabolomics for deciphering the transient chemistry of wines and spirits**

Régis Gougeon, Institut Universitaire de la Vigne et du Vin, Université de Bourgogne, France O12

11h30-11h50 **Metabolomic and transcriptomic networks supporting nitrogen use and remobilization efficiencies in two oilseed rape genotypes**

Sylvain Dechaumet, Institut de Génétique, Environnement et Protection des Plantes, Université de Rennes, France O13

11h50-12h05 **Comparative study of primary metabolism in diverse fleshy fruit species**

Léa Roch, UMR1332, Biologie du Fruit et Pathologie, Université de Bordeaux, France OJ4

12h05-12h20 **Valorization of waste from the Malagasy hemp industry by metabolomics tools**

Alexandra Berlioz-Barbier, BRUKER, Lyon, France OS3

12h20-12h30 Session flash 2 (3 présentations)

Modérateurs: Floriant Bellvert et Pascal de Tullio

Keep improving chemical identification using tandem mass spectrometry data in a pharmaceutical context

Youzhong Liu, Department of Mathematics and Computer Science, University of Antwerpen, Belgique F4-P4

A NMR-based metabolomics study of minced pork meat inoculated with Brochothrix thermosphacta, Leuconostoc gelidum and Pseudomonas fragi

Emilie Cauchie, Food Science Department, Université de Liège, Belgique F5-P5

Cold shock metabolomic response of bacteria from clouds

Cyril Jousse, Plateforme d'Exploration du Métabolisme, MetaboHUB Clermont-Ferrand, Université Clermont Auvergne, France F6-P6

12h30-13h20 Déjeuner

13h20-14h20 Session poster 2

14h20-15h05 Conférencier invité

Chairwoman: Estelle Pujos-Guillot, Clermont-Ferrand, France

Deciphering the role of mitochondrial dysfunction in diseases: What can we learn from metabolomics?

Prof. Christine Des Rosiers, *Institut de cardiologie de Montréal et Université de Montréal*, Canada OP4

15h05-16h00 Session Santé 2

Chairwomen: Justine Bertrand-Michel, Toulouse, France et Justine Leenders, Liège, Belgique

15h05-15h25 **A Plasma Metabolomic Signature Involving Purine Metabolism in Human Optic Atrophy 1 (OPA1)-Related Disorders.**

Cinzia Bocca, Equipe Mitolab, Institut MITOVASC – UFR Santé Université d'Angers, France O14

15h25-15h45 **Identification de biomarqueurs dans le trouble de déficit d'attention avec sans hyperactivité.**

Camille Dupuy, Imagerie et cerveau, Université de Tours, France O15

15h45-16h00 **Communication sponsor WATERS**

OS4

16h00-16h30 Pause-Echanges informels-Visite des stands

16h30-17h25 Session Environnement et microbiologie 3

Chairmen: Cédric Bertrand, Perpignan, France et Guillaume Meiffren, Lyon, France

16h30-16h50 **The hidden life of flavonoids: a new role in heavy metals tolerance and in plant adaptation to extreme metal environment.**

Massimiliano Corso, Université Libre de Bruxelles, Belgique O16

16h50-17h10 **Mercaptomic: towards the profiling of reactive metabolites in toxicology**

Emilien jamin, Toxalim – Institut national de la recherche agronomique, Toulouse, France O17

17h10-17h25 **Investigation of the effects of a wastewater treatment plant effluent on the marine bivalve *Mytilus galloprovincialis* through a metabolomics approach**

Thibaut Dumas, Hydrosciences Montpellier (HSM), Université de Montpellier, France OJ5

17h25-18h05 Session Membres d'honneur

Chairmen: Fabien Jourdan, Président du RFMF et Sandrine Aros, Présidente du RFMF Junior

17h25-17h45 **NMR-based metabolomics workflow for quality and effect assessment of alternative plant-based diets in rainbow trout.**

Catherine Deborde, Plateforme Métabolome Bordeaux, MetaboHUB, CGFB, INRA, France OH1

17h45-18h05 **Response to uranium stress within *Microbacterium* bacteria: metabolomic analysis by NMR and LC-MS.**

Alain Paris, UMR 7245 - Molécules de Communication et Adaptation des Micro-organismes (MCAM) – Museum National d'Histoire Naturelle, France OH2

18h10-19h30 Assemblée générale du Réseau Francophone de Métabolomique et de Fluxomique junior

20h00-23h00 Repas de Gala et remise des prix

Palais Provincial de Liège

Vendredi 25 mai 2018

9h45-10h30 Conférencier invité

Chairman: Patrick Giraudeau, Nantes, France

Biomedical NMR metabolomics across time scales

Prof. Bénédicte Eléna-Herrmann, *Institut for Advanced Bioscience, Université de* Grenoble, France OP5

10h30-11h55 Session Santé, Développement et Agro-ressources

Chairmen: Marianne Fillet, Liège, Belgique et Cédric Delporte, Bruxelles, Belgique

10h30-10h50 **A new comprehensive GC/GC-HRTOFMS approach in metabolomics**

Nicolas Di Giovanni, Organic and Biological Analytical Chemistry Group, Université de Liège, Belgique O18

10h50-11h20 Pause

11h20-11h40 **Intérêt des méthodes en « omique » pour accéder aux modifications précoces du métabolisme énergétique chez la chèvre laitière en *peri-partum*.**

Céline Domange, Modélisation Systémique Appliquée aux Ruminants, AgroParisTech, France O19

11h40- 11h55 **La métabolomique comme outil d'étude de l'évolution de *Pseudomonas aeruginosa* au cours des infections pulmonaires chroniques dans la mucoviscidose**

Oriane Moyne, Laboratoire TIMC-IMAG équipes TheRex et EPSP, Université de Grenoble, France OJ6

11h55-12h05 Remise du prix de thèse 2018

Lauréate : Baninia Habchi, pour ses travaux de thèse portant sur la «Mise en évidence des perturbations métaboliques liées à l'exposition aux toxiques présents dans l'environnement ou l'aliment par spectrométrie de masse à ultra haute résolution FTMS combinée avec des outils chimiométriques »

12h05-12h25 Communication du prix de thèse 2018

12h25-12h40 Clôture des Journées et présentation des 12éme journées

Ateliers

Atelier 1 : Présentation et application de PepsNMR, un package R pour le prétraitement de spectres 1 H-NMR.

Personnes encadrant la formation :
Manon Martin - Correspondant atelier - manon.martin@uclouvain.be
Bernadette Govaerts

Public envisagé et les prérequis :
- Public : étudiants, ingénieurs et chercheurs travaillant en RMN.
- Prérequis : notions de prétraitement en métabolomique RMN, notions de R.

Objectif de la formation :
L'objectif de cette formation est entre autres d'expliquer (ou rappeler) les objectifs des différentes étapes du prétraitement en RMN, partant de signaux FID jusqu'à une table finale de spectres RMN 1D prêts à être analysés par des méthodes chimiométriques. Cette formation permettra également de présenter les outils de prétraitement originaux disponibles dans le package R PepsNMR (e.g. suppression signal de l'eau, correction de la ligne de base, warping, bucketing, etc.) ainsi que des méthodes d'évaluation de la qualité du prétraitement. L'ensemble de ces outils sera mis en œuvre par les participants sur des données métabolomiques.

Compétences acquises à la sortie de la formation :
- Compréhension globale des différentes étapes de prétraitement des données RMN 1D
- Capacité d'appliquer le package de prétraitement PepsNMR à d'autres jeux de données

Durée de la formation : 2,5 heures.

Date et lieu : Mardi 22 mai 2018, de 12h30 à 15h, à la salle Philo 2 (Université de Liège, Faculté de Philo et Lettres, Place du XX août)

Nb de places max : 30 personnes max.

Attention les participants devront venir avec un ordinateur avec R installé sur les machines, installation des packages PepsNMR, MBXUCL. Les instructions d'installation des packages seront envoyées aux participants préalablement à la formation.

Atelier 2 : Outils d'annotation de données MS/MS : applications pratiques

Personnes encadrant la formation :
Etienne Thévenot (CEA, Saclay)
David Touboul (CNRS, ICSN)
Alexis Delabrière (CEA, Saclay)
Yann Guitton (LABERCA, Nantes) - Correspondant atelier - yann.guitton@oniris-nantes.fr
Emilien Jamin (INRA, Toulouse)
Grégory Genta-Jouve (Université Paris-Descartes)

Public envisagé et les prérequis :
- Public : étudiants et permanents confrontés à l'annotation de données MS/MS
- Prérequis : connaissances basiques en spectrométrie de masse tandem et chromatographie

Objectif de la formation :
L'objectif est d'acquérir un nouveau savoir-faire dans le domaine de l'annotation de spectres MS/MS. Cet atelier fait suite à celui organisé durant SMMAP2017 qui a attiré plus de 60 participants et qui était basé sur la description des méthodes d'annotation in silico des données MS/MS. Le but est cette fois de proposer un atelier plus interactif avec la mise en pratique des outils permettant aux participants de s'affranchir de la barrière de découverte des interfaces et du paramétrage parfois complexe.

Compétences acquises à la sortie de la formation :
Une compréhension générale de l'utilisation des outils d'annotation MS/MS sera acquise par les participants avec un focus particulier sur 2 ou 3 outils (logiciel de fragmentation in silico et outil de classification des données MS/MS par homologie) afin de permettre une mise œuvre efficace dans les laboratoires.

Durée de la formation : 2,5 heures.

Date et lieu : Mardi 22 mai après-midi de 12h30 à 15h, salle Lumière (Université de Liège, Faculté de Philo et Lettres, Place du XX août)

Atelier 3 : La quantification en métabolomique

Personnes encadrant la formation :
Justine Bertrand-Michel - Correspondant atelier - justine.bertrand-michel@inserm.fr
Marlène Lacroix
Cécile Canlet

Public envisagé et les prérequis :
Public : étudiants, Ingénieurs et chercheurs
Prérequis : notion de métabolomique et de spectrométrie de masse

Objectif de la formation :
L'objectif est de sensibiliser les participants aux notions de quantification en métabolomique ciblé ou non ciblée en spectrométrie de masse et en RMN 1D. Il sera présenté les différents modes de quantification (absolue, relative), les pré-requis, les limitations... Des modes opératoire seront présentés et discutés.

Compétences acquises à la sortie de la formation :
Acquérir et comprendre les notions théoriques de la quantification en métabolomique, et avoir un regard critique sur sa pratique.

Durée de la formation : 2,5 heures.

Date et lieu : Mardi 22 mai de 15h15 à 17h45, salle Philo 2 (Université de Liège, Faculté de Philo et Lettres, Place du XX août)

Atelier 4 : méta-métabolomique, discussion autour des méthodes d'analyse des données. Focus sur les aspects multiblock, multitableaux.

Personnes encadrant la formation / animant la table ronde:
Cyril Jousse - Correspondant atelier - cyril.jousse@uca.fr
Jean-Charles Martin,
Binta Dieme,
Gabriel Markov - Correspondant atelier - gabriel.markov@sb-roscoff.fr
Julien Boccard

Public envisagé et les prérequis :
- Public : Etudiants, personnel technique et chercheurs.
- Prérequis : notions de métabolomique et d'écologie.

Objectif de la table ronde:
PREMIER TEMPS (45 minutes) : Présentation des outils et méthodologies d'analyse des données en métabolomique.
DEUXIEME TEMPS (1 heures) : Discussion sur les objectifs et potentialités dans le cadre de données de méta-métabolomique.
TROISIEME TEMPS (45 minutes) : Discussion autour d'un groupe de travail pour le déploiement d'un workflow d'analyse des données en méta-métabolomique.

Compétences acquises à la sortie de la formation :
Pour l'ensemble des participants : une compréhension générale des outils et methodologies.
Pour le groupe de travail : démarrage d'un plan d'action en faveur du développement d'un workflow.

Durée de la formation : 2.5 heures.

Date et lieu : Mardi 22 mai de 15h15 à 17h45, salle Lumière (Université de Liège, Faculté de Philo et Lettres, Place du XX août)

Table ronde Junior : Interventions et discussions autour des problématiques propres aux juniors dans les domaines de la métabolomique et de la fluxomique

Personnes animant la table ronde :
Grégory Genta-Jouve
Sandrine Aros

Public envisagé et les prérequis :
-Public : adhérents de – 33 ans au 1er janvier 2018
-Prérequis : aucun

Objectif de la formation :
Discussion / échanges autour des problématiques rencontrées par les juniors (dans les domaines de la métabo / fluxo) pour s'apporter des solutions ou conseils.

Compétences acquises à la sortie de la formation :
- Avoir des éléments d'informations pour optimiser la construction de son réseau professionnel et de son projet scientifique en métabolomique et fluxomique.
- Avoir une vision plus élargie des questions et problèmes rencontrés par les juniors.

Durée de la formation : 2 heures.

Date et lieu : Mardi 22 mai de 18h à 20h, salle Lumière (Université de Liège, Faculté de Philo et Lettres, Place du XX août)

http://www.rfmf.fr/rfmf-junior.html


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JOURNÉES
SCIENTIFIQUES

Résumés

Communications Speakers invites (OP)

OP1

Opening new fields of natural products research by metabolomics tools

Young Hae Choi

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Natural products are the basis of our life providing food, shelter, fuel, medicines, clothes, agrochemicals, cosmetics, and so on. Furthermore, they are still the most valuable resource of bioactive compounds in modern life sciences, accounting for over 50% of the new medicines currently on the market. This is mainly due to the chemical diversity offered by these natural resources. However, this enormous diversity and the dynamic ranges of metabolites levels has posed a real challenge to natural product research particularly when attempting to profile all metabolites in a given organism or biological material. The first step of natural product utilization is profiling existing chemicals but this is a big obstacle for the research. One of the possible solutions to get the full information may lie in metabolomics, an emerging field of “omics” technology aimed at the comprehensive profiling of (ideally) all the metabolites in an organism providing a view of the whole metabolic network. As an essential element of systems biology, the possibility of capturing holistic information of the complex metabolic system in an unbiased manner has been a dream of natural products researchers for a long time.

In the last decades, metabolomics has developed rapidly, being applied in a vast range of life sciences such as functional genomics, chemotaxonomy, quality control of medicinal plants, crop improvement and pest management or as a tool in the bioassay-guided fractionation of extracts.

Having the ambitious goal of obtaining information on all metabolites creates the need to count on the most advanced technology and techniques for pre-analytical treatment, detection and data handling of information as well as fine tuning in terms of full compound identification and their localization.

Although metabolomics is initially expected to fulfill the profiling of natural products, additional scientific benefits are found by extensive works in these days e.g. proving synergism, interaction between organism and deconvolution of physiological roles of metabolites in nature.

In this presentation, I will review the current metabolomics methods and applications based on past research activities and the potential application of metabolomics, highlighting its role in the future of natural products research.

OP2

Metabolomics at 5000m and other stories: Applications of metabolomics to understanding human physiology.

Jules Griffin,

Department of Biochemistry, University of Cambridge, Cambridge, UK

Diet is a major contributor to metabolic disease risk and we have been using a combination of both targeted and untargeted metabolomics/lipidomics to examine the interaction between diet and disease in human populations to better understand diet's role. To investigate how diet influences the development of non-alcoholic fatty liver disease we applied direct infusion mass spectrometry of lipids in plasma to study the association between the lipidome and hepatic steatosis assessed by ultrasound in volunteers from the UK-based Fenland Study (n=1507), and relate associations with food intake. A cluster of triacylglycerols (TAGs) containing saturated and monounsaturated fatty acids with 16-18 carbons were associated with hepatic steatosis, higher consumption of carbohydrate and variations in the gene for protein phosphatase 1, regulatory subunit 3b (PPP1R3B), which regulates partly glycogen synthesis. In an alternative metabolomic approach to human disease we have applied targeted metabolomics to profile core metabolism in patients with aortic stenosis and hypertrophic cardiomyopathy. Models based on random forest analysis readily separated the two disease groups, highlighting a reduction in fatty acid metabolism in aortic stenosis in the form of the accumulation of long chain carnitines in blood plasma. Finally, I will discuss a third human study where we investigated how humans adapt to high altitude by profiling volunteers during their ascent to Everest Basecamp. Here we find that alterations in the activity of the transcription factor PPAR-α, an important nutritional sensor, relate to the balance between carbohydrate and fatty acid metabolism, and provide a selective advantage in those of Sherpa ethnicity when compared with a largely Caucasian population.

OP3

From multivariate to multiblock: tackling data structures in Metabolomics

Serge Rudaz

School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland

Due to the ever-increasing number of signals that can be measured, life sciences datasets not only become gradually larger, but also more intricate in their structures. Challenges related to making use of this wealth of data include extracting relevant elements within massive amounts of signals possibly spread across different tables, reducing dimensionality, summarizing dynamic information in a comprehensible way and displaying it for interpretation purposes. Metabolomics constitutes a representative example of fast moving research fields taking advantage of recent technological advances to provide extensive sample monitoring. Since several years, the integration and visualization of multivariate datasets constitute key issues for effective analysis leading to valuable biological or chemical knowledge. Factorial methods based on the computation of latent variables or components, such as principal component analysis (PCA) and partial least squares (PLS) regression constitute potent tools to provide compact data representations and diagnostic tools for the detection of relevant variables. Nevertheless, most of these approaches lack the ability to fully exploit very high dimensionality or complex data structures such as (1) multifactorial, (2) longitudinal and (3) multiblock setups. As presented in this lecture through examples, accounting properly for the structure and inherent properties of metabolomic datasets is mandatory for harnessing their complexity and provide relevant information. In that perspective, chemometrics has a central role to play in the choice of an appropriate methodology to cope with multivariate datasets originating from experimental design, high-order data structures involving time-resolved measurements and multiblock data collections extending metabolite coverage.

OP4

Deciphering the role of mitochondrial dysfunction in diseases: What can we learn from metabolomics?

Christine Des Rosiers

Institut de cardiologie de Montréal et Université de Montréal, Canada

Biosketch:

Dr. Christine Des Rosiers is a Professor in the Department of Nutrition of the *Université de Montréal* and Director of the Montreal Heart Institute Research Centre Metabolomic Laboratory and Platform. She is a founding member of the Society for Heart and Vascular Metabolism and is currently serving as President since 2015. The focus of her research is on the role of metabolic alterations in the pathogenesis of disease, particularly heart disease. She has over 25 years of research experience in metabolic investigations using stable isotopes and mass spectrometric-based methodology. She specifically gained recognition for the development of these methods for the metabolic and functional phenotyping of the *ex vivo* working mouse heart. More recently, she has taken the direction of metabolomic initiatives as part of multidisciplinary translational projects aiming at the discovery of biomarkers of disease development or treatment response in various conditions, which include heart disease, but also diabetes, as well as mitochondrial and inflammatory diseases.

OP5

Biomedical NMR Metabolomics Across Time Scales

Bénédicte Elena-Herrmann[1,2]

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Nuclear Magnetic Resonance (NMR) is a versatile technique that can contribute to biological investigations across time scales as a powerful metabolic phenotyping platform.

Real-time NMR investigation of metabolites kinetics in hybrid cell-free protein synthesis systems will first illustrate how the detailed and dynamic NMR metabolic fingerprints can provide a unique analytical platform for synthetic biology [1]. We will then explore translational studies in cancerology where longitudinal investigations of biological fluids are employed to probe the dynamic changes of the host metabolism associated with targeted therapies or to predict at baseline the clinical response to chemotherapy [2]. Molecular epidemiology investigations carried out in prospective context will finally illustrate how metabolic profiles can reflect cancer risks in large human cohort studies.

References:

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[2]. Jobard E., Trédan O., Bachelot T., Vigneron A. M., Aït-Oukhatar C. M., Arnedos M., Rios M., Bonneterre J., Diéras V., Jimenez M., Merlin J.-L., Campone M. & Elena-Herrmann B. *Oncotarget* 8(48): 83570–83584 (2017).

Mots-Clés : NMR, public health

Communications Orales

Les résumés de l'ensemble des communications orales figurant sur le programme

(Senior, Membres d'honneur, Junior et Flash)

O1

Study of the environmental impact of Bti vs. chemical insecticides by metabolic foot-printing approach.

Patil Chandrashekhar [1], Amani Ben Jrad [1], Hikmat Ghosson [1], Delphine Raviglione [1], Marie-Virginie Salvia [1], Cédric Bertrand [1]

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Mosquitoes can carry infectious diseases from person to person and from place to place. Presence and establishment of invasive mosquito species such as Aedes aegypti and Aedes albopictus is rapidly increasing in the European environment. The most efficient means to fight against mosquitos is the use of insecticides. A lot of chemical insecticides were developed. First of all, organochlorine compounds such as DDT were used. However, most of these substances were then removed from the market as they showed severe risks for the human and animal health as well as for the ecosystem.

The European Directive in 1998 led to the increasing use of biological insecticides such as cry proteins produced by the bacterium Bacillus thuringiensis israelensis (Bti) that kill mosquito larvae after being ingested. Considering the interest in Bti as more environmentally sustainable bioinsecticide, it is important to examine in detail environmental fate and impact of Bti. The available tool such as half-life, t1/2, does not consider the biodegradation and biotransformation phenomenon of complex formulations. To address this challenge, 'Environmental Metabolic Footprinting' (EMF), giving an idea of the resilience time was recently developed in the laboratory (Patil et al. 2016; Salvia et al, 2017) to evaluate the impact of synthetic, botanical and microbial insecticides on soil and sediment matrix respectively.

The project 'EnvFate' aims to employ an EMF approach, to dynamically characterize environmental markers of Bti pollution found among the sediment matrix meta-metabolome. Metabolome characterization will require to develop and optimize extraction and detection protocols using LC-MS platform. In addition, the metabarcoding approach will allow to understand microbial community responses to the Bti pollution. Emphasis will be placed on better standardisation, data interpretation and evaluation that will build confidence in the value of "omics technologies – this being essential to increase their (regulatory) use. These activities will advance our understanding of environmental risks associated with Bti, and pave the way for the development and adaptation of new environmental monitoring tools.

We will present sensible preliminary findings from metabolomics data. The changes in the meta-metabolome after Bti and -cypermethrin treatment is investigated at various time intervals and compared with control in order to evaluate the resilience time of the sediment.

O2

Etude par métabolomique et protéomique de la réponse de la bactérie marine Pseudoalteromonas lipolytica TC8 à un stress cuprique

Laurie Favre [3;2;1], Annick Ortalo-Magné [1], Kerloch Lionel [1], Carole Pichereaux [4], Benjamin Misson [5], Cédric Garnier [5], Jean-François Briand [1], Gérald Culioli [1]

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[4] Fédération de Recherche FR3450 – CNRS – France
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La Rade de Toulon concentre le plus important port militaire français, un port de ferries ainsi qu'un port de plaisance. Il s'agit d'un site maritime multi-contaminé pour lequel les polluants métalliques constituent la source majeure de contamination (1). Une telle anthropisation des écosystèmes marins impacte les organismes en termes de croissance, de développement, d'immunité et de reproduction (2). Le cuivre, présent à hautes concentrations dans les revêtements antifouling, est ainsi particulièrement connu pour provoquer des dégradations irréparables dans les écosystèmes marin (3). Néanmoins, certains organismes, et notamment certaines classes bactériennes, semblent s'accommoder mieux que d'autres de la présence de hautes teneurs en ce métal. Afin de mieux appréhender l'effet d'une exposition à d'importantes concentrations en cuivre sur la réponse physiologique (croissance, profils métabolique et protéique) de bactéries marines, une souche issue d'un biofilm naturel prélevé en zone contaminée (Pseudoalteromonas lipolytica TC8) a été sélectionnée. L'étude a débuté par la détermination des concentrations en cuivre effectives inhibant sa croissance ou provoquant sa mort. Dans un second temps, une étude conjointement menée par métabolomique et protéomique a été réalisée afin d'évaluer l'impact sur le métabolome et le protéome de P. lipolytica TC8 de concentrations élevées en cuivre (sublétales) en fonction de son mode de culture (biofilm ou planctonique). En présence de cuivre, la plus grande variation du métabolome est observée au niveau des cultures planctoniques. Cela se traduit au niveau membranaire par une diminution drastique de la production d'ornithine lipides (OLs) et une augmentation des phosphatidyléthanolamines (PEs).

L'étude comparative des métabolomes en absence de cuivre ayant préalablement révélée des différences significatives de production d'OLs et de PEs entre les deux modes de culture, l'hypothèse d'un changement de métabolisme induit par le cuivre des cellules planctoniques vers un phénotype biofilm a été émise. Au niveau du protéome, la présence de cuivre dans le milieu de culture a un effet significatif sur l'abondance de transporteurs membranaires de type RND ou ABC ainsi que sur celle des ATPases de type P ou encore d'enzymes ayant un rôle de protection contre le stress oxydatif ; de telles protéines étant connues pour être impliquées dans l'homéostasie cellulaire du cuivre.

(1) Tessier et al., Mar Pollut Bull 2011, 62 (10), 2075–2086
(2) Menezes-Oliveira et al., Sci Total Environ 2013, 458 –460, 361–366
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O3

Métabolomique des interactions plantes/ bactéries dans la rhizosphère : activité modulatrice de la plante hôte sur le métabolisme secondaire bactérien

Laura Rieusset[1], Claire Prigent-Combaret [2], Gilles Comte [2]

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Le sol adhérant aux racines des plantes abrite une communauté bactérienne importante et diversifiée. La plante libère, en effet, au niveau de ses racines, des exsudats qui vont sélectionner des bactéries capables de les utiliser. En retour, certaines bactéries sont capables d'entretenir des relations de coopération avec la plante, en stimulant sa croissance et en la protégeant contre des pathogènes. Ces bactéries phytobénéfiques dites PGPR (Plant Growth-Promoting Rhizobacteria) peuvent également interagir entre elles au sein de la communauté.
Le but de notre projet est d'évaluer l'impact des métabolites racinaires d'une plante hôte sur le métabolisme secondaire de bactéries PGPR appartenant au groupe des Pseudomonas fluorescents.

Pour répondre à ces objectifs, nous avons procédé à une extraction des métabolites secondaires de 3 génotypes de blés différents et testé l'impact de ces extraits sur la croissance et le métabolisme secondaire de plusieurs souches PGPR.
Les données obtenues après comparaison des métabolomes des bactéries seules ou en présence des extraits, ont permis de mettre en évidence une modulation du métabolisme secondaire bactérien par les métabolites racinaires du blé. Ces résultats montrent que la plante hôte serait capable de moduler la production plusieurs composés bactériens présentant des activités antimicrobiennes, sidérophores ou encore inconnues. De façon intéressante, il semble que les génotypes n'aient pas le même effet sur toutes les souches, suggérant une spécificité d'interaction entre les génotypes de blé et les souches de Pseudomonas fluorescents.

O4

Metabolic reprogramming in IDH1 Mutant Acute Myeloid Leukemia Cells

Lucille Stuani [1], Marie Sabatier [1], Estelle Saland [1], Lara Gales [2], Tony Palama [2;3], Floriant Bellvert [2;3], Pierre Millard [2], Jean-Emmanuel Sarry [1], Jean-Charles Portais [2;3]

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The cytosolic form of NADP+-dependent isocitrate dehydrogenase (IDH1) is one of the key metabolic enzymes critically important in cellular defense against oxidative damage, maintenance of the cytosolic redox state, and for numerous anabolic pathways required to support cell growth. We hypothesized that metabolic reprogramming is redirected to markedly increase multiple catabolic pathways to sustain cell proliferation in IDH1 R132H mutant AML cells. To address the role of catabolism, we have used an integrative approach combining metabolomics, fluxomics using ^{13}C-substrate-labeled cultures, transcriptomics and enzyme activity profiling coupled to the use of engineered cell lines and patient specimens in vitro and in vivo. We have observed that metabolic reprogramming initiated by IDH1 mutation leads to marked increases in catabolism (glucose, pyruvate, glutamine, fatty acids) that along with the enhancement of wild-type IDH enzyme activity contributes to provision of a-KG to sustain 2-HG biosynthesis and replenish other Krebs cycle intermediates required for biosynthetic reactions, oxygen consumption and ATP production by oxidative phosphorylation. Nutrient starvation or inhibition of metabolic pathways modulates 2-HG production that does not necessarily correlate with IDH1 mutant cell proliferation and survival. Catabolic flexibility increases mitochondrial FA oxidation and increases Krebs cycle flux and OxPHOS activities/dependency in IDH1 mutants. Accordingly, we find that IDH1 mutant cells are more resistant to chemotherapies. Our studies provide the scientific rationale for clinical trials of innovative combinatory targeted therapies to selectively treat this subset of patients, especially those insensitive to newly-FDA approved IDH mutant-specific inhibitors.

O5

Exudative Age-Related Macular Degeneration: from metabolomics to the identification of lactate as a key functional and targetable metabolite for personalized medicine

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Exudative age-related macular degeneration (AMD) is the most severe form of this pathology and the leading cause of blindness in the aging population. It is characterized by the occurrence of a choroidal neovascularization (CNV) and may be related to an inflammatory status. Treatments are mainly based on regular intravitreal injection of anti-VEGF to stabilize CNV. Nevertheless, despite such important advances, some clinical issues remain to be addressed. Among those, the personalization of the therapeutic strategies and the discovery of new therapeutic approach are essential. In order to study CNV occurrence and evolution, we decided to apply a NMR-based metabolomics approach on a murine laser-induced CNV model and on patient's cohorts. Metabolomics led us to identify some metabolites linked to CNV developments in both human and murine samples. These metabolites could be considered not only as markers of the pathology but also as putative target for a new treatment of AMD. Among those, lactate emerges as a key metabolite in both settings. In the mice model, this metabolite could be associated with CNV while higher lactate levels were detected in patients and associated with bleeding phases. Mechanistically, we demonstrated that lactate, initially produced in the eyes increases at the systemic level and plays a critical role in the onset of the inflammatory and angiogenic phases. Pharmacological normalization of lactate levels by blocking pyruvate deshydrogenase kinase or by modulating LDH activity, inhibits CNV formation, demonstrating the therapeutic potential of our finding. Moreover, CNV inhibition by anti-angiogenic drugs led also to a reduction of lactate level, providing unprecedented options for AMD patient follow-up during treatment. All together, we demonstrated that metabolomics is a suitable tool to deep insight AMD and our findings identify lactate as a functional, traceable and targetable molecule and open new perspectives for optimizing and personalizing AMD treatment.

O6

Consequences of blunting the mevalonate pathway in cancer identified by a pluri-omics approach

Corcos Laurent[1]

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We have previously shown that the combination of statins and taxanes was a powerful trigger of HGT-1 human gastric cancer cells' apoptosis 1. Importantly, several genes involved in the "Central carbon metabolism pathway in cancer", as reported in the Kyoto Encyclopedia of Genes and Genomes, were either up- (ERBB2, SLC16A3, GCK, LKB2, MYC) or down- (P53, PDK, MDH1, OGDH) regulated in response to the drug association. In the present study, we conducted non-targeted metabolomics and lipidomics analyses by complementary methods and cross-platform initiatives, namely mass spectrometry and nuclear magnetic resonance, to analyze the changes resulting from these treatments. We identified several altered biochemical pathways involved in the anabolism and disposition of amino acids, sugars and lipids. Using the Cytoscape environment with, as an input, the identified biochemical marker changes, we distinguished the functional links between pathways. Finally, looking at the overlap between metabolomics/lipidomics changes and transcriptome alterations, our analyses pointed to correlations between gene expression modifications and changes in metabolites/lipids within several metabolic pathways, including the "Alanine, Aspartate and Glutamate metabolism", the "Pantothenate and Acetyl-CoA biosynthesis", the "Butanoate metabolism", the "Sulfur metabolism" and the "Arginine biosynthesis" pathways. Taken together, our results demonstrated that this combination of robust biochemical and molecular analytical approaches was efficient to identify both altered metabolic pathways and overlapping gene expression changes in human gastric cancer cells engaging into apoptosis following blunting the cholesterol synthesis pathway.

O7

Inferring new biochemical reactions and new metabolite structures to cope with metabolic pathway drift in emerging model organisms

Gabriel Markov [1], Jean Girard [2], Meziane Aite [3], Arnaud Belcour [4], Camille Trottier [3], Charlotte Marteau [5], Cédric Leroux [6], Ludovic Delage [2], Simon Dittami [2], Pierre Sauleau [5], Erwan Corre [7], Jacques Nicolas [4], Catherine Boyen [2], Catherine Leblanc [2], Anne Siegel [3], Jonas Collén [2]

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Inferring genome-scale metabolic networks in emerging model organisms is challenging because of incomplete knowledge on their biochemistry and incomplete conservation of biochemical pathways during evolution, that limits the possibility to automatically transfer knowledge from well established model organisms. Using an integrative approach combining both genomic and metabolomic data in the model red alga Chondrus crispus, we show that even metabolic pathways considered as conserved like sterol or mycosporine-like amino acids synthesis pathways undergo substantial turnover in terms of enzymes, catalytic activities for orthologous enzymes, and succession of steps necessary to form final metabolites. This phenomenon, that we here formally define as ”metabolic pathway drift”, is consistent with findings from other areas of evolutionary biology, indicating that a given phenotype can be conserved even if the underlying molecular mechanisms are changing. Therefore, specific bioinformatic tools are necessary to infer *ab initio* new biochemical reactions and new metabolic structures that can be checked further experimentally. Here we present a proof of concept with Pathmodel, a new program developped to formalize the logical reasoning necessary to infer new reactions and new molecular structures in the sterol and mycosporine-like amino acid biosynthesis pathways, based on previous biochemical knowledge on those pathways in other organisms.

O8

Getting more from less: optimizing the investment of analytical resources in metabolomic studies by using a scoring algorithm

Julian Pezzatti [1], Víctor González-Ruiz [2;1], Santiago Codesido [1], Yoric Gagnebin [1], Julien Boccard [1;2], Serge Rudaz [1;2]

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Metabolomic sciences aim at the study of low molecular weight molecules in different biological matrices. Although the investigation of the whole metabolic content can be achieved by means of untargeted approaches, the annotation of relevant metabolites is necessary for an optimal interpretation of the biological information present in the studied systems [1]. To make possible the annotation of features, databases gathering different chemical properties of standards are often measured in-house to achieve the reliability of the identification process.
LC-HRMS approaches are used in the context of untargeted workflows acquisitions. Taking into account the fact that human metabolites span a wide range of polarity values, RPLC is the option of choice for the mid- to apolar metabolites and HILIC for the polar or ionized compounds [2]. This combination of LC techniques is also generally implemented with electrospray ionization in both positive and negative modes. Similar strategies are often used to approach the chemical complexity represented by biological samples.
Although the application of all the implemented LC-HRMS techniques will always provide the most comprehensive set of chemical information from the specimens, the simultaneous deployment of all the analytical methods will be seldom possible due to the limitations in terms of time, human and material resources. Therefore, which combination of LC and ionization techniques is the most informative one becomes a pivotal question to optimize the analytical investment. Herein, we introduce an ad-hoc developed algorithm allowing to answer this question by integrating different measurable parameters of the analytical response obtained for each analyte on each technique.
To demonstrate the validity of the algorithm, a chemical library comprising 500 human endogenous compounds was analyzed under five different LC-HRMS conditions. Derringer's functions were established for every detected compound based on three main criteria, namely: retention, sensitivity (signal to noise ratio) and peak shape. Scores obtained from the desirability functions allowed to note the different LC-HRMS acquisition performance according to their suitability to analyze each compound. By applying the scoring function, it was possible not only to find the optimal combination of LC methods to maximize the output-to-investment ratio of the analyses, but also to select univocally annotated features thanks to the scores for further data treatment. With the help of two or three complementary methods out of the five analytical methods and an appropriate features selection, the amount of identified metabolites obtained decreased by only 10% while the analysis time dropped by more than 40%.

O9

Volumetric absorptive microsampling for targeted metabolomics of whole blood

Miranda Kok[1], Gwenaël Nys[1], Marianne Fillet[1]

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Volumetric absorptive microsampling (VAMS) enables the collection of small and accurate quantities of biological fluids. Therefore, this sampling technique is of great interest for volume-limited samples or serial collection of samples.
Here, we present and discuss the potential of VAMS for targeted mass spectrometry (MS)-based metabolomics. In total, 24 amino acids and 12 organic acids were selected as target metabolites. Two ultra-high performance liquid chromatography (UHPLC) methods coupled to tandem MS have been developed and optimized for the separation and quantitation of these metabolites. A reversed-phase UHPLC-MS/MS method was used to analyze organic acids, whereas hydrophilic interaction chromatography (HILIC)-MS/MS was selected for the determination of amino acids.

VAMS devices were used to collect 10 L of whole blood via a simple finger prick. After collection, the samples were dried for two hours before sample preparation. A design of experiments was conducted to find an extraction solvent providing the maximum recovery of the target analytes from the dried VAMS samples. Overall, the optimum extraction solvent was acetonitrile-water in a proportion of 60:40 (v/v), resulting in the detection of all target metabolites in whole blood with good repeatability.
Furthermore, the stability of the analytes in dried whole blood supported on VAMS devices was investigated. We showed that the amino and organic acids were stable for at least 26 hours when stored at room temperature. This is in contrast to the instability of these compounds in blood, thereby showing great possibilities of VAMS in metabolomics studies.

O10

Improved NMR methods for ^{13}C NMR based fluxomics

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Measuring the carbon isotopic content of different metabolites is a powerful tool to quantify fluxes in living systems. Spanning the different forms of life, the approach has found applications in the study of bacteria, yeast, plants, or humans. In the latter case, the altered metabolism of cancer cells has attracted significant interest. Two main techniques used for such analysis are mass spectroscopy and NMR, and recent trends integrate both methods. Whereas the former has the main advantage of tremendous sensitivity, allowing the determination of isotope distribution of ever smaller samples, determining the exact position of the ^{13}C nucleus in a given metabolite remains challenging. NMR spectroscopy on the other hand suffers from low sensitivity, but has – at least in principle – the possibility to determine the ^{13}C content at the individual positions of a metabolite.

We introduce the pure shift method in the heteronuclear 2D J-resolved (2DJ) pulse sequence that was previously proposed for 13C fluxomics. The measurement of ^{13}C incorporation in the aliphatic side chains of the branched chain amino acids (BCAA) Ile, Leu and Val, residues obtained from a bacterial culture grown on U-^{13}C labeled glucose with unlabeled Thr underscores the superior resolution of the pure shift 2DJ experiment, and allows quantitative elucidation of the biosynthetic pathway of the BCAA in the absence of protein over-expression, thereby providing novel biochemical insight.

We further explore how 15N-based NMR experiments can be used in the field of fluxomics when rapid quantification is of prime importance. Borrowing from protein NMR, these novel experiments can quantify the control exerted by the first enzyme of the pentose phosphate path-way (G6PDH) on the glycolytic and pentose phosphate flux in Escherichia coli. Estimation of these fluxes is based on the absolute quantification of the four isotopic species for the (Cα, CO) two-carbon block of leucine. We demonstrate both a significant gain in completeness of information and of time.

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O11

From a metabolomics research study to ISO17025 method accreditation

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Although many guidelines for validating targeted methods are available and provide actors with performance indicators (e.g. pesticides, pharmacological drugs), such equivalent guidelines for untargeted method validation are not available yet, which certainly explains their limited application for routine or official control purposes. The main difference between non-targeted and targeted approaches is related to the multivariate nature of fingerprints in the former case, compared to single or multi-chemical species analysis in the latter one. In targeted strategies, results and method performance are evaluated compound-by-compound using univariate statistics, while multivariate analysis (which are generally referred to a chemometric methods) are required to evaluate relevance of patterns arising from fingerprinting approach. Guidelines for biomarkers/models discovery and validation are therefore urgently required by the scientific community. In that context a preliminary metabolomics research work by LC-HRMS dedicated to the investigation of biomarkers related to forbidden growth promoters administration in bovine enabled highlighting a set of biomarkers and establishing a prediction model [1]. The present work describes the validation process implemented and proposes performance criteria (selectivity, robustness, stability, suspicion threshold definition, false positive and false negative rates) in agreement with EU expectations (Dec 2002/657) in the objective of validating a screening method. Further, as discussion, we describe the process toward ISO17025 accreditation as well as practical aspects in relation with routine implementation of the method. [1] Dervilly-Pinel, Chereau, Cesbron, Monteau, & Le Bizec, Metabolomics (2015)

O12

MESSAGE IN A BOTTLE: METABOLOMICS FOR DECIPHERING THE TRANSIENT CHEMISTRY OF WINES AND SPIRITS

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Metabolomics applied to vine and wine sciences, has mostly developed over the last ten years. To that respect, FT-ICR-MS has clearly shown unprecedented capability based on ultra high re-solving power and mass measurement accuracy [1]. The introduction of the concept of oenolomics, exemplified by the metabologeography expression of cooperage oak wood in bottle-aged wines [2], further paved the way for breakthrough investigations of specific chemical fingerprints of wines, considered as transient molecular memories of vineyard-related environmental parameters and/or enological practices, which may have driven their original composition [3]. We thus reported a remarkable and straightforward discrimination of wines according to either the level of SO2 added to the must at pressing, or the type of stopper used at bottling, the latter being directly correlated to distinct oxygen ingresses during ageing [4]. Through some examples, we show how FT-ICR-MS can decipher the extent of the yet-unknown chemistry of wines, and even spirits [5], and how the combination with separation techniques can favor the identification of specific signatures. Finally, we show how network-based approaches can open new perspectives for the identification of the transient chemistry of winemaking [6].

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O13

Metabolomic and transcriptomic networks supporting nitrogen use and remobilization efficiencies in two oilseed rape genotypes

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Winter oilseed rape (Brassica napus L.), the main European oil crop, is a very high nitrogen (N)-fertilizer consuming species characterized by a rather low N use efficiency (NUE). Therefore, oilseed rape production is suffering significant energetic and environmental penalties. Despite high capacity for mineral N absorption from soil, organic nitrogen remobilization (NRE) within the plant is considered inefficient, especially during leaf senescence and nutrient recycling between source and sink tissues. Leaf ageing is associated with metabolic/catabolic reconfigurations to provide mobile nutrient metabolites and energy to support translocation processes (Dechaumet et al., 2018). Moreover, N fertilization regimes largely infers NRE performances together with already observed genotypic variability for this trait (Girondé et al., 2015). Our study focused on the holistic approach of metabolite and gene networks being involved in nutrient recycling processes considering both N availability and genotype diversity. A non-targeted metabolomic approach by UPLC-HRMS has been combined to a transcriptomic analysis of 60 000 genes by DNA microarray on two NRE-contrasted genotypes under high and low N regimes and along three stages of leaf development during the reproductive phase where nutrient should be reallocated to yield elaboration. Besides metabolite and gene atlas development, a comparative analysis using correlation networks unravel distinct regulatory patterns between the two genotypes with metabolic signatures specific to remobilization processes and N starvation.

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O14

A Plasma Metabolomic Signature Involving Purine Metabolism in Human Optic Atrophy 1 (OPA1)-Related Disorders.

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PURPOSE: Dominant optic atrophy (DOA; MIM [Mendelian Inheritance in Man] 165500), resulting in retinal ganglion cell degeneration, is mainly caused by mutations in the optic atrophy 1 (OPA1) gene, which encodes a dynamin guanosine triphosphate (GTP)ase involved in mitochondrial membrane processing. This work aimed at determining whether plasma from OPA1 pathogenic variant carriers displays a specific metabolic signature.

METHODS: We applied a nontargeted clinical metabolomics pipeline based on ultra-high-pressure liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) allowing the exploration of 500 polar metabolites in plasma. We compared the plasma metabolic profiles of 25 patients with various OPA1 pathogenic variants and phenotypes to those of 20 healthy controls. Statistical analyses were performed using univariate and multivariate (principal component analysis [PCA], orthogonal partial least-squares discriminant analysis [OPLS-DA]) methods and a machine learning approach, the Biosigner algorithm.

RESULTS: A robust and relevant predictive model characterizing OPA1 individuals was obtained, based on a complex panel of metabolites with altered concentrations. An impairment of the purine metabolism, including significant differences in xanthine, hypoxanthine, and inosine concentrations, was at the foreground of this signature. In addition, the signature was characterized by differences in urocanate, choline, phosphocholine, glycerate, 1-oleoyl-rac-glycerol, rac-glycerol-1-myristate, aspartate, glutamate, and cystine concentrations.

CONCLUSIONS: This first metabolic signature reported in the plasma of patient carrying OPA1 pathogenic variants highlights the unexpected involvement of purine metabolism in the patho-physiology of DOA.

O15

Identification de biomarqueurs dans le Trouble de Déficit d'Attention avec sans Hyperactivité

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Le Trouble de Déficit d'Attention avec ou sans Hyperactivité (TDAH) est une pathologie neurodéveloppementale très hétérogène touchant 5% des enfants scolarisés et se caractérisant par des troubles de l'attention, de l'hyperactivité et de l'impulsivité. Son diagnostic se fait uniquement à l'aide d'échelles comportementales et est complexifié par l'hétérogénéité phénotypique et temporelle des patients.

A l'heure actuelle aucune étude ne décrit de biomarqueurs du TDAH. L'identification de biomarqueurs reste donc un challenge important pour diagnostic et le suivi thérapeutique des patients.
Associé à la recherche de biomarqueurs circulants, l'étude du métabolisme central est déterminante pour mieux comprendre la physiopathologie du TDAH. A ce titre, l'utilisation de modèles animaux est pertinente pour étudier le métabolome cérébral. Le modèle de rat le mieux caractérisé est la souche SHR/NCrl comparée à leur témoins WKY/NHsd. Ce modèle présente à la fois les trois symptômes et des anomalies fonctionnelles similaires retrouvés en clinique.
L'objectif de cette étude est d'identifier des biomarqueurs métaboliques, au niveau central et périphérique, chez les rats SHR/NCrl. Pour cela, dix régions cérébrales (cortex orbito-frontal, cortex pré-limbique, cortex moteur 2, cortex pariétal, striatum dorsal, striatum ventral, amygdale, hippocampe, cervelet médian et cervelet latéral) sont prélevées, ainsi que des prélèvements périphériques (sang, urines et fèces), puis analysés par chromatographie liquide couplée à la spectrométrie de masse haute résolution (LC-HRMS). Les données obtenues sont traitées par des analyses multivariées (PCA, OPLS-DA), univariées (test de Student) et les voies métaboliques discriminates sont recherchées.
L'étude en composante principale montrent clairement que les deux groupes de rats ont des métabolomes différents. Les voies métaboliques de l'arginine, de la carnitine, du tryptophane, de l'histamine et des pyrimidines sont retrouvées altérées chez les rats SHR/NCrl comparés aux témoins. Comparativement aux données de la littérature qui décrit largement l'implication de la voie des catécholamines, nos résultats montrent des altérations métaboliques d'autres neuro-transmetteurs comme la sérotonine ,le glutamate et l'aspartate ainsi que d'autres métabolites telles que la carnitine ou les pyrimidines.

Les résultats de ce travail sont un premier pas vers une meilleure compréhension de la physopathologie de ce trouble. A l'avenir, des études de métabolomique sur des échantillons périphériques (urines, sang, fèces) seront réalisées afin de faire un lien entre le métabolome central et périphérique dans ce modèle animal, mais aussi d'appliquer cette méthode à la clinique pour améliorer le diagnostic.

O16

The hidden life of flavonoids: a new role in heavy metals tolerance and in plant adaptation to extreme metal environment

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Heavy metals (HM) are widely distributed in nature and represent the most frequent soil contamination source worldwide. Hyperaccumulators are plants that possess the ability to ac-cumulate high concentrations of trace elements in above-ground tissues. Arabidopsis halleri is a model species to study adaptation to extreme metal conditions since it presents variable Cd, Zn and Pb hyper -accumulation and -tolerance traits and is genetically closely related to the model species A. thaliana. Arabidopsis halleri populations growing on metal-contaminated (M) sites usually display enhanced HM tolerance compared to populations collected on non-contaminated (NM) sites.
We recently performed a comparative ionomic (ICP-MS), transcriptomic (RNA-Seq) and metabolomic (HPLC-MS) analysis in A. halleri populations displaying contrasting phenotypes induced by HM. Results highlighted flavonoids-related genes and metabolites to account for HM tolerance and accumulation variability in A. halleri populations and A. thaliana knockout mutants for flavonoids genes. Flavonoids are phenolic compounds characterized by two benzene rings linked by a heterocyclic pyrane ring and primarily occur in plants as O-glycosides. Flavonoids are dietary components with antioxidant properties which play a pivotal role in human health and plants stress responses. Using UV-spectroscopy and NMR techniques I showed that, in addition
to their antioxidant properties, flavonoids are able to form a complex with HM (Cd, Pb, Zn and Cu) in vitro and I hypothesize a major evolutionary role for flavonoids in HM tolerance and transport in both Arabidopsis species.
Heavy metals (HM) are widely distributed in nature and represent the most frequent soil contamnation source worldwide. Hyper accumulators are plants that possess the ability to accumulate high concentrations of trace elements in above-ground tissues. Arabidopsis halleri is a model species to study adaptation to extreme metal conditions since it presents Cd, Zn andPb hyper -accumulation and -tolerance traits and is genetically closely related to the model species A. thaliana. A comparative transcriptomic analysis in A. halleri populations displaying contrasting phenotypes highlighted flavonoids-related genes to account for HM tolerance and accumulation variability and forms the basis of the current project. Flavonoids are phenolic compounds with antioxidant properties which play a pivotal role in human health and plants stress responses. My data support favonoids ability to form a complex with HM (Cd, Pb, Zn and Cu) in vitro and a role in tolerance to HM in both Arabidopsis species. However, no information is available on: (i) flavonoids' ability to chelate HM and affect HM transport in vivo and (ii) flavonoids' evolutionary role in metal hyperaccumulators. The project aims at addressing these questions through biochemistry, transcriptomic, metabolomic and imaging analyses. The role of flavonoids on HM accumulation and tolerance will be dissected in A.thaliana (wild-type and mutants) and A. halleri (knock out lines). Ionomic, transcriptomic and metabolomics analyses will be used to identify new transport and detoxification pathways controlled by flavonoids during HM stress. Using fluorescent probes, flavonoids and HM will be tracked in vivo in wild-type and flavonoids-mutants. Flavonoids' ability to bind HM in vivo and to act as antioxidant will be also tested. This study will decipher flavonoids' role in plants during HM stresses and adaptation to metal-environment

O17

Mercaptomic: towards the profiling of reactive metabolites in toxicology

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Human exposure to chemical contaminants and other toxic substances, particularly through diet, is nowadays a public health issue and a major societal concern. The link between food-toxic effect and disease occurrence requires, above all, the most complete characterization of exposure. In food safety, current exposure assessment approaches are based on food consumption data crossed with food contamination data or biomonitoring data. In both cases, this allows evaluating exposure only in a targeted way on a few families of compounds. However, based on our previous results in exposomic [1], food or environmental toxicology should focus on the exposure to a mixture of compounds (contaminant cocktails), mostly at low doses, and in an untargeted way to detect/identify unknown compounds. Among these numerous known and unknown metabolites representative of our exposure, it seems a priority to focus on toxic compounds.

In this context; we developed an untargeted method using high resolution mass spectrometry coupled to liquid chromatography to specifically profile electrophilic metabolites which are most of the time toxic, due to their chemical reactivity towards nucleophilic sites present in bio-macromolecules such as DNA, RNA or proteins. The main line of cellular defense against these electrophilic compounds is the conjugation to the tripeptide glutathione, which is then cleaved and methylated in the kidney to yield the mercapturic acid conjugate as the final metabolite excreted into urine. Interestingly, mercapturic acid conjugates display a characteristic neutral loss in MS/MS experiments. Thus, using the ”all ions MS/MS” mode (MSE) of a Synapt G2-Si mass spectrometer it is possible to detect all the metabolites displaying this characteristic loss, and therefore highlight the reactive metabolites.

As a proof of concept, this approach has been applied to the study of different groups of rats fed diets containing various oils and heme iron. According to our previous results on lipid peroxidation [2] these diets led to the production of different aldehydes conjugated to mercapturic acid. The most famous is DHN-MA which corresponds to the mercapturate conjugate of 4-hydroxynonenal (4-HNE), which is commonly used as a biomarker of lipid peroxidation [2]. Using our methodology, we were able to detect without a priori, dozens of mercapturate conjugates, including DHN-MA and other known conjugated aldehydes. Furthermore, our approach also allowed the detection of conjugates of unexpected aldehydes, and of other chemical classes, for which putative identifications have been proposed based on complementary structural analyses. Interestingly, multivariate statistical analyses of the HRMS signals carried out on the mercapturate conjugates yield a better characterization of the studied animal groups compared to results obtained from a classic untargeted metabolomic approach.

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O18

A new comprehensive GC/GC-HRTOFMS approach in metabolomics

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Using an optimized and validated GC GC-HRTOFMS method we developed for the metabolic profiling of human serum, in combination with strict QA/QC system, we were able to highlight sets of biomarkers capable to discriminate between various inflammation phenotypes (high, low, remission, and control) representative of inflammatory bowel diseases.

During this proof-of-concept study, two main challenges of untargeted metabolomics were especially considered.

First, the issue of data handling large datasets and low number of samples compared to variables was solved by the definition of a workflow of data preprocessing and processing. It included the creation of a study template, the rigorous selection of good chromatographic quality features, the multiplication of statistical techniques submitted to re-sampling and test validation, and performance models designed to assess the ability of the candidate biomarkers to discriminate. In practice, 94 injections were made over 4 weeks, consisting of 70 study samples along with 16 QC samples and 8 reinjections due to QC system rejection. The all chromatograms template included 524 verified features that were then reduced to less than two hundreds after selection of the ones having an analytical variation under 30%, based on the QC samples. This resulted in the finding of 36 robust biomarkers that positively discriminated between the different phenotypes of inflammation, including high and low inflammation, remission, and healthy statutes.

Second, the identification of unknown compounds was enhanced by using state-of-the-art high-resolution (HR) time-of-flight mass spectrometry and allowed to name and characterize putative biomarkers with higher degree of confidence. This is a mandatory step for further development of analytical chemistry in clinical applications, its use in routine laboratories and integration of the results obtained in biological pathways interpretation.

In conclusion, this study has shown the usefulness of optimized and fully controlled GC/GC in clinical research and the major role high resolution is to play in order to fully exploit the potential offered by state-of-the-art analytical techniques.

O19

Intérêt des méthodes en ” omique ” pour accéder aux modifications précoces du métabolisme énergétique chez la chèvre laitière en peri-partum.

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La période de fin de gestation et de début de lactation est critique dans la vie des ruminants pendant laquelle une quantité importante de l'énergie est allouée aux fonctions de reproduction, développement du fœtus puis à la production de lait, donc préférentiellement à la survie de l'espèce au détriment de la survie de l'individu. Des ruptures potentielles d'équilibre énergétique peuvent perturber le bien-être, les performances de production et, à terme, la santé des animaux. Même si un traitement palliatif adéquat lors de l'apparition des premiers signes cliniques peut suffire, il est néanmoins préférable d'éviter le développement de ces maladies avant l'observation des premiers signes cliniques. Il a été montré que les saponines, en interférant avec le microbiote ruminal, contribuaient à réorienter les fermentations ruminales vers une diminution du rapport C2/C3. Nous avons donc émis l'hypothèse que les saponines, en favorisant la production de propionate, principal précurseur de glucose chez le ruminant, pourraient palier un éventuel bilan énergétique négatif chez la chèvre laitière en peri-partum. Deux groupes de 12 chèvres (témoins ou recevant 25g/j de saponines pendant 4 semaines) ont été suivis durant 7 semaines en peri-partum en confrontant leurs profils métaboliques plasmatiques obtenus après analyse 1H RMN. En effet, la métabolomique est particulièrement bien adaptée à la mesure d'un grand nombre de métabolites (empreinte métabolique), à l'échelle systémique. Cela permet ainsi d'accéder à des déséquilibres métaboliques (fonction du stade physiologique, challenge nutritionnel,...) ayant lieu de façon plus précoce ou à comparer des différences d'empreintes métaboliques plus subtiles que par des analyses classiques. Du fait de la complexité de nos jeux de données (longitudinales avec peu d'individus, contenant des variables qualitatives et quantitatives), une analyse canonique de corrélations nous a permis dans un premier temps, de mettre en évidence les corrélations fortes entre le stade physiologique, l'effet des saponines et les concentrations relatives de certains métabolites. Dans un deuxième temps, en utilisant des modèles tenant compte de la variabilité individuelle de réponse à l'ajout de saponines (modèle linéaire avec un effet aléatoire), nous avons pu détecter les métabolites dont les concentrations étaient modifiées par l'ajout des saponines en peri-partum. Les premiers résultats, confrontés aux données de la littérature, permettent d'identifier les variations de plusieurs métabolites d'intérêt, impliqués dans certaines voies du métabolisme énergétique, dont la methylhistidine et l'octanoylcarnitine. Suite à ces résultats préliminaires, nous proposons d'approfondir nos analyses en confrontant les profils métaboliques plasmatiques, reflet du métabolisme systémique de l'animal, avec les profils métaboliques ruminaux, afin de conforter et de mieux identifier l'impact des saponines et la variabilité individuelle de ces effets.

OH1

NMR-based metabolomics workflow for quality and effect assessment of alternative plant-based diets in rainbow trout.

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Development of sustainable diets for aquaculture is crucial due to the decreasing availability of ingredients of marine origin. Nevertheless, the sustainable diets based on plant-based feed-stuffs have numerous impacts on fish nutrition processes. In this work NMR-based metabolomic approaches were developed and tested on plasma of rainbow trout, Oncorhynchus mykiss, fed from the first feeding and up to 14 months with plant-based or marine-based diets and a commercial diet as control. At the end of the experiment fish were sampled 6h and 48h after the last meal. The blood was collected and the plasma prepared. In parallel, extraction of soluble compounds of diets was done. Plasma and polar extracts of diets were analysed using a Bruker 500 MHz spectrometer. Plasma CPMG and zgpr 1H-NMR profiles were processed separately with NMRProcFlow[1] tool (chemical shift calibration, baseline correction, peak realignment and non-uniform bucketing, Signal-to-Noise Ratio determination). Each spectral region of interest was determined either with intelligent bucketing or variable-size bucketing. CPMG and zgpr bucket variables were combined and used for statistical analyses with BioStatFlow (www.biostatflow.org). Biomarkers of diets in plasma were detected, and some of them were also found in the NMR profiles of diets. This approach will be used for trouts fed with less contrasted alternative diets.

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OH2

Response to uranium stress within Microbacterium bacteria: metabolomic analysis by NMR and LC-MS

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Accumulation of toxic metals and radionuclides in the environment represents a public health and wildlife concern. Bacteria resistant to these elements represent an attractive source of biotechnological developments for bioremediation. Four Microbacterium strains were isolated from radionuclide- and metal-rich environments including naturally rich sites (uranium-rich soil from Limousin, France) or anthropogenically polluted sites (Chernobyl exclusion zone, Ukraine). The four strains exhibited contrasted uranium-tolerance capabilities, from highly tolerant to sensitive. Each strain was exposed or not to 10 M uranyl nitrate (U) and the cells were collected and extracted after 4 h and 24 h exposure. A metabolomic analysis was conducted by NMR from 1D 1H NOESY experiments recorded on a 600 MHz instrument equipped with a cryoprobe and by UHPLC-MS on a high resolution ESI-Q-TOF instrument. The 1H NMR data were treated by an untargeted approach involving bucketing of the 1H NMR spectra or by deconvolution, using the package Batman under R environment. The LC-MS data were preprocessed with the package XCMS on R, which allows peak detection, automatic retention time alignment peak matching and, last, adduct/isotope-related redundancy exploration. Data generated from 1H NMR or LC-MS were submitted to multivariate analysis using principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and regularized canonical correlation analysis (rCCA) using sparse-PLS2 regression, with the R package mixOmics. A clear hierarchy between the different controlled factors was observed thanks to either rCCA or PLS-DA; the main part of variance being is explained by the ”strain” effect with a sub-grouping of tolerant strains opposed to sensitive ones. Interestingly, a specific metabolic adaptation of strains to U–exposition including short/long duration was noticed. Supra integration of the biomarking set coming from both 1H NMR and LC-MS data generators is tentatively done using robust rCCA (package groc on R) to isolate for every strain candidate metabolic pathways supporting tolerance or explaining higher sensibility towards uranium.

OJ1

High-resolution mass spectrometry based non-targeted metabolomic analyses for the study of the toasting/ tannin potential interaction in oak wood

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Wine ageing in oak barrels is a traditional process that represents an essential step to optimize the organoleptic properties of wines. Indeed, oak wood extractible compounds include essentially volatile compounds related to wines oaky character and mainly non-volatile compounds such as ellagitannins and other compounds as triterpenes, coumarins, lignins and polysaccharides. In order to provide an innovative tool that could allow for a better management of wines qualitative expression via oak barrel ageing, the characterization of the oak wood extractable metabolome has been considered by high-resolution mass spectrometry with the main objective to uncover new molecular evidences. For the purpose of this study, 12 oak wood samples with three different total tannin potentials (expressed in total ellagitannin content after IR spectroscopy analysis on unheated wood) and different toasting levels were considered. Acidified hydroalcoolic oak wood extracts were analyzed via LC-MS-QTof and FT-ICR-MS. Metabolic profiles were converted into matrices in order to perform both multivariate statistical analyses (PCA, PLS-DA) and univariate analysis (Wilcoxon-Mann-Whitney) to highlight the most discriminant compounds. The non-targeted analysis of the metabolic profile of wood extracts allowed clear discrimination of woods tannin potentials regardless of the toasting impact and vice versa. A yet unknown diversity of molecular markers related to the tannin potential and the toasting effect were identified. These markers could be used to achieve a fine classification of staves in order to guarantee more homogeneous and reproducible oak wood profiles during barrel making.

OJ2

Gut microbiota and faecal levels of short chain fatty acids differ upon blood pressure levels in man

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Introduction and aims: Arterial hypertension (HT) is a worldwide public health issue. The pathophysiology of primary HT - the most common form of HT - remains largely unknown. Recent observations in rodents and patients suggest that gut microbiota (GM) may influence blood pressure (BP) homeostasis, namely via carbohydrate fermentation end-products.
Methods: After informed consent, male patients and volunteers were prospectively recruited and categorized into 3 groups according to 2013 European Society of Hypertension criteria based on 24-hour BP measurements (Spacelabs 90207): (i) HT (24-h BP 130 and/or 80 mmHg or in the presence of an antihypertensive treatment); (ii) borderline (24-h BP
Results: The cohort included 55 males (mean age: 55.5 10.5 years): 39 with HT (21 treated with antihypertensive medications), 7 with borderline BP, and 9 with normotension. No significant difference was observed between groups concerning age, BMI, rate of smokers and weekly alcohol consumption. Eight participants had diabetes and all were in the HT group. History of cardiovascular or gastroenterological disease did not differ as well as the frequencies of antibiotic treatment during the previous 3 months before data collection. GM from HT and borderline patients showed significantly increased abundance of Prevotella genus and reduced presence of Bacteroides genus in comparison to controls. SCFA levels were significantly different among groups, with stool levels of acetate, butyrate and propionate reaching 63.2 ± 5.6, 19.8 ± 2.8 and 16.7 ± 2.4 (relative values) for untreated HT patients versus 16.0 ± 4.7, 4.4 ± 1.4 and 3.6 ± 1.3 (relative values) for normotensive individuals, respectively. No significant difference was observed in serum and urine metabolomes. By contrast, regression lines for stool metabolomes of patients highlighted correlations with mean (MBP), systolic (SBP) and diastolic (DBP) BP levels. Stool samples from untreated HT, borderline and controls patients correlated with MBP levels, with R^2 coefficient reaching 0.86, 0.94 and 0.94, respectively. Similar R^2 values were obtained regarding SBP and DBP linear regressions.
Conclusions: Our pilot study supports an association between GM composition and BP levels, with significant impacts on stool abundance of some fermentation-derived SCFA.

OJ3

About the use of surface-enhanced Raman scattering to monitor the cellular release of neurotransmitters

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Dopamine (DA) is a catecholamine neurotransmitter of the central and peripheral nervous system. Its role is of utmost importance, as it can be emphasised by the diseases that arise when DA blood levels are dysregulated. For instance, in Parkinson's disease, DA levels are downregulated while they are upregulated in schizophrenia [1]. Therefore, sensitive and reliable analytical tools are requested to quantify DA in complex sample matrices.

Among the family of vibrational spectroscopy tools, surface-enhanced Raman scattering (SERS) is a variant of conventional Raman spectroscopy that exploits the electromagnetic properties of rough metallic surfaces to increase the Raman signal about 103 to 1011 times. Nowadays, two kinds of SERS substrates are predominantly used: suspensions of metallic nanoparticles and solid SERS substrates. While the synthesis of the former can easily be carried out in every laboratory at minimal costs and with little specific equipment, the latter experience more reproducible results due to the rigorous control of the nanomaterial size and placement [2]. In order to combine the advantages of both kinds of substrates, the fabrication of polymeric substrates entrapping nanoparticles has recently emerged [3]. During the last decades, the use of SERS has expanded thanks to the great capabilities of the technique, i.e. the non-destructivity, the sensitivity, the rapidity and the green character of SERS. Particularly, an increasing number of publications is dealing with the analysis of body fluids, tissues, cells or cell culture media [4]. In this context, this work was dedicated to the study and comparison of the quantitative performances of different kinds of SERS substrates for DA. The emphasis was put on DA quantification in the culture medium of PC-12 cells, since these cells synthesise and release DA and are consequently a model for DA neurosecretion [5]. The SERS substrates that were investigated were suspensions of gold nanoparticles (AuNPs), and solid SERS substrates made of metallic nanoparticles embedded in a polymeric network.

On one hand, proteins contained in the culture medium adsorbed onto the surface of AuNPs, preventing the aggregation of AuNPs. Consequently, a pre-aggregation step of the AuNPs was undertaken to circumvent this SERS-deleterious stabilisation. The optimised sample preparation allowed to quantify DA from 0.5 to 50 ppm (2.64 to 264 M), with an individual sample analysis time below one minute. Furthermore, the developed methodology enabled to examine the effect of different cations and their concentrations on the DA exocytosis from PC-12 cells. Finally, the increase of DA synthesis and release from PC-12 cells exposed to dexamethasone was monitored.

On the other hand, the trapped configuration of the nanoparticles inside a polymeric matrix conferred protein adsorption resistance on the nanoparticles. Several polymers and kinds of metallic nanoparticles were tried out and the best combination led to DA quantification from 2.5 to 25 ppm (13.2 to 132 M) in the culture medium of PC-12 cells. Moreover, these SERS substrates could be placed in the culture medium of cells in order to follow the release of neuro-transmitters in real time, enabling to sense the normal and impaired cellular communication in response to diverse stimuli.

In conclusion, this research project demonstrated the great potential of SERS for the analysis of neurotransmitters released by cells under stimulation.

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OJ4

Comparative study of primary metabolism in diverse fleshy fruit species

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In addition to model fruit species such as tomato or grapevine, several species (peach, apple, kiwifruit, clementine, strawberry, pepper, cucumber and eggplant) were studied to provide a reasonable range of fruit traits: growth dynamics, climacteric character, maturation duration, phloem-transported sugars and starch storage level. We focused on primary metabolism, which provides energy and biosynthetic precursors to support fruit growth and ripening, essential for the quality of fruit and its biomass.

In order to visualize the global composition difference between the nine species, major and minor polar metabolites (sugars, organic and amino acids), were identified in the edible part of the fruit, using 1D and 2D NMR. Common major metabolites, total proteins and starch were quantified in all species across at least nine stages of development using high-throughput microplate targeted measurements. In addition, monosaccharides of cell wall polysaccharides and cellulose were quantified as they largely contribute to fruit biomass. Overall these quantifications explained 40% to 90% of the dry matter depending on the stage of development. For all these compounds, differences in composition between species already observed during the early stages of development were exacerbated in mature fruits, in contrast to intermediate stages with more inter-species similarities.

Solanaceae species were studied in more details for a range of metabolites involved in central metabolism determined with NMR metabolomic profiling or MS/MS targeted analyses. In addition, enzyme capacities was explored in order to estimate their developmental dynamics. Similarities and differences between Solanaceae species were visualized using multivariate and univariate analyses, including ANOVA-PCA and HCA.

The quantitative compositional data will be complemented and combined with phenotypic data. They will also be used for the parameterization of metabolic models in order to highlight essential regulation steps.

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OJ5

Investigation of the effects of a wastewater treatment plant effluent on the marine bivalve Mytilus galloprovincialis through a metabolomics approach

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Conventional wastewater treatment plants (WTP) discharge numerous organic contaminants in aquatic environments such as a large diversity of pharmaceutically active compounds (PhACs), pesticides, polycyclic aromatic hydrocarbons, plasticizer, and many others (Fent et al., 2006; Kolpin et al., 2002). Unfortunately, wastewater volume has been rising proportionate to the quickly growing human worldwide population (Sato et al., 2013). Because of a strong demo-graphic pressure, the coastline is affected by the discharge of contaminants through the development of sea outfalls WTP but also by the contributions of catchment areas. Scientific evidences highlight potential ecotoxicological risk and negative effects of contaminants usually detected in WTP effluents and in aquatic environments (Bolong et al., 2009; Fent et al., 2006). The challenge is today to assess the effects of such cumulative pollution on aquatic organisms. In the last decades, effective techniques have been developed to obtain considerably more complete and specific information on the biochemical responses of organisms to toxic exposures. The omic approaches allow researchers to more deeply investigate and unravel unknown toxicological effects and mechanisms of environmental contaminants (Weckwerth, 2011). Among them, metabolomics is a powerful approach to assess the health status of organisms based on the identification of low molecular weight metabolites (50-1500 Da), whose production and levels vary with the physiological, developmental, or pathological state of cells, tissues, organs or whole organisms (Courant et al., 2014; Lin et al., 2006). In this context, we propose to elucidate potential effects of a WTP effluent on the marine bivalve Mytilus galloprovincialis (Mediterranean mussel) through a metabolomics approach. For that purpose, an exposure has been performed in controlled laboratory conditions. After seven days of acclimatization, mussels from a control group (n=35) were exposed to solvent (MeOH) while mussels from an exposed group (n=35) were exposed to an extract of WTP representing a final dilution of 1/20th to the WTP discharge to mimic a real dilution in the environment. Seven days following the exposure, mussels were dissected and their sex determined. Metabolic fingerprints were generated from digestive gland by LC-HRMS analysis. Afterwards, univariate and multivariate tests were applied to highlight disrupted metabolites in male and female following exposure. Male mussels showed numerous metabolites altered in response to WTP effluent exposure, belonging mainly to amino acids, purine and pyrimidine metabolism. A modulation of these metabolites could have potential effects on several biological processes, such as energetic metabolism, DNA synthesis, osmoregulation, byssus formation and reproduction. On the other hand, female mussels also showed many impacted metabolites. Some of them were common to male, while many others seemed to be specific of female response. Statistical analyses were performed in order to determine the variability of gender response to the exposure, both quantitatively and qualitatively.

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OJ6

La métabolomique comme outil d'étude de l'évolution de Pseudomonas aeruginosa au cours des infections pulmonaires chroniques dans la mucoviscidose

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L'infection pulmonaire chronique par Pseudomonas aeruginosa (Pa) est considérée comme la principale cause de morbidité et de mortalité liée à la mucoviscidose. Au cours de cette infection persistante, Pa s'adapte à l'environnement pulmonaire caractéristique de ces patients et évolue avec son hôte pendant des décennies. Bien que plusieurs études aient tenté d'évaluer les mécanismes génétiques d'évolution chez cette bactérie, il demeure difficile aujourd'hui d'expliquer les relations entre les mutations accumulées dans le génome bactérien et l'expression de phénotypes cliniquement pertinents, ou encore de corréler ces mutations avec l'état de santé du patient. Afin de mieux appréhender le niveau d'expression phénotypique des processus de régulation biologique, nous proposons d'étudier l'évolution du métabolisme de Pa dans ces infections chroniques. Nous avons constitué une banque longitudinale d'isolats cliniques de Pa prélevés depuis les expectorations de 32 patients adultes atteints de mucoviscidose et suivis au CHU Grenoble-Alpes entre 2010 et 2015. Sur la base d'un profilage génétique, nous avons sélectionné, pour chaque patient, deux à quatre isolats représentant différents stades d'évolution du clone ayant initié l'infection chronique.

Les 66 isolats bactériens ont ensuite été mis en culture dans un milieu chimiquement défini visant à reproduire les conditions nutritionnelles rencontrées par la bactérie dans les poumons des patients. Les empreintes métabolomiques ont ensuite été acquises par HPLC-HRMS sur un appareil Orbitrap (Q Exactive, ThermoFisher), et sont actuellement en cours de traitement. Une étude préliminaire menée sur trois isolats prélevés en 2010, 2012 et 2014 chez le même patient a permis la détection de 1893 variables, dont 173 annotées putativement. L'analyse en composantes principales a permis de mettre en évidence un profil discriminant les isolats selon leur date de prélèvement. Les 66 isolats ont par ailleurs été caractérisés d'un point de vue phénotypique. Les profils de résistances aux antibiotiques ont été testés sur gélose, et la cytotoxicité sur cellules épithéliales et immunitaires a été mesurée en cinétique par microscopie automatisée. Enfin, la sévérité de l'atteinte respiratoire des patients infectés a été modélisée d'après les données cliniques. L'objectif de notre étude est en premier lieu de mettre en évidence des adaptations convergentes du métabolisme de souches de Pa infectant différents patients. Nous étudierons également les corrélations entre les signatures métaboliques observées et l'expression de phénotypes cliniquement pertinents, ou encore l'état de santé des patients.

F1-P1

MetWork: a Web Server for in-silico Metabolization of Natural Products

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MetWork is a tool developed to provide a fast set of annotations to a molecular network when at least one metabolite is previously identified. Its algorithm is based on two scripts to generate data: metabolization and fragmentation and controlled by two scripts to compare generated data with experimental LC-MS/MS data. Metabolization applies a set of chemical reactions that could happen in the organism studied on an input metabolite with known structure, the products of the reactions provide new molecules to evaluate. Fragmentation simulates the mass spectrum of the generated molecules using CFM-ID software. Spectrum comparison gives an estimation of the generated structure to correspond to a metabolite of the network with a ranking based on dot product. MetWork could accelerate and optimize natural products analysis by highlighting putative structures in molecular network. The solution is based on Python and Django web framework and will be proposed as a service on a web platform.

F2-P2

Metabolomics as a tool to identify phenolic compounds involved in spatial distribution of flax leaves

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Flax (Linum usitatissimum) is an important oilseed crop cultivated and commercialized in various parts of the world. Though flaxseed chemical composition is deeply studied, little is known about that of the flax leaves. Therefore, this study aims at identifying the secondary metabolites contained in flax leaves and reveals their spatial distribution through different foliar populations. LC-HRMS was firstly applied for a high-throughput analysis of the methanol/water extract of flax leaves, followed by a targeted isolation of compounds of interest using preparative HPLC. A total of sixteen compounds belonging to coniferyl alcohol derivatives, apigenin derivatives and luteolin derivatives were isolated and their complete characterization was performed by 1D and 2D NMR experiments. The spatial distribution of these compounds was further studied using a LC-MS-based metabolomic approach. PCA analysis showed a clear discrimination between different foliar populations from basal to apical leaves.

F3-P3

Influence of psychosocial stress on salivary metabonomic profile

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Different biomarkers used for assessing emotional states of individuals are documented in the literature. Most of methods use plasma as biofluid which collection can occur a stress and requires qualified personnel.
Our study aims at monitoring stress, cognitive (over)load and mental fatigue using non-invasive techniques. For this purpose, we used saliva as the biological matrix since its sampling is easy and less likely to induce stress to the subjects.

Samples are studied following a double strategy: a targeted approach combined with a non-targeted one. The targeted method concerns analytical quantification of different salivary biomarkers associated to emotional states such as 3-Methoxy-4-hydroxyphenylglycol (MHPG), a norepinephrine metabolite.

On the other hand, we studied the evolution of the salivary metabonomic profile. 17 young male students are submitted to a modified version of the Trier social Stress Test (TSST) protocol to induce a psychosocial stress. Saliva samples are collected from subjects and processed using 1HNMR spectra. Evolution of the salivary metabonomic profiles is studied by a multivariate analysis (PCA and PLS-DA) approach and permits to assess the correlation between the fluctuation of emotional states and the variability of metabolites content of this biofluid.

Preliminary results showed a variation of the metabonomic profiles after the stress step of the TSST with a back to the initial profile trend following the 30 minutes resting step. Considering these results, based on the NMR spectra, we identify potential biomarkers involved in metabonomic profiles changes due to stress (e.g. mannose, sacharopine, or formate).

F4-P4

Keep improving chemical identification using tandem mass spectrometry data in a pharmaceutical context

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Background Mass spectrometry (MS)-based structural elucidation of small molecules plays an important role during pharmaceutical development and in support of investigations for marketed products. Nowadays, sophisticated MS instruments enable the automatic fragmentation of thousands of compounds with high sensitivity, scan speed, resolution and good mass accuracy consequently. However, the interpretation of fragmentation (MS/MS) spectra still requires manual intervention of MS experts, and can take up to 70% of total time spent on analysis. The lack of automatic, versatile and reliable MS/MS data processing pipelines has become the biggest bottleneck in de novo structural elucidation in pharmaceutical companies. Our study tackles this challenge by developing a user-friendly workflow combining both publicly-available and in-house developed structure elucidation smart algorithms.

Method The de novo identification workflow is expected to consist of three steps: 1. MS1 and MS2 spectra of unknown compounds are submitted to SIRIUS software for elemental formula annotation. A hypothetical fragmentation trees is built in which nodes represent formulas of the fragments and edges represent fragmentation events. This step also removes unexplained mass peaks and assigns exact masses to fragments. 2. The pre-processed MS2 spectra are identified with publicly-available identification tools such as Metfrag, MAGMa or CSI:FingerID. Alternatively, library searching is performed. Our in-house spectral library contains about 2 000 manually-curated drugs-related compounds measured on different LC-MS instruments, and is constantly updated. The matching between spectra is performed via fragmentation tree comparison. 3. Each computational approach (including spectral library searching) generates a list of structure candidates, ranked from the most to least probable. However, true candidates do not always appear at the top of list due to database limitations or unsuitable scoring systems. We have developed in our laboratory a substructure recommendation tool called MESSAR (MEtabo-lite SubStructure AutoRecommender). Using all information of MS2 spectra (fragment masses, mass differences and neutral losses), this tool re-ranks the candidate structures based on whether they contain recommended substructures. MESSAR has improved considerably the rank of true structures in several validation datasets. Preliminary results Our workflow was implemented in R (v3.4.3) and python (v3.6.5), and was tested on 50 spectra of known pharmaceutical compounds (validation dataset). The candidate rankings were evaluated against their molecular similarities (using tanimoto coefficients) with true compounds, namely higher-ranked candidates should be more similar in structure to true compounds. Although exact structure identification is not feasible in many cases, MAGMa or CSI:FingerID combined with MESSAR top-ranked structures similar to the true compound. In other words, recommended structures from our workflow can support the manual interpretation in pharmaceutical development.

F5-P5

A NMR-based metabolomics study of minced pork meat inoculated with Brochothrix thermosphacta, Leuconostoc gelidum and Pseudomonas fragi.

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Introduction: In Europe, the losses of initial meat production represent 20% and more than half of this occurs at animal production, slaughtering, processing and distribution steps [1, 2, 3]. Among the rea-sons for food loss and waste, spoilage by bacteria that contaminate the food matrix and are able to develop during transformation steps and storage is a major issue [4, 5]. In order to control food waste, studies have highlighted the importance of monitoring the microbial diversity of food [3]. As such, the combination of metabolomics data with other complementary approaches (classical microbiology and quality parameters) can gives the opportunity to gain deeper insights into, and have a better comprehension of the spoilage mechanisms [6, 7]. The aim of the current study was to assess meat spoilage through the evolution of bacterial counts and changes in the metabolic profile of minced pork meat using Proton Nuclear Magnetic Resonance (1H-NMR) based metabolomics.
Materials and Methods: Microbiological assessment, pH measurements, gas composition and metabolomics analysis were carried out in minced pork meat samples stored under food wrap (FW) and under modified atmosphere packaging (MAP, 70% O2 – 30% CO2) at 4, 8 and 12 C during 13 days. All samples were irradiated and then inoculated separately with three dominant bacterial strains isolated from previous aging tests: Brochothrix thermosphacta, Leuconostoc gelidum and Pseudomonas fragi. Analysis were carried out at day 0 and at day 13 for metabolomics analysis, and each day for all others measurements. For all conditions, non-inoculated samples are also analysed. Spectral data were treated and analysed using discriminant analysis.
Results: The best metabolomics signature of samples is obtained by Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA) and reveals a clear discrimination between: (1) the packaging conditions, (2) the temperature storage, (3) the inoculated and non-inoculated samples, and the type of strain inoculated. Comparison of sterile meat at day 0 and at day 13 revealed also a good separation of the two metabolomics groups, according to the meat degradation over time. By multivariate analysis (PCA and PLS-DA) of samples at the end of the storage period (day 13) it can be observed that the type of strain inoculated had a more impact on the metabolome of meat that the packaging conditions. Moreover, the results evidenced a noticeable increase in acetate and glycerol for B. thermosphacta; betaine and lactate for L. gelidum; threonine and glycine for P. fragi.
Discussion: These results support the use of NMR-based metabolomics as a valuable tool to provide information on pork meat spoilage and to follow intrinsically the evolution of the metabolomics pattern linked to a specific strain in a complex bacterial ecosystem.
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F6-P6

Cold shock metabolomic response of bacteria from clouds

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In the air, microorganisms face harsh conditions of life (including exposure to UV and oxidants, osmotic and cold shocks). After aerosolization, their journey can last several days in the atmospheric compartment. Into cloud droplets, microbial communities consist of 103–104 bacteria and archaea and 102–103 eukaryote cells per milliliter [1]. Alpha-, Beta- and Gamma-Proteobacteria (Pseudomonadales in particular) are core members of the cloud microbiota. Some maintain metabolic activity and respond to environmental conditions until being back to ground. In our studies, we examined the metabolome of Pseudomonas species, P. syringae and P. grami-nis, isolated from cloud water when exposed to low temperature [2] or oxidants (H2O2)[3], respectively. Non targeted analyses of cell extracts by LC/MS and NMR (Metabolic Profiler c) allowed identifying specificities in the cellular functioning in the cold and at high concentra-tions of oxidants, likely contributing to survival in clouds. The response of cold shocked cell suspensions exhibited notably cryoprotectants and antioxidants compounds, along with central metabolism regulations resulting in modifications of the profiles of amino acids, carbohydrates, lipids and short peptides in the whole metabolomes. These observations provide new insights into metabolic functioning of micro-organisms in the atmosphere and clouds, and on their capacity to survive in these inhospitable environments.

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- Jousse et al. (2018). Metabolomic study of the response to cold shock in a strain of Pseudomonas syringae isolated from cloud water. Metabolomics 14:11. https://doi.org/10.1007/s11306-017-1295-7
- Wirgot et al. (2017) H2O2 modulates the energetic metabolism of the cloud microbiome. https://doi.org/10.5194/acp-2017-581.

Key words: Cloud, bacteria, stress, metabolomics, Pseudomonas, Metabolic Profiler

Communications par affiche

Les résumés de l'ensemble des communications par affiche

NB : les résumés des posters 1 à 6 sont repris dans la section précédente (communications flash F1-P1 à F6-P6)

P7

Towards the deciphering of the mechanism of action of an experimental drug against Chagas disease, using multi-omics platform

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Trypanosoma cruzi (T. cruzi), a kinetoplastid protozoan parasite, is the etiologic agent of Chagas disease. The disease affects millions of people and represents a major burden for the socio-economic development of affected countries (mainly Latin America). The mobility of patients infected with Chagas disease makes it a global public health burden. So far, no vaccine exists and current drugs suffer from significant drawbacks, which limit their use in disease endemic areas. In the foreseeable future, the very few drugs currently in clinical development do not offer new alternatives to patients. Thus, there is a need to identify and develop novel drug candidates with new mechanisms of action to overcome the current situation and eventually feed the Chagas clinical trials pipeline.
The objectives of our study were to identify metabolites and proteins markers of the efficacy of an experimental drug, S205, and to determine the related metabolic pathways to formulate hypotheses on its unknown mode of action. To this aim, we focused on uninfected and T. cruzi infected rat myoblast cells, in presence or not of the drug targeting intracellular amastigotes form of the parasite (intracellular phase in the life-cycle of trypanosomes). The drug effect on cells was analyzed after 24h of exposition. We analyzed polar extracellular metabolites by 600 MHz NMR and intracellular lipids and proteins were determined by LC-HRMS using a Q-Exactive (Thermo) and a Q-TOF (Bruker Maxis HD), respectively.

Through our profiling approach, we identified potential markers for infection and drug mode of action. Indeed, the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) protein of T. cruzi was identified as a potential target of the drug S205 by proteomics and metabolomics has shown that the drug affects the energy metabolism and hence, through the impact on Acyl-CoA syn-thesis, the formation of lipids. Thus, S205 seems to act through a specific target and both technologies applied (proteomics and metabolomics) complement each other in the description of the mechanism.
We have shown the relevance of proteomics and metabolomics approaches to contribute to the elucidation of drug mechanism of action as a complement to classical methods. The methods are applicable even to complex systems such as mammal cells infected with parasites.

P8

Quick annotation of flavone glycosides from the Sideritis hyssopifolia using in silico metabolization

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Annotation of small molecules remains a challenge in untargeted metabolomics. During the last decade, the development of molecular networking, available through the freely available platform GNPS enables the annotation of known compounds using spectra libraries. The low number of spectra present in this database is a limit towards the identification of unknowns, even when slight structural changes occur (oxidation, reduction, hydroxylations, etc...). In the present study, we applied the newly developed *in silico* metabolization tool to explore the chemical space of isoscutellarein glycosides. Following LC-MS/MS data dependant analysis (DDA) and construction of a molecular network using the GNPS platform, a single ion corresponding to isoscutellarein-7-O-β-d-glucopyranosyl was identified in the network. This compou nd was further metabolized *in silico* using known biochemical transformations and for each produced metabolite corresponding to a *m/z* present in the network, a MS/MS spectrum was generated and searched in the experimental chromatogram. After run completion, 10 ions of the cluster were successfully identified. The structures of the compounds were secured after their isolation using reversed phase HPLC.

P9

Analyse métabolomique non ciblée du méconium humain

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Le développement de certaines pathologies dans l'enfance peut résulter de processus ou d'expositions particulières durant la fenêtre périnatale. Dans ces travaux, nous cherchons à décrire l'exposome prénatal par l'étude de méconiums humains, i.e. les premières selles du nouveau-né. Le méconium représente en effet une matrice accumulatrice et inerte, formée dès le deuxième mois de grossesse : il intègre donc les processus internes de la mère et du fœtus (métabolisme, microbiote), ainsi que les différents facteurs (alimentation, polluants/contaminants...) auxquels la mère est exposée pendant la grossesse. Le but de cette étude était de développer et valider un protocole d'analyse métabolomique non ciblé, ce type d'approche étant peu décrite dans la littérature pour des échantillons de méconiums. Ce protocole est développé sur un système de chromatographie liquide couplée à la spectrométrie de masse à haute résolution (LC/HRMS). Grâce à différents méconiums collectés en maternité, nous avons tout d'abord développé et com-paré différentes méthodes d'extraction de métabolites avant analyse par LC/HRMS. L'acquisition des données a ensuite été réalisée sur un LTQ-Orbitrap après séparation sur une colonne chro-matographique de type HILIC avec détection en mode négatif. Les données brutes générées ont été traitées par XCMS sur le portail W4M dédié au traitement, à l'analyse et à l'annotation des données métabolomiques. A l'aide de cet outil et de la base de données spectrales du labora-toire, environs 90 métabolites ont pu être annotés avec succès. Les principales familles annotées correspondent à des acides aminés, des alcools ou encore des acides carboxyliques. Des analyses complémentaires seront réalisées en parallèle en HILIC avec une détection en mode positif, ainsi qu'en C18 afin de compléter cette liste de métabolites.

Les méthodes développées et validées seront appliquées à des échantillons de méconiums issus d'une cohorte mère-enfant. Des approches complémentaires de protéomiques et de séquençage d'ADN16s seront réalisées pour avoir un reflet global de cet exposome prenatal. La composition globale du méconium sera corrélée à différents facteurs maternels (lieu de vie, alimentation...etc.), ainsi qu'à la santé de l'enfant dans les 5 premières années de vie.

P10

Huntington's disease : metabolomic studies by NMR analyzes on drosophila model

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This study is designed to identify metabolites at two different stages of Huntington's disease modeled in *Drosophila melanogaster*.
Transgenic flies have been obtained to express a pathogenic polyglutaminated huntingtin fragment (exon1 with 93 polyQ repeats). Pan-neuronal expression of this transgene (*htt-93Q*) induces neuropathology that recapitulates most of the characteristics of the human disease. In particular, the neuropathology is progressive, older flies having more severe symptoms. Therefore, 10-day and 16-day-old flies were sampled. The 10th day corresponds to the pre-symptomatic stage, the 16th day to the presence of the disease symptoms. After sample preparation, 1H-NMR spectra of six sets of adult Drosophila, expressing the transgene or not, sampled at 10 and 16 days, were performed on a BRUKER 700 MHz NMR spectrometer with cryoprobe. For assignment pur-poses, COSY, TOCSY, 13C-HSQC and HMBC spectra were acquired. Multivariate statistical analyzes (Principal Component Analysis or PCA and predictive methods as PLS and OPLS) were performed with the 1D NMR spectra. A series of discriminant metabolites were hence detected and unambiguously identified. An efficient statistical model was built with the most discriminant metabolites.
We demonstrate in this study that the Huntington's disease could be identified by the analysis of only a few metabolites. These metabolites allow the prediction of the Huntington's disease even before the appearance of its symptoms. Identification of metabolic pathways disrupted by the disease will help to consider future therapeutic targets.

P11

Annotation of oak leaf metabolome for genetic and ecological studies

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Tree leaves produce numerous specialized metabolites which affords protection against abiotic stress (UV, drought, frost) and defence against natural enemies (leaf herbivores, pathogens). In the context of global change, exploring the variation of these important biochemical traits within species is paramount to understand their evolutionary history and ecological role to ensure sustainable management of populations. In Oaks, resources to study the genetics underlying natural variation as well as genetics x environment interactions are well developed, however the specialized metabolome of leaf oak has been scarcely explored. In this work, we initiated the annotation of over 120 molecules from the oak (*Quercus spp*.) leaf specialized metabolome. Specialized metabolites of leaf methanolic extracts were first separated with a reversed-phase column and detected using a LTQ/Orbitrap-MS system operated in positive mode. We then annotated the molecules using retention times and accurate masses of protonated molecules. We confirmed our annotations with MS2 and MS3 experiments using CID fragmentations, as well as comparison with commercial standards when available. Two main specialized metabolite families were highlighted: flavonols and hydrolysable tannins. Flavonols showed a structural diversity in terms of aglycones and aglycone decoration. Hydrolysable tannins were characterized by a lesser diversity. These annotations will serve as a basis to the study of natural variation and the ecological role of the specialized metabolome.

P12

Field-based metabolomics of Vitis vinifera L. Stems Provides New Insights for Genotype Discrimination and Polyphenol Metabolism Structuring

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Grape accumulates numerous polyphenols with abundant health benefit and organoleptic properties that in planta act as key components of the plant defense system against diseases. Considerable advances have been made in the chemical characterization of wine metabolites particularly volatile and polyphenolic compounds. However, the metabotyping (metabolite-phenotype characterization) of grape varieties, from polyphenolic-rich vineyard by-product is unprecedented. As this composition might result from the complex interaction between geno-type, environment and viticultural practices, a field experiment was setting up with uniform pedo-climatic factors and viticultural practices of growing vines to favor the genetic determinism of polyphenol expression. As a result, UPLC-MS-based targeted metabolomic analyses of grape stems from 8 Vitis vinifera L. cultivars allowed the determination of 42 polyphenols related to phenolic acids, flavonoids, procyanidins and stilbenoids as resveratrol oligomers (degree of oligomerization 1 to 4). Using a partial least-square discriminant analysis approach, grape stem chemical profiles were discriminated according to their genotypic origin showing that polyphenol profile express a varietal signature. Furthermore, hierarchical clustering highlights various degree of polyphenol similarity between grape varieties that were in agreement with the genetic distance using clustering analyses of 22 microsatellite DNA markers. Metabolite correlation network sug-gested that several polyphenol subclasses were differently controlled. The present polyphenol metabotyping approach coupled to multivariate statistical analyses might assist grape selection programs to improve metabolites with both health-benefit potential and plant defense traits.

P13

Non-target analysis for the evaluation of surface water contamination: influence of sample preparation prior to UHPLC-HRMS analysis

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Identifying organic contaminants present in aquatic environments is of major concern for wa-ter quality monitoring. During the last 15 years, application of suspect and non-target analyses to study the presence of emerging contaminants and their transformation products in aquatic environment has increased to meet this challenge. The sample preparation is the first crucial step to be optimized before using these approaches for qualitative evaluations of water contamination. The main objectives of our study – based on a metabolomics workflow strategy – were to evaluate the quality of the data obtained with the different sample preparation tested (repeatability, num-ber of relevant signals, polarity range of the analytes detected, ...). A large surface water sample (15 L) was collected from an urbanized river. Different solid phase extraction conditions (SPE, n=3 per condition) were tested towards UHPLC-HRMS (Ultra High Performance Liquid Chro-matography - C18 column - hyphenated to High Resolution Mass Spectrometry) analysis. The SPE protocols varied according to the extraction phase (Oasis HLB® or multilayer cartridge), the elution solvent (methanol (MeOH) or MeOH and dichloromethane (DCM)) and sample pH (pH 3 or 7). To ensure data quality, analytical blanks, SPE blanks and quality control (QC) were also injected. The data were processed based on a metabolomic workflow using XCMS before comparison of features between sample preparation methods. Data were sorted out depending on (i) signal-to-noise ratio (S/N) compared to analytical blank, (ii) S/N compared to SPE blanks, analytical repeatability (obtained by coefficient of variation calculation for QC features), and (iv) extraction repeatability. Only signals with a fold change higher than 50 were kept and further processed to structural elucidation. Data treatment revealed the Oasis HLB® extraction at pH 7 with MeOH elution is the least repeatable one, unlike the multilayer extraction at pH 7 with MeOH elution which is the most repeatable. General comparison of the data revealed that the number of features detected for each method varies, with a higher number of features for the two SPE with Oasis HLB® extraction phase at pH 7 (elution with MeOH or MeOH and DCM). The extraction with Oasis HLB® – especially at pH 7 – revealed a higher detection of features with a m/z above 600 Da compared to the multilayered cartridge which seems better to recover compounds with a m/z between 50 and 200 Da. Based on the features retention time distribution comparison, the multilayer cartridge allowed the extraction of more polar compounds than the other SPE. The structural elucidation of features with a fold change higher than 50 highlighted presence of pharmaceuticals in the water sample (e.g. celiprolol – a betablocker, lamotrigine – an antiepileptic). To our knowledge, this methodology is poorly applied to evaluate analytical repeatability and has never been carried out to assess sample preparation methods for water suspect and non-target screening studies. Such an approach allows to better assess the quality of the data, especially for structural elucidation

P14

Criblage et quantification par UHPLC-MS/MS d'hormones végétales de la famille des strigolactones

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Les strigolactones sont des molécules produites par les végétaux qui non seulement stimulent la germination des plantes parasites, mais ont une action hormonale dans la plante-même [1,2]. Les strigolactones (SLs) jouent de multiples rôles dans le développement végétal et contribuent aussi à la mise en place de symbioses avec les micro-organismes du sol pour améliorer la nutrition de la plante [3]. Cependant leur rôle et leur synthèse ne sont pas encore complètement connus, ainsi que leur spécificité d'action suivant leur structure chimique. Les SLs possèdent une struc-ture chimique tétracyclique avec un squelette tricyclique ABC conjugué à un buténolide D par une liaison éther d'énol. Actuellement une vingtaine de SLs naturelles ont été identifiées. Elles se différencient par leurs décorations sur le squelette ABC tandis que le cycle D (buténolide) est invariant [4]. Dernièrement, d'autres composés dits " strigolactone-like " (SLs-like) ont été découverts possédant une action hormonale semblable et probablement biosynthétisés à partir d'un précurseur des SLs, ils s'en rapprochent d'ailleurs structurellement (présence du cycle D) [5].
Notre travail avait deux objectifs, i) développer et valider une méthode de quantification absolue sur 4 des strigolactones principales du pois, et ii) proposer une méthode de criblage et de quan-tification relative sur l'ensemble des SLs et SLs-like déjà connues et leurs isomères. i) Grâce à une synthèse originale d'étalons SLs marqués 3 fois au deutérium ou non marqués, nous avons mis au point l'extraction, la purification et la quantification de ces molécules par étalonnage interne. Les extraits sont purifiés par SPE en 2 fractions spécifiques, et analysées par LC-MS/MS triple quadripôle en mode MRM sur 2 transitions spécifiques. La validation de notre méthode montre que la quantification à partir de la somme des transitions MRM des deux adduits MH+ et MNa+ permet une meilleure précision de mesure [6]. ii) La méthode classique de crible MS/MS des SLs (recherche des précurseurs de l'ion fragment cycle D en mode ascendant) est efficace mais manque de sensibilité. C'est pourquoi nous proposons d'analyser toutes les transitions MRM existantes de la littérature sur les 31 SLs et SLs-like connues (soit 116 transitions) et validons la présence d'un composé ou son isomère seulement si un signal est présent sur au moins deux transitions, dont une spécifique. Par ce profilage, nous avons pu ainsi mettre en évidence, dans quatre espèces végétales très différentes, des SLs et SLs-like putatives qui n'avaient pas été détectées par crible classique.

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P15

Identification et quantification par LC-MS de composés phénoliques dans la pulpe et la peau de 4 variétés de pommes de terre: Solanum tuberosum. Complémentarité des approches MS/MS et HRMS.

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La pomme de terre est la principale denrée alimentaire non céréalière du monde [1]. Elle contient en plus de l'amidon des métabolites de type phénoliques, caroténo'ides et glyco-alcalo'ides. Ces derniers assurent au sein de la plante des fonctions spécifiques indispensables à sa survie comme : la pigmentation des fleurs (pollinisation), des fruits (dissémination), des semences (viabilité), la protection contre la radiation UV (anti-radicaux), la défense contre des microor-ganismes pathogènes (antiseptique), etc... [2]. Les propriétés de ces composés sont très utilisées dans l'industrie agro-alimentaire, pharmaceutique et cosmétique (antioxydants, antimicrobiens, filtres solaires, pesticides ...). D'où le défi de pouvoir caractériser ces métabolites dans différentes variétés et organes pour ensuite orienter, améliorer et valoriser au mieux ces composés dans les process industriels.
Notre étude a pour objectif de caractériser les métabolites de la pulpe et la peau dans quatre variétés de pomme de terre par chromatographie en phase liquide à haute performance couplée à un spectromètre de masse en tandem en basse et haute résolution. Nous avons ainsi identifié et quantifié 16 composés phénoliques polaires, 21 anthocyanes et 9 flavonoïdes dans ces échantillons dont les majoritaires ont pu être validés en haute résolution.

Nos investigations montrent une grande hétérogénéité dans la composition entre les variétés mais aussi, entre la peau et la pulpe au sein d'une même variété de pomme de terre. Il semble que la pulpe de la variété MS présente une plus grande quantité d'anthocyanes, soit 1200 fois supérieure à la quantité présente dans les pulpes des autres variétés. De même l'analyse démon-tre une richesse de la peau dans tant au niveau de sa composition et que de la teneur de ces composés, ce qui lui conférerait un intérêt tout particulier dans l'alimentation humaine du fait de leurs bienfaits anti-oxydants.

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P16

Elucidation de la structure de la lithiase urinaire par imagerie IR-TF

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Les lithiases urinaires sont des concrétions minérales qui se forment au niveau des reins. En fonction de leur taille et de leur composition, leur présence peut être un signe avant-coureur de maladies graves. Elles sont généralement analysées par spectroscopie Infrarouge à transformée de Fourier (IR-TF). La structure de la lithiase apporte des informations de l'évolution de la lithiase au court du temps. Celle-ci a des conséquences cliniques qui permettent d'améliorer la qualité des soins apportés au patient. Or, cette structure n'est pas élucidable par l'analyse con-ventionnelle en IR-TF car la lithiase est broyée pour pouvoir l'analyser ne donnant donc qu'une information moyenne de la composition. Le couplage de l'imagerie avec la spectroscopie IR-TF apporte un réel intérêt pour élucider la structure de la lithiase urinaire car c'est une technique non destructive et relativement rapide (temps d'analyse moyen par lithiase < 3h).

Pour réaliser l'étude, onze lithiases avec des compositions chimiques différentes ont été sélectionnées puis analysées par imagerie IR-TF en réflexion (DRIFTS). Les images ont été acquises avec une résolution latérale de 5.5 µm et une résolution spectrale de 4 cm-1. Les données hyper-spectrales obtenues ont été prétraitées avec une correction de ligne de base de type Whittaker. Les spectres des différents composés et les cartes de distribution afférentes ont été obtenus par décomposition des images à l'aide de la MCR-ALS.

Les images IR permettent d'identifier la totalité des composés présents dans les lithiases avec une corrélation spectrale de plus de 0,90 pour l'ensemble des molécules. Dans certains cas, l'imagerie permet de mettre en exergue la présence de composés qui n'avaient pas été détectés par spectroscopie IR conventionnelle. Les images obtenues par MCR-ALS permettent de discriminer l'oxalate de calcium monohydraté (Whewhellite) de l'oxalate de calcium dihydraté (Weddellite) qui étaient pour alors difficilement discernables par spectroscopie. L'identification des protéines est également possible lorsqu'elles sont présentes à plus de 10% de la surface de la lithiase. L'imagerie IR montre ainsi un fort potentiel dans l'évaluation qualitative de la composition et de la structure de la lithiase urinaire et pourrait être transposée à d'autres applications métabolomiques.

P17

Metabolomics enabled the identification of pre-frailty sub-phenotypes in elderly

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Context: Ageing is a dynamic process depending on intrinsic and extrinsic factors and its evolution is a continuum of transitions, involving multifaceted processes at multiple levels. It is recognized that frailty and sarcopenia are shared by the major age-related diseases thus contributing to elderly morbidity and mortality. They are major health issues in aging populations, given their high prevalence and association with several adverse outcomes. Pre-frailty is still not well understood but it has been associated with changes in several physiological systems, including inflammation as well as changes in the balance of micronutrients and vitamins. Due to the complex phenotypes and underlying pathophysiology, the need for robust and multidimensional biomarkers is now essential to move towards more personalized care and prevention. **Objective**: The objective of the present study was to better characterize the complexity of pre-frailty phenotype using untargeted metabolomics in order to identify specific biomarkers, and study their stability over time. **Research design and methods:** The approach was based on the NUAGE project (FP7 EU programme; clinicaltrials.gov, NCT01754012) that regrouped 1,250 free-living elderly people (65-79 y.o., men and women), free of major diseases, recruited within five European centres. Half of the volunteers were randomly assigned to an intervention group (1-year Mediterranean type diet). Presence of frailty was assessed by the criteria proposed by Fried *et al* (Fried et al. *J Gerontol A Biol Sci Med Sci*, 2001). In this study, a sub-cohort consisting in 212 subjects (pre-frail and non-frail) from the Italian and Polish centres were selected for mass spectrometry-based untargeted metabolomics. Metabolic profiles were determined from serum samples at T0 (baseline) and T1 (follow-up). All data were processed under the Galaxy web-based platform Worflow4metabolomics, guaranteeing their reproducibility (Giacomoni *et al. Bioinformatics*, 2015). Univariate statistical analyses were performed to identify discriminant metabolites regarding pre-frailty status. Predictive models were then built using linear logistic regression and ROC curve analyses were used to evaluate multivariate biomarkers. **Results**: Presence of sub-phenotypes of pre-frailty both at the gender level and depending on the pre-frailty progression and reversibility were revealed by untargeted metabolomics. Additionally, early markers, able to predict the evolution towards pre-frailty within one year, were identified for both genders. Moreover, some of these early biomarkers were found to be still relevant for classification of a 'light pre-frail' phenotype after its clinical appearance. **Conclusion**: These results open the door, through multivariate strategies, to the possibility of monitoring the disease progression over time at a very early stage. Longitudinal analysis of individual time trajectories to detect early deviations of health status would indeed contribute to a better disease prevention.

P18

Corsaire, plate-forme de métabolomique du Grand Ouest

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Corsaire est une fédération de plateaux analytiques dédiée à l'analyse de petites molécules biologiques. Elle couvre les domaines de la métabolomique, la fluxomique, l'isotopomique et l'analyse structurale. La biologie marine, l'agronomie, la santé et l'écologie chimique sont le cœur de l'activité de Corsaire. Ses approches analytiques vont de l'approche non ciblée pour la recherche de biomarqueurs, à l'approche ciblée pour l'analyse qualitative et quantitative de molécules d'intérêt. Pour ce faire, la plate-forme dispose d'un large parc analytique, composé d'équipements de RMN et de spectrométrie de masse, associés à différentes techniques séparatives adaptées à des matrices biologiques de nature et d'origines variées. Un accompagnement de la procédure analytique est proposé depuis la prise en charge des échantillons biologiques, l'extraction, les phases séparatives, l'acquisition des données brutes et, au besoin, leur traitement chimiométrique.

P19

Interactions between commensal *Escherichia Coli* and the host: impact on the metabolomic profile of the host

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Escherichia coli is a common and widespread inhabitant of human gastrointestinal tract, the prevalence in human gut is more than 90%. *E. coli* generally appears soon after birth and can reach levels higher than 109 CFU/g feces. In adulthood, *E. coli* among other *Enterobacteriaceae* represents the predominant aero-tolerant bacteria and its abundance is increased in high fat diet (HFD), diet correlated with a low-grade inflammation. Most works concerning *E. coli* focus on pathogenic strains and its impact on the host. However, data coming from our laboratory reveals that commensal *E. coli* contributes to intestinal homeostasis. The objective of this work is to better understand the interplay between commensal *E. coli* and the host in physiological conditions and if a HFD modifies *E. coli* impact. This study shows how subdominant microbiota members alone *E. coli* could impact the host metabolism; and how fat consumption may determine its impact. We have mono-colonized previous germ-free (GF) mice with two different *E. coli* commensal strains: i) the CEC strain, able of reinforcing the mucus barrier, ii) the characterized probiotic strain Nissle1917. GF, monoxenic and conventional mice were fed a standard diet or a HFD. Urine, plasma and caecal samples have been analyzed using 600 MHz proton NMR spectroscopy. NMR data were subjected to multivariate statistical analyses, Principal Component Analysis (PCA). PCA score plots showed a clear separation between the two diets for the three matrices. For urine and caecal samples, with standard diet, metabolic fingerprints are different between axenic, monoxenic and conventional mice. The two groups of monoxenic mice are not discriminated on the PCA score plot. With HFD, the four groups of mice are well separated on the PCA score plot for urine and caecal samples. For plasma samples, the four groups of mice cannot be discriminated using PCA analysis with standard diet or HFD. These data reveal that the metabolomic trajectory of the host is more sensitive to bacterial status over a HFD. The identification of discriminant metabolites between groups is in progress. The metabolic pathways disrupted by the bacterial status will be studied using the MetExplore software.

P20

Caractérisation de composés bio-actifs issus de la diversité microALgale par métaboLomique différentielle et Déréplication assistée par mise en REseAu Moléculaire (ALL DREAM)

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Face aux préoccupations du grand public sur l'impact des pesticides sur la santé et l'environnement, la législation Européenne a incité au développement de nouvelles stratégies permettant d'évaluer et de limiter les impacts potentiels des différentes pratiques agriculturales. Ainsi, le développement de nouvelles pratiques dites " vertes " telles que l'utilisation de produit de biocontrôle est en plein essor. Dans ce contexte, la biomasse issue de la production de micro-organismes photosynthétiques peut être une alternative à mettre en avant. En effet, les micro-algues constituent, au sein du milieu marin, une source variée de molécules bioactives. Les métabolites issus de ces dernières sont actifs sur une très large gamme de cibles biologiques et peuvent être valorisés de diverses manières : bio-pesticide, cosmétique, pharmaceutique, etc. Le projet " ALL DREAM " a pour but de développer un outil innovant adapté à l'analyse de la di-versité chimique des micro-algues et à l'évaluation de leur potentiel comme agent de biocontrôle. Ce projet propose d'unir, d'une part, des analyses différentielles des empreintes métaboliques et, d'autre part, des cartes de diversité moléculaire établies par l'étude des " réseaux moléculaires ". Le " réseau moléculaire " (molecular networking) est une approche bio-informatique innovante qui permet de caractériser des classes de métabolites en fonction de leurs données spectrales et d'étudier leurs degrés de similarité. Grâce à l'utilisation de réseaux moléculaires, des données et mélanges complexes peuvent être comparés in silico et annotés. Cette approche facilite l'étude du métabolome des organismes vivants et la caractérisation par déréplication des métabolites actifs étudiés. Pour ce projet, nous avons sélectionné une dizaine d'espèces de micro-algues faisant partie des classes des chlorophycées et phaeophycées. Ces dernières nous ont permis d'évaluer la faisabilité de l'approche à la fois du point de vue chimique que biologique. Avant l'acquisition des empreintes métaboliques par LC-HRMS, une attention particulière a été portée :

- Au développement d'un protocole d'extraction des échantillons permettant d'accéder à une large gamme du métabolome,
- A une méthodologie d'acquisition des empreintes métaboliques afin que celle-ci soit la plus reproductible et robuste possible.

Dans le cadre de la recherche de métabolites biocides, un test herbicide ciblé sur des thylako'ides ainsi qu'un test antioxydant ont été réalisés. Ces derniers sont, par la suite, optimisés en microplaques et couplés à l'approche métabolomique. Ce poster présentera

donc la méthodologie de préparation des échantillons et d'acquisition des empreintes métaboliques. Mais aussi, les premiers résultats de caractérisation métabolique couplée à l'activité biologique des extraits des micro-algues modèles.

Références bibliographiques :

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P21

Identifying metabolites with CE-MS: Improved reproducibility from improved migration modeling

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Capillary electrophoresis (CE) is well known for its usefulness as a separation technique, particularly for easily ionizable or polar molecules. As such, it can in principle provide additional information for identification. When coupled to mass spectrometry (MS) and in a similar way to RPLC, which relies on retention times, CE can achieve identification with the help of a corresponding parameter, migration times. The latter are noticeably influenced by factors (such as the electroosmotic flow) that are difficult to control. But *electrophoretic mobility* is an intrinsic property of the substance, and can be used for identification – when computable from migration time [1].
Even then, one wants the raw electropherogram converted to mobility scale before peak picking, to apply automated detection in a robust way. Given the volume of data generated by current CE-MS it is not easy to manually transform whole electropherograms to mobilities. We recently presented an original software tool, ROMANCE, developed to take care of this issue. ROMANCE has been shown to generate reproducible peaks in the mobility scale,that serve the sought-for purpose of identification [2].

This has raised the issue of *exactly* how reproducible these peaks are. The transformation from migration time to mobility corrects for the uncontrolled experimental conditions, but it might introduce additional sources of error. They manifest as variability in both peak area and peak position, the latter being key for identification. The critical point turns out to be the model-derived relation between migration time and mobility. This is necessarily an approximation of the actual physical processes underlying the migration. We considered a common scenario (CE with electric field ramping) in which the standard, well known relation between migration time and mobility is not valid. A corrected expression was derived.

We ran experimental tests on a set of common metabolites. Converting the electropherograms into mobilities with the usual formula, we found the error in observed mobilities to lie between 15-18% of their known value. By using the newly derived formula it was lowered down to the 0-2% range. In conclusion, a good understanding of the migration physics is fundamental for identification in CE-MS.

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P22

Coupling metabolome and microbiome analyses for deciphering the chemical dialogue between the brown alga Taonia atomaria and its epiphytic bacterial communities

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In marine ecosystems, macroalgal surfaces are prone to be colonized by complex communities of microorganisms including bacteria, archaea, microalgae, protozoa, and fungi. This colonization process, called biofouling, is generally initiated by the attachment of bacteria and leads to the formation of biofilms, i.e. complex tridimensional structures of microbial cells embedded in an exopolymeric matrix (Flemming *et al*. 2016). Through the evolution, marine macroalgae and microbes have developed a wide range of associations, such as parasitism, mutualism or commensalism, and ultimately the surface of macroalgae constitutes a privileged area of chemical interactions between the algal host and its associated microbiome (the whole forming a biological system called ”holobiont”) (Hollants *et al*. 2013, Wahl *et al.* 2012). Advances in technologies for microbiome sequencing and metabolomics have enabled these research fields to reach a stage of maturity. Thus, integration of microbial and metabolomics data from such a holobiont system would allow a better understanding of: (i) the impact of compounds released by the host at its surface on the epiphytic communities, and (ii) the microbial influence on the host through production and/or degradation of biologically-active molecules.

In the present study, we investigated by a multi-omics approach the seasonal co-variation between the surface metabolome and the epibacterial communities of the Mediterranean brown macroalga *Taonia atomaria*. The LC-MS based metabolomics and the 16S rDNA metabar-coding studies showed a similar seasonal trend. Furthermore, a particular attention was paid to few metabolites known to regulate the epibiosis by inhibiting the adhesion of specific bac-teria (Othmani *et al.* 2016) but also on compounds implied in the seasonal discrimination between the surface extracts. Among these last metabolites, several betaines together with diacylglycerylhydroxymethyl-N,N,N -trimethyl-*b*-alanine (DGTA) derivatives and dipeptides, identified through molecular networking, were the most discriminant. These data were then connected to the metabarcoding dataset using multi-block PLS-DA to identify compounds inhibiting some specific bacterial OTUs or involved in the shaping and the seasonal variation of epiphytic communities.

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Doxorubicin-induced metabolic alterations in H9C2 cells and effects of preincubation with dexrazoxane, a cardioprotective agent

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Introduction

Currently, doxorubicin treatment in oncology is limited by its irreversible cardiotoxicity whose gravity depends on cumulated dose and can range from subclinical myopathy to patient's death. The main toxicological mechanism is the production of reactive oxygen species (ROS), leading to an oxidative stress impairing mitochondrial function and membrane integrity. It results in cardiomyocytes death either by apoptosis or necrosis, clinically expressed by a progressive heart failure. Nowadays, the main strategy to deal with this cardiotoxicity is the co-administration of dexrazoxane, a cardioprotective agent that acts by reducing ROS production through iron chelation. However, this strategy shows limited efficacy and there is a need for new cardioprotective tools. The goal of this research is to study doxorubicin-induced metabolic alterations and the protective role of dexrazoxane in order to highlight possible new targets to counteract doxorubicin cardiotoxicity.

Material and methods

Rat cardiomyoblasts H9C2 (2-1) (ECACC 88092904) were cultured in DMEM according to ECACC guidelines. For all exposure procedures, cells were first seeded with a density of 30.000 cells/cm2 and were kept growing during 48 hours before any exposure. For oxidative stress and metabonomic investigations, cells were randomly assigned into 4 groups : a control group (CTR), a 0,3 µM doxorubicin-exposed group (DOX), a 3 µM dexrazoxane-exposed group (DEX) and a group pre-incubated with 3 µM of dexrazoxane during 30 min before 0,3 µM doxorubicin exposure (DEX+DOX). Oxidative stress was assessed after 2 hours of exposure with a quantitative fluorimetric DCFH-DA assay of reactive oxygen species (ROS) production and a 1H-NMR-based metabonomic study was carried out after 24 hours of exposure on both culture media and extracted intracellular polar metabolites. Acquired 1H-NMR spectra were baseline and phase-corrected and TSP-calibred. Spectral area from 0,08 to 10 ppm was subdivised into integrated sub-regions of 0,04 ppm wide. The water peak was deleted and each subregion integral was normalized to spectrum total area. Multivariate data analysis were performed on data and discriminant metabolites were identified using several databasis.

Results

Results of DCFH-DA assay describe a clear increased production of ROS when H9C2 cells are exposed to doxorubicin. This production is proportional to doxorubicin concentration in a linear way. Dexrazoxane preincubation with a dose 10 times higher than doxorubicin dose can reduce significantly the ROS production. The metabonomic study highlighted some metabolic alterations due to doxorubicin exposure: a switch from mitochondrial aerobic energy metabolism to cytosolic anaerobic metabolism (increase of lactate, phosphocreatine and glutamine production and secretion, decrease of UDP-glucose level), a cell response to oxidative stress by an increased intracellular taurine level, modification of amino acids metabolism.

The metabonomic study also highlighted metabolic effects of dexrazoxane pre-incubation : recovery of mitochondrial aerobic metabolism (decrease in lactate, glutamine levels and increase in UDP-glucose level), activation of choline metabolism (phosphocholine, glycerophosphocholine and serine increased levels) for biological membranes synthesis, activation of creatine phosphorylation and an increased secretion of succinate. Interestingly, choline metabolism activation may be linked to cell survival and growth pathways. Concomitantly, succinate secretion may promote proliferation pathways by simulating GPR91 receptor. Choline metabolism and GPR91 could be potential targets for improving cardioprotection during doxorubicin exposure and are therefore attractive for further investigations.

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P24

NMRProcFlow: An easy GUI tool dedicated to 1D NMR spectra processing (1H & 13C) for Metabolomics

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Although metabolomics by 1D NMR spectroscopy has become a common approach, multiple challenges in spectra and data processing remain to be solved. Unlike techniques coupled with mass spectrometry such as LC-MS, GC-MS or CE-MS„ 1D NMR spectroscopy has only one dimension on which we can rely, and apart from very well-mastered and very reproducible use-cases, the implementation of 1D NMR spectra processing workflows within a Virtual Research Environment (VRE) and operating automatically in order to be widely used by non-expert users has not yet reached full maturity. Indeed, the expert eye is often required and even crucial to disentangle the intertwined peaks and the best way is to proceed interactively with a 1D NMR spectra viewer.

To fulfill this need, we have been developing NMRProcFlow [1], an interactive 1D NMR spectra processing (1H & 13C) dedicated to metabolomics. It has been built by involving NMR spectroscopists eager to have a quick and easy tool that greatly helps spectra processing and that can be of used also by new-comers. For each of the two major metabolomics approaches, namely Metabolic Fingerprinting and Targeted Metabolomics, the workflow covers all steps from spectral data up to data matrix output. Moreover, the possibility of visualising the experimental factor levels within the NMR spectra set through a spectral viewer makes the tool valuable to create links between the experimental design and subsequent statistical analyses, and thus facilitates interactions between biologists and NMR spectroscopists. In addition, NMRProcFlow allows experts to build their own spectra processing workflows, in order to become models applicable to similar NMR spectra sets, i.e. stated as use-cases.

NMRProcFlow is accessible online (http://nmrprocflow.org), or alternatively, a virtual machine for local installation can be downloaded.

NMRProcFlow is now implemented as a data pre-processing, analysis and visualization tool in the PhenoMeNal [2] Application Library and also as a Galaxy Interactive Environment (IE) offering a more complex user interface (GUI) that what is offered by standard Galaxy tool inte-gration.

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https://phenomenal-h2020.eu ; https://portal.phenomenal-h2020.eu/help/NMR1d-Workflow

Combined metabolomic and proteomic profiling of maize leaf to reveal metabolic responses to cold temperatures

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In Northern-Europe, maize field early-sowing is used to maximize yield and may lead to moderate damages of seedlings due to chilling. However, detailed studies of the metabolic responses to low, non-freezing, temperatures remain rare. In the present experiment, eighteen genetically-diverse maize hybrids (Zea mays, dent panel lines crossed to the flint inbred-line UH007) were sown in a growth chamber and cultivated at optimal temperature for three weeks and then three decreasing temperature steps. The youngest ligulated leaf was harvested for biochemical studies. Metabolite profiles were determined using proton nuclear magnetic resonance (1H-NMR) of polar extracts and liquid chromatography mass spectrometry (LC-ESI-QTOF-MS) of semi-polar extracts to measure biological variation of primary and specialized metabolites in maize leaves. Starch and total proteins were also measured. Proteome profiles were determined with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Multivariate and univariate statistical analyses were used to compare the profiles between the experimental conditions and reveal different response patterns. The metabolites, metabolite signatures or proteins differentiating the temperatures could be used as biological markers for temperature response when their identification steps are completed.

Keywords: metabolomics, proteomics, maize leaf, environmental changes.

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P26

Targeted and untargeted metabolomics using LC-HRMS on cereal grains contaminated by fungi from endemic areas for Kashin-Beck Disease.

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Cereal grains are sometimes unfit for consumption due to contamination by filamentous fungi during harvest or storage. The main fungi contaminations are due to *Aspergillus, Penicillium, Fusarium, and Alternaria* that might produce secondary metabolites including mycotoxins. The latter are a large group of compounds toxic at several extend (according to their nature and concentration) for humans and animals. The major mycotoxin groups are of public health concern and include aflatoxins, fumonisins, ochratoxins, deoxynivalenol, trichothecenes and zearalenone (Lee and Ryu, 2017).
Herein we analyzed grains (mainly barley, but also maize, wheat, and tsampa (roasted barley flour)) that were collected in different areas of Tibet (3) and Inner Mongolia (1). These areas were endemic (EA) osteochondropathy named Kashin-Beck Disease (KBD) and other areas were not (NEA). KBD has a high prevalence from South East Siberia to North West part of China. Currently it remains mainly active on the Tibetan plateau. KBD expresses by painful and deformed joints of the four members with limited motion and shortening of long bones. If causes and molecular mechanisms of KDB are poorly understood, mycotoxins have been involved and T-2 toxin (trichothecenes sub-family often found in some *Fusarium spp*.) has been reported. T-2 toxin is indeed able to promote degradation of cartilage proteoglycans. (Mathieu and Hissenkamp, 2008; Guo et al, 2014; Tian et al, 2012)

The cereal grains included in the study were microbiologically tested for fungi and most of them were positive for the genus *Alternaria* in the EA samples. These fungi also produce several mycotoxins. The grains were grounded to flour and an extraction commonly used for mycotoxins was performed (Varga et al, 2014). Then, a solid-phase extraction followed by LC-HRMS in positive mode analysis was performed. Data were treated in two different ways: (i) a targeted approach were several mycotoxins were systematically searched (altertoxins, alternariol, tentoxins, fumonisins, trichothecenes, australifungin, ...) based on literature, customized Agilent mycotoxin databank and on the m/z values, and (ii) an untargeted metabolomics approach of the sample was performed using the Workflow4Metabolomic (W4M) platform.

The targeted analysis of the extracts revealed only few mycotoxins. Australifungin was suspected but was discarded, as MSMS fragmentation pattern was highly different from the pattern of standard australifungin. No fumonisin was detected. AAL toxin E has been detected in several samples of the EA zone but has not been confirmed since no standard was available. Interestingly, the untargeted metabolomics approach using multivariate analysis, principally PCA and PLS-DA analyses, could cluster the samples based on the areas, and for example could discriminate EA and NEA on the first component (component 1) in Tibet areas. Furthermore, Biosigner tools highlighted several unidentified metabolites that strongly discriminated EA and NEA. Further investigations (such as molecular networking) should be driven on the identification of these metabolites and their potential link with KBD.

In conclusion, metabolomics investigations could discriminate the cereal grains from endemic and non-endemic areas for KBD in Tibet and Inner Mongolia. However, only few known mycotoxins could be definitely identified in the samples but nowadays none of them could be linked to KBD.

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P27

How does E. coli's metabolic network deal with a genetic "bug" affecting the first step of the branched chain amino acids pathway?

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Escherichia coli K-12 MG1655 strain (F- lambda- ilvG- rfb-50 rph-1) is a model organism for scientific research and a workhorse for biotechnology, which carries frameshift mutations in its genome. Among them, a reported frameshift mutation concerns *ilvG* gene (ilvG-) which encodes the acetohydroxy-acid synthase II (AHAS; EC 2.2.1.6), leading an inactive gene product. The unique known consequence of this genetic "bug" is the valine sensitivity, characterized by a growth defect of *E. coli* K-12 in minimal medium supplemented with valine. Here, we wondered if the lack of AHAS II enzyme induced other disorders at the metabolic level despite of the presence of the two isoenzymes, AHAS I and AHAS III. By using an exometabolome comparative analysis using 1D-H NMR between the wild-type strain and a strain repaired for this specific mutation, we identified the accumulation of three metabolites, namely propionate, DHIV (2,3-dihydroxy-isovalerate) and DHMP (2,3-dihydroxy-3-methylpentanoate). The two latter metabolites are intermediates of the branched chain amino acids (BCAA) pathway, both being the substrate of dihydroxy acid dehydratase, for the formation of valine and leucine, and for the formation of isoleucine respectively. Surprisingly, the reaction catalyzed by the dihydroxy-acid dehydratase takes place downstream of that catalyzed by the AHAS, making the accumulation of DHIV and DHMP not obvious to explain. Propionate is reported to be produced from ketobutyrate in anaerobiosis by pyruvate formate lyase. The propionate accumulation observed in this study in aerobic condition suggests the existence of an unknown metabolic process in connection with the absence of AHAS II. Combining metabolomics approaches based on both MS and NMR, and molecular biology tools, we pinpointed local metabolic reorganizations explaining the accumulation of these metabolites in culture supernatants. This works demonstrates the flexibility of this metabolic node in E. coli which contributes to the adaptative ability of metabolism to genetic modifications.

P28

Introducing HDiSpeC: A new desktop software for live Molecular Networking and t-SNE projections of MS/MS spectra

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Molecular Networking (MN) applications are expending fast through the development of the Global Natural Products Social (GNPS) Platform [1] becoming a standard in the metabolomic community. This paradigm is based on the observation that structurally related compounds share comparable fragmentation fingerprint. A score of similarity between ion fragmentation patterns is provided using a modified cosine calculation. The idea of vector-based spectral similarity calculation has been widely used and discussed, especially in mass spectral library searching applications [2].
The major drawback of this architecture is that considering too much cosine scores to create edges between nodes might result in crowded networks without a clear separation between clusters. Using a cosine score threshold and a topK ranking algorithm as filtering steps, only the most relevant cosine scores are selected. However, depending on the filtering values selected, numbers of cosine scores are arbitrarily deleted and a lot of information is ignored. Of course, it is possible to find out an optimal set of parameters that could reduce the impact of defects previously mentioned but this step is time-consuming and should be optimized for each particular data set.

The problem of creating a representation of a high-dimensional dataset like MS/MS spectra can be solved by using algorithms developed for dimensionality reduction and pattern recognition purposes. A recent technique known as *t-distributed stochastic neighbor embedding* (t-SNE) has rapidly established itself as a method of choice for summarizing high-dimensionality datasets [3]. This multivariate embedding method gives a particular attention for local details by using non-linear outputs to represent the entire data space [4].

Our new software provides two ways of overcoming GNPS weaknesses. First, we give users the choice between two different representations of the initial dataset, one based on a classical GNPS-like molecular networking and another one based on the t-SNE algorithm. These two views of the same data can give complementary information and we often see clusters in one view that is not present in the other or at least not as large. Second, all parameters except those used for cosine score calculations can be tuned in real time and new networks can be generated within a second for small datasets.

With the development of this unified interface, we fulfilled the need of a dedicated, user-friendly, local software for MS/MS comparison and spectral network generation, removing the requirement of several different software (FTP client, browser and molecular interaction networks visualizer).

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P29

Metabolomic study for the evaluation of the treatment of major depression by ultrasound neurostimulation

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Major depression is a global public health problem. Antidepressant treatment represents the classical approach, although 50% of patients do not benefit from improvement. Alternative therapies have been proposed including transcranial magnetic stimulation or deep brain stimulation, but these techniques have various limitations.
Ultrasound neurostimulation (USNS) has recently been proposed for the non-invasive stimulation of brain tissue and is attracting increasing interest.

This work proposes to evaluate the efficacy of the USNS in an unpredictable mild chronic stress mouse model using behavioral tests associated with a PET imaging study and a metabolomic study.

The metabolomic study was carried out by LC-HRMS using an approach targeting a chemical library of 500 metabolites and on different brain regions that have been targeted by ultrasound treatment (cortical regions: cingulate, limbic and prelimbic) as well as deeper regions (amygdala and hippocampus) because of their implications for major depression and their interconnectivity. The results obtained show that treatment with USNS produces alterations in the metabolome of all brain areas studied.

In particular, the alanine, aspartate and glutamate pathway is altered in all regions and we observed increased levels of glutamic acid in the PrL / IL cortex (FC = 0.37) as well as a decrease in glutamine in the Cg and hippocampus after USNS.
Beyond the conclusion that the USNS represents a promising approach for the treatment of major depression; this study reinforces the idea that metabolomics is an approach of choice to evaluate the effect of new therapeutics by dissecting mechanisms of action.

P30

GC-MS metabolomic analysis of human plasma in spondyloarthritis

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Rationale: Spondyloarthritis (SpA) is a chronic, progressive inflammatory rheumatic disease of multifactorial origin affecting primarily young adults. Currently available treatments are mainly nonspecific and symptomatic and a better understanding of SpA pathogenesis is needed to improve early diagnosis and identify new therapeutic approaches. This study was aimed at discovering biomarkers that might help identify patients at an early stage of disease or healthy subjects at risk of developing it, with an untargeted metabolomic approach.
Methods: Human plasma samples were obtained from 2 cohorts of patients, one discovery cohort (20 patients and 21 healthy controls) and one validation cohort (40 patients and 20 healthy controls). Plasma was treated with acetonitrile/isopropanol/water 3/3/2 (*v/v/v*) and the dried extracts derivatized with MSTFA. Gas chromatography was performed with an Uptibond 5 Premium 30m x 0.25mm x 0.25 µm analytical column (Interchim, Montluçon, France) with a temperature gradient from 60 to 325 C in 40 min. Mass spectrometry analysis was carried out with a TSQ 8000 instrument (Thermo Fisher Scientific, Villebon-sur-Yvette, France) in full-scan mode in the range 50-600 *m/z* with electron ionization. The data was analyzed with Workflow4Metabolomics.

Results: In the discovery cohort, 344 compounds were detected among all samples. The Partial Least Square – Discriminant Analysis (PLS-DA) model identified significant differences between individuals with SpA and healthy controls (R2X=0.230, R2Y=0.996, Q2Y=0.719). Multivariate analysis and binary classifiers such as support vector machine provided a set of 4 discriminant compounds which were not identified in the Golm metabolome database. Among those compounds, 2 of them belong to the same chemical family of exogenous molecules and were found overexpressed in patients. The formal annotation of these compounds is ongoing and the relative quantification in the validation cohort also suggests an overexpression in individuals with SpA. Conclusion: The results confirm the interest of untargeted metabolomics as a useful approach in the understanding of the pathogenesis of SpA and to discover new potential biomarkers of clinical interest.

P31

Metabolomic analysis to characterise fruit maturity at harvest in apple and kiwifruit

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Kiwifruit and apple are by far the two largest fruit export crops from New Zealand. Both species are harvested at maturity and can then be stored for a long periods of time at chilling temperatures. Delivering high quality product is the major issue for New Zealand fresh fruit exporters. Fruits must have good storage performance to reduce losses and ensure perfect quality for delivery to the consumer.
One of the most important factors affecting good storage is ensuring fruit are at the correct maturity at harvest. Soluble solids content (SSC) has been used as a measure of maturity and as a harvest index for kiwifruit in New Zealand since 1980 (*1*). However, attainment of a minimum SSC is not a reliable indicator of storage performance, and comparisons of harvest date and between orchards have shown that other factors must be involved. New measures have to be found to provide a strong prediction of maturity and storage performance. With these new measures, fruit lines with good storage potential will be channelled into longer-term storage, and lines with less storage potential will be marketed earlier. Our aim was so to identify new biochemical attributes of fruits to give an improved determination of maturity at harvest and a strong predictive value for eating quality post storage.

Gold-fleshed kiwifruit (Actinidia chinensis var. *chinensis* 'Zesy002'; commonly known as Gold3) and apple (*Malus x domestica* 'Royal Gala'), from four different vines and trees respectively, were harvested at four different times, each two weeks apart, with Harvest 3 corresponding to commercial harvest. LC-MS-based metabolomics analysis combined with multistatistical analysis was carried on both species to gain an overview of fruit metabolism according to harvest time. Clear differences in metabolic fingerprints were observed between harvest times for fruits. The important metabolites responsible for the discrimination were identified. Related metabolic pathways were identified and tests of corresponding transcripts and enzyme activities are in progress.

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P32

Mise en évidence de métabolites chez Gammarus fossarum exposé à deux composés pharmaceutiques par nanoLC-HRMS

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La présence de substances pharmaceutiques dans les milieux aquatiques est aujourd'hui avérée et constitue une source d'exposition directe et indirecte, via la chaine trophique pour des organismes supérieurs (poissons, oiseaux), voire l'homme via les espèces sauvages commercialisées.

L'étude menée ici a pour but d'apporter des réponses quant à la métabolisation due à l'exposition à des composés pharmaceutiques chez un invertébré modèle largement utilisé comme espèce sentinelle de la contamination des milieux aquatiques, *Gammarus fossarum*. En particulier, le projet vise la mise en évidence d'une potentielle différence de métabolisation des individus de sexe différents susceptibles de conduire à des différences de sensibilités toxicologiques.

Dans ce travail de métabolomique environnementale, l'objectif est donc d'étudier par nanochromatographie liquide couplée à la spectrométrie de masse haute résolution (nanoLC-HRMS), en conditions de laboratoire, l'exposition de *Gammarus fossarum* à l'anxiolytique oxazépam et l'antiépileptique carbamazépine. Des gammares mâles et femelles ont été exposés au mélange des deux composés pharmaceutiques (1000 ng.L-1) pendant 14 jours. Ils ont été analysés à l'échelle d'un seul individu grâce à une méthode incluant une extraction de type QuEChERS suivie d'une analyse par nanoLC-QqToF (nano RSLC U3000 Thermo ® - Maxis Plus Bruker ®). Les empreintes moléculaires obtenues ont été investiguées à l'aide de la plateforme Galaxy Work-flow/4/Metabolomics. Le logiciel MATLAB a été utilisé pour la correction des dérives expérimentales (lock mass et QC) ainsi que pour le retraitement des données par la méthode ANOVA-PCA.L'exploitation des données, dont la reproductibilité est améliorée à la fois par la normalisation par la lock mass mais également par le lissage apporté par les QCs, met en évidence un effet de l'exposition au mélange des deux substances pharmaceutiques ainsi qu'un effet du genre. Les résultats montrent par ailleurs un effet d'interaction significatif entre les deux facteurs étudiés ce qui suggère que l'effet de l'exposition au mélange de médicaments dépend du genre de l'individu exposé. L'identification des signaux discriminants potentiels métabolites ou biomarqueurs sont en cours par des analyses MS/MS.

P33

Etude métabolomique par RMN du proton de l'effet du stress osmotique chez Brachypodium dystachion

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La disparition des ressources fossiles et minières relance et active la recherche de solutions alternatives telles que la production d'énergies renouvelables à partir de biomasse végétale. Brachypodium est une des plantes modèles utilisées pour la recherche sur les biocarburants, et également pour la recherche sur la production et l'amélioration des céréales (Brkljacic et al., 2011). Le rendement des cultures est fortement dépendant des conditions environnementales et est susceptible d'être plus impacté si les conditions météorologiques sont défavorables, en particulier lors de périodes de sécheresse. Dans un contexte de changement climatique, il devient nécessaire d'améliorer la compréhension des stratégies utilisées par les plantes pour s'adapter au stress hydrique. L'approche métabolomique par RMN du proton est largement utilisée pour les études de stress biotiques et abiotiques chez les plantes (Kim et al., 2011). Ainsi, cette approche sera utilisée pour étudier les changements de contenu métabolique des différents organes de Brachypodium dystachion (feuilles, tiges, racines) soumis ou non à un stress osmotique (mimant un stress hydrique) pendant 1 et 10 jours. L'analyse en composante principale (ACP) montre une discrimination entre les groupes témoins et les groupes stressés à J1 et à J10 pour chaque organe. Chez les plantes stressées, des variations en contenu métabolique communes aux trois organes ont été observées, avec notamment une augmentation du contenu en certains acides aminés (asparagine, glutamate, GABA), en acide malique et en certains glucides (saccharose et raffinose), et une diminution du contenu en aspartate, glutamine et acide formique.

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Precautions of a revisited protocol for 1D 1H-NMR profiling of plant samples from extract preparation to spectra processing. Preliminary results of an inter laboratory experiments.

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The main objective of the present work is to minimize uncontrolled variability in plant sample preparation before NMR profiling, taking into account sample composition, specificity in terms of pH and paramagnetic ion concentrations, and spectrometer performance. Therefore, we implemented additional steps in a plant extract-preparation SOP previously published[1] and automation of routine spectrometer qualifications (shimming, temperature control) for each sample before spectra acquisition. In this poster, we report on an inter laboratory comparison of plant derived samples by 1H-NMR spectroscopy across three different sites utilising instruments from two manufacturers with different probes and magnetic field strengths of 9.4 T (400 MHz, ECZ-S JEOL, double resonance ROYAL probe), 11.7 T (500 MHz, AVANCE III Bruker, double resonance broadband probe BBI) and 14.1 T (600 MHz, AVANCE III Bruker, Triple resonance probe TXI).

Comparability of the datasets from the three laboratories was exceptionally good in terms of spectral quality. The coefficient of variation of the half-width and the signal-to-noise ratio of two selected peaks were comprised between 5 and 10% depending on the spectrometer. The three collections of spectra were processed separately with NMRProcFlow [2] tool (Fourier transformation, phasing, chemical shift calibration, baseline correction, peak realignment, non-uniform bucketing, Signal-to-Noise Ratio determination). Each spectral region of interest or bucket was determined either with intelligent bucketing or variable-size bucketing. The resulting buckets were subjected to multivariate analysis with BioStatFlow application (www.biostatflow.org). This proof of concept on a medium-size collection of sixty samples of durum wheat kernel extracts showed that the data collected at the three different sites on instruments of different field strengths and manufacturers yielded the same discrimination pattern of the biological groups.

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Réorganisation du métabolome de la graine de lin lors de son développement : intérêt de la fusion de données lors de l'analyse métabolomique par RMN et LC-MS

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Les composés du lin (*Linum usitatissimum*) sont largement utilisés dans différents secteurs industriels.La graine de lin est fortement valorisée pour son huile, mais d'autres composés comme les polyphénols (lignanes notamment) peuvent également être utilisés en cosmétique ou en nutraceutique. D'où l'intérêt de pouvoir accéder au contenu de tous les métabolites de la graine de lin. L'analyse globale du contenu de la graine au cours de son développement présente des difficultés techniques en raison de la présence simultanée de métabolites de différentes natures chimiques et à des concentrations variées. De ce fait, la RMN est un outil de choix dans les approches non ciblées pour des études à haut débit de type métabolomique car elle est non sélective et non destructive. La spectrométrie de masse est complémentaire à la RMN car elle donne accès aux métabolites plus faiblement concentrés grâce à sa très grande sensibilité. Dans ce contexte, un protocole d'extraction a été optimisé pour réaliser des analyses par RMN et par spectrométrie de masse à partir d'un même extrait végétal disponible en faible quantité. La réalisation de l'ensemble des analyses sur le même extrait a permis l'utilisation de méthodes d'analyses de données combinées2 de type multiblock.Ces analyses ont ici permis d'accéder à une meilleure connaissance de la réorganisation du métabolome de la graine de lin au cours de sa formation.

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Impact of Botryosphaeriaceae on metabolism of two V. vinifera subspecies – Identification of infection and resistance biomarkers of ancestral grapevine V. vinifera subsp. sylvestris

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Botryosphaeria Dieback is one of the main grapevine trunk diseases and shows a considerable impact on the production and the durability of the French and world vineyards. To date, no treatment is available to limit the progression of this disease. It is associated with the development of fungi of the Botryosphaeriaceae family, including *Neofusicoccum parvum* and *Diplodia seriata*, which produce wood-degrading enzymes as well as phytotoxic metabolites. Their link with the characteristic leaf symptoms of the disease is not yet clear. Similarly, the responses of the grapevine following the fungal infection are still poorly described, especially the changes in its metabolism. On the other hand, the ancestor of our European grapevine, the subspecies sylvestris, shows some resistance to fungal diseases that we could exploit.For this purpose, we carried out both a targeted metabolomic study to quantify by GC-MS the primary metabolites in grapevine (sugars, amino acids and organic acids) as well as an untargeted study of secondary metabolites by LC-MS / MS. In particular, we have been able to highlight biomarkers of fungal infection as well as different metabolic profiles between the two grapevine subspecies linked to their difference in resistance to the pathogen.

Evaluation de biomarqueurs d'une exposition gestationnelle au bisphénol S par une approche globale stéroïdomique sur le modèle ovin

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Le bisphénol S (BPS) est largement utilisé dans l'industrie plasturgique comme substitut du bisphénol A (BPA), interdit en France dans tous les contenants alimentaires depuis le 1er janvier 2015. La présence du BPS est ubiquitaire comme en témoigne la détection quasi systématique du BPS dans des urines collectées en Asie ou aux Etats-Unis (*Environmental Science & Technology* 2012, 46: 6860). Cette prévalence élevée et croissante de l'exposition humaine au BPS est préoccupante en raison de son potentiel oestrogéno-mimétique et anti-androgénique, notamment durant la période à risque de la gestation. Par ailleurs, chez l'homme (*Environmental Science & Technology* 2017, 51: 2456), du BPS a été détecté dans le sang de cordon ombilical, attestant ainsi de la capacité du BPS à passer la barrière placentaire. L'équilibre stéroïdien est indispensable au maintien de la gestation et au développement embryonnaire et fœtal. Il joue également un rôle important dans la programmation fœtale. Des effets du BPS sur la stéroïdogenèse, similaires à ceux du BPA ont été mis en évidence in vitro et ex vivo. Ces perturbations de l'homéostasie des stéroïdes sexuels pendant la phase de programmation de la gestation pourraient être impliquées dans l'incidence accrue de troubles exprimés chez l'adulte. Dans ce contexte, notre hypothèse est que la signature stéroïdomique de l'unité materno-foeto-placentaire pourrait constituer un biomarqueur précoce des effets délétères du BPS. Pour mettre à l'épreuve cette hypothèse, une approche globale de type stéroïdomique a été mise en œuvre sur un modèle de brebis gravide, pertinent vis-à-vis de l'homme en termes de physiologie de la gestation. Les brebis gravides ont été traitées quotidiennement, du 68ème au 96ème jour de gestation, avec du BPS (à la dose de 5 mg/kg/j ou de 50 g/kg/j) ou du solvant par voie intramusculaire. Les tissus fœtaux collectés en fin d'expérience ont été analysés sur les mâles uniquement (1) pour évaluer l'exposition au BPS et à son principal métabolite le BPS-Glucuronide (mesure par couplage LC-MS/MS) et (2) par une approche stéroïdomique pour déterminer une trentaine d'hormones stéroïdes sous formes libre et conjuguée (mesure par couplage GC-MS/MS). Des analyses statistiques sont en cours afin de retraiter les résultats obtenus et in fine d'évaluer l'impact du BPS sur la signature stéroïdomique de l'unité materno-foeto-placentaire.

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Multi-platform data integration for improved metabolite coverage: application to chronic kidney disease

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Chronic kidney disease (CKD), one of the major public health issue, is a renal disorder characterized by progressive loss of kidney function leading to end-stage renal disease and high risk of cardiovascular morbidity and mortality. Due to the irreversibility of the disease progression, hemodialysis and transplantation are mandatory at end-stage. New biochemical insights are needed for early detection and better understanding of the metabolites and biochemical pathways involved in CKD. For that purpose, untargeted metabolomics, one of the omics technologies that aims to comprehensively analyse the metabolic complexity of biological systems, allows an extensive coverage of metabolites (mass < 1000 Da) for phenotype modifications assessment caused by pathologies. As no technique offers an exhaustive monitoring of all metabolites in a biofluid, the use of multiple analytical platforms is needed.

Reversed-phase chromatography (RPLC) and hydrophilic interaction chromatography (HILIC) coupled to high resolution mass spectrometry (HRMS) are complementary techniques commonly used for their coverage of apolar and polar metabolites, respectively. A strategy based on the combination of these two analytical approaches was applied to plasma samples collected from a clinical study designed to evaluate the metabolic impact of CKD. The cohort was composed of 56 control samples, and 104 patients at several disease stages, including 35 dialysed patients before their mid-week dialysis session. Sampling was also performed just after dialysis session to assess impact of hemodialysis. Each sample and quality control (QC) was analysed by RPLC and HILIC coupled to quadrupole time-of-flight mass spectrometer (QTOF-MS) in negative and positive electrospray ionization mode.

More than 300 annotated compounds were investigated thanks to the fusion of datasets generated from multiple platforms using an in-house database of about 700 metabolites. The major sources of variability observed in the dataset were related to biological modifications due to the pathology stages. OPLS models allowed to define 65 increased and 7 decreased metabolites showing high correlation with CKD stages. Thanks to pathways analysis, 9 of these discriminant metabolites were found to be involve in tryptophan and serotonin metabolisms indicating potential link to the pathology. Furthermore, study design allowed to illustrate the beneficial effect of dialysis by eliminating most of unwanted metabolites, while keeping most of necessary metabolites but also the adverse effect on subgroup of metabolites. The workflow and the multivariate analysis of the dataset developed in this study allowed patient stratification according to CKD stages and hemodialysis state, leading to generate biological hypotheses based on the metabolic profiles.

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Steroidomic Profiling For The Analysis Of Seminal Liquid: The ”PNR 50” Case Study

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Steroids are essential hormones in animal organisms involved in a great diversity of pathologies. The monitoring of such compounds could allow observing and understanding immune responses, circadian rhythms, foetal development and gonadal functions. However, various organs can produce steroids thus possibly misleading the interpretation of the content of a given clinical sample. This could be the case of steroids implied in male fertility. Androgens are reported to regulate spermatogenesis, but also to be dysregulated in prostate cancer and in sperm abnormalities. Their production from Leydig cells and the metabolism in prostate gland could be followed by their levels in semen, rather than blood in which variations would be hidden by other sources of endogenous steroids' regulation.
This work presents a preliminary study of a cohort of 50 young male conscripts in the context of the ”PNR 50” project aimed at evaluating male reproductive health across Switzerland. Serum and seminal liquid were collected and two sample preparations were evaluated, namely protein precipitation and Supported-Liquid Extraction. Then RPLC was carried out on a 1.7 µm Kine-tex C18 column (2.1 x 150 mm) with a gradient of H2O/ACN +0.1% FA to separate steroids prior to High Resolution Mass Spectrometry analysis. A hybrid quadrupole Orbitrap MS (Q-Exactive Focus) and a maXis 3G QTOF were used. Retention time prediction of steroids was performed using the Dynasti software (1) and an in-house steroids database. Peak picking was performed with Progenesis software (Waters). Semen quality parameters were also recorded to evaluate fertility using a computer-assisted sperm analyser (CASA).

The steroid profile analysis allowed 32 steroids to be annotated (level 1, 2 and 2+ according to reference guidelines for ID features in metabolomics) in seminal fluid and 19 in serum, including 7 steroids present in both matrices. CASA parameters were not found to be correlated to either serum or seminal fluid steroids using multivariate data analysis; indeed no formal correlation was established between serum and seminal liquid steroid profiles, highlighting the relevance of multi-compartmental studies for such specific biological questions. Further analysis are mandatory in seminal fluid to obtain a better understanding of the production, secretion and complex regulation of steroid hormones.

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2 - Sumner, L. W. et al (2007). Proposed minimum reporting standards for chemical analysis. *Metabolomics*, 3 (3), 211-221.

P40

Metabolomic profiling of the oviductal fluid across the estrous cycle in the cow*

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Although crucial for gamete transport, fertilization and early embryo development, there is very limited knowledge on the metabolic content of the oviductal fluid (OF) and its regulation across the estrous cycle in domestic mammals. Bovine oviducts ipsi- and contralateral to the side of ovulation were collected at a local slaughterhouse and classified into 4 stages according to the ovarian and corpus luteum (CL) morphologies (n=18-25 cows/stage): just after ovulation (postov), at mid- and late-luteal phase (mid-lut and late-lut), and before ovulation (preov). Concentrations of progesterone and 17b-estradiol were assayed in pools of OF (3-4 pools/stage and side; 4-10 cows/pool) by GC-MS/MS and their content in metabolites quantified by n-clear magnetic resonance spectroscopy. Comparisons between groups were made with a two-way ANOVA followed by Bonferroni post-tests.
A total of 38 metabolites were identified, among which the amino acid glycine and the energy substrates lactate and myoinositol were the most abundant at all stages. Little difference was seen in metabolite contents between ipsi- and contralateral OF. In ipsilateral OF, concentrations of glucose-1-phosphate were the most affected by the stage of the estrous cycle and were 5 to 6 times less abundant at preov and postov than at mid- and late-lut. At the opposite, glycine, alanine, valine, leucine and succinate were more abundant at postov than at mid- or late-lut in ipsilateral OF. This is the first metabolomic profiling of oviductal fluid in domestic mammals. These results may be helpful to optimize cultured media for gamete maturation, in vitro fertilization and embryo production.

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Analyse métabolomique du mucus cervical de brebis en H1-RMN*

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L'essentiel des inséminations dans l'espèce ovine est réalisé en semence fraiche, conservée à 15°C dans un dilueur à base de lait, car la congélation altère la capacité des spermatozoïdes à franchir le col de l'utérus. Les taux de réussite à l'IA classique avoisinant à peine 20%, l'utilisation de semence congelée nécessite une laparoscopie pour déposer les spermatozo'ides dans l'utérus par voie chirurgicale, une pratique peu envisageable en routine. Or, la fertilité de la semence fraiche diminue rapidement après seulement 8h de conservation, ce qui impose de fortes contraintes logistiques aux centres de production et limite leurs possibilités de développement national et international. Le projet OUEB (Optimisation de l'Utilisation de la semence de Bélier, programme financé par Apis-Gene) ambitionne d'acquérir de nouvelles connaissances, dans le but de rationaliser des travaux ultérieurs d'amélioration de la technique d'insémination chez les ovins. Les objectifs du projet sont, d'une part, d'identifier les paramètres spermatiques modifiés après congélation et au cours d'une conservation à 24h et, d'autre part, de caractériser le mucus cervical et ses interactions avec les spermatozoïdes. Une des hypothèses est que la viscosité du mucus empêche physiquement des spermatozoïdes de moins bonne qualité de passer. La caractérisation fine de la composition de ce mucus cervical permettrait donc de mieux comprendre ses interactions avec les spermatozoïdes.
Les mucus cervicaux de 20 brebis de race Ile de France ont été collectés à J0 au cours 3 cycles naturels et 3 cycles induits (avec un traitement hormonal pour la synchronisation des chaleurs), correspondant à la phase œstrale du cycle. Un total de 113 échantillons ont été analysés en H1-RMN (spectromètre Bruker Avance 600 MHz avec cryosonde), ce qui a permis l'identification de 47 métabolites, dont 30 métabolites ont pu être quantifiés avec la méthode ERETIC. Une différence significative (test T) pour la quasi-totalité des métabolites entre les mucus de cycles naturels et les mucus de cycles induits, a été mise en évidence. Les raisons de cette différence sont en cours d'exploration, notamment grâce à des analyses en protéomique, lipidomique et glycomique de ces mêmes mucus cervicaux. Sur la base d'autres données phénotypiques, ces différents mucus ont été classés selon leur viscosité. Il en ressort une fréquence significativement plus élevée de mucus avec une viscosité basse en cycles induits par rapport aux cycles naturels (respectivement 61,9% et 40% ; (test Chi^2) $p< 0.05$). En revanche, sur la base des métabolites identifiés, la viscosité n'est pas un facteur discriminant puisque l'analyse en PLS-DA ne permet pas de séparer les échantillons.

Ces analyses seront également réalisées sur les mucus cervicaux prélevés en phase lutéale afin de comparer l'évolution de la composition du mucus cervical au cours du cycle œstral.

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Untargeted metabolic profiling of environmental volatile compounds by Headspace-Solid Phase Micro Extraction-Gas Chromatography-Mass Spectrometry: an introduction to environmental ”volatilomics”

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***Key words**: Volatilomics, Headspace, Solid Phase Micro Extraction, GC-MS, Environment*

Pesticide use is permanently increasing for several reasons. However, these chemical compounds are threatening human health and environment. Thus, natural pesticides are recently emerging as an alternative solution. In fact, as these biopesticides are issued from nature, they are supposed less harmful versus the environment. However, the evaluation of biopesticides' environmental impact remains an actual challenge. In fact, classical methods e.g. half-life are limited, due to the complexity of biocontrol products. Thus, new omics-based methods are being developed in order to adapt new environmental evaluation protocols.

In this context, Environmental Metabolic Footprinting (EMF) was recently developed using Liquid Chromatography-Mass Spectrometry (LC-MS) based metabolomics approach [1],[2], aiming to introduce the ”resilience time” as a new integrative evaluation concept. It corresponds to the time required for the dissipation of the compound and its effect on the matrix microbiome.

More recently, a new approach was considered in order to study the volatile compounds in environmental samples. In fact, pesticide degradation can lead to volatile by-products. Moreover, volatile microbial compounds can signalize biodiversity disruptions. Thus, novel Headspace-Solid Phase Micro Extraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS) based analytical method is being developed, in order to analyze the environmental ”Volatilome” (xen-ovolatilome + microbial volatilome). A kinetic study is performed in order to evaluate the volatilome changes over the time. It could supplement the information given by LC-MS-based EMF. Furthermore, this methodology shows several advantages. In fact, no laborious and com-plex steps for sample preparation are needed. Therefore, this method reduces time, solvents and chemical reagents consumption, which agrees with green chemistry and reduce analysis coast.

Preliminary tests were performed with sediment samples. These sediments were treated with *Bacillus thuringiensis israelensis (Bti*), a complex bio-insecticide, and the -Cypermethrin, a standard chemical insecticide. For two days kinetics study (day 0 and day 8), HS-SPME-GC-MS analyses were applied, with no complex extraction needed, as mentioned above. They were followed by unsupervised descriptive multivariate statistical analysis in order to analyze the changes in volatilome. The results demonstrate sufficient sensitivity to discriminate between *Bti*treated samples and control samples. Moreover, some Variables Importance in Projection (VIP) are detected by supervised explicative multivariate analysis. More optimization is planned to enhance the reliability for complex and long-scale kinetic studies, in order to set a novel, simple and cheap method to be applied in EMF concept.

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[2] Environmental Metabolic Footprinting (EMF) vs. half-life: a new and integrative proxy for the discrimination between control and pesticides exposed sediments in order to further charac-terise pesticides' environmental, Salvia M. V., Ben Jrad A., Raviglione D., Zhou Y., Bertrand C., *Environmental Science and Pollution Research*, 28 June 2017. https://doi.org/10.1007/s11356-017-9

P43

Take the fast track to electrophoretic mobility: enhancing the robustness of your CE-MS metabolomic data with ROMANCE

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Although LC– and GC–MS are the gold standards in metabolomics, the separation of charged or very polar molecules remains challenging so far. CE can provide very efficient separations for such compounds, but the variability of the migration times usually makes it to be regarded as lacking the robustness required for metabolomic applications [1]. The use of *effective electrophoretic mobility* (μeff) has been proposed as a strategy to circumvent this problem because it only depends on the nature of the separation electrolyte [2], but its application is limited by tedious and time-consuming manual calculations. ROMANCE (RObust Metabolomic Analyses with Normalized CE) is a computationally efficient tool allowing the automated conversion of batches of large CE-MS files from the time-scale into the *electrophoretic mobility scale*.

ROMANCE is an open-source software which has been developed in Scala. It can handle mzML files containing either MS or MSn data as well as profile or centroid spectra. ROMANCE can run on any operating system and its multi-core architecture allows parallel processing of multiple CE-MS files. Herein, we show how the use of *electrophoretic mobility* improves data robustness and comparability in inter- and intra-laboratory conditions by analyzing data from two different CE-MS platforms.

In a first step, ROMANCE was used to convert files coming from a standard mix of compounds analyzed by different operators, in the same laboratory and on separate days. When the analyses were compared by using the μeff-scale, the variability of the electrophoretic mobilities of the peaks (expressed as %RSD) ranged between 0.9 and 1.2%, significantly lower to the 1.3% – 4.5% obtained when the original time-scale is used. Moreover, for concentration-sensitive detectors, peak areas become also normalized and independent from the migration time when this transformation is applied. Secondly, CE-MS results obtained from different experimental setups (comprising different MS, CE capillary geometries and applied pressures) were studied. The correlation improved in the case of the μeff-scale with regard to the time-scale, allowing the direct comparison of the transformed electropherograms obtained in distinct laboratories. Finally, de-proteinized and fortified human serum samples were analyzed. After x -axis transformation with ROMANCE, detected features were annotated by matching their measured accurate m/z and eff values to a library of reference standards generated in a separate laboratory. 67 features were annotated with a μeff error below 3%, showing the ability of ROMANCE to allow CE-MS-based metabolomics data to be exchanged. After conversion with ROMANCE, the resulting files have normalized x -axis which only depends on the BGE. Most of the variability from experimental factors is corrected, resulting in comparable and pseudo-aligned electrophoretic runs which can be then submitted to further steps of a metabolomics workflow with greater confidence. This approach allows straightforward CE-MS-based identification of metabolites in a similar way to LC-MS analyses.

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First insight on metabolomics workflows and differences between dinoflagellate: characterization and discrimination of modern and ancient strains and their algal growth.

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Depuis les années 1950, les apports trophiques en milieux côtiers ont été largement perturbés par une augmentation des apports en azote et phosphore liés aux activités agricoles et urbaines Dans le but d'étudier les éventuelles réponses adaptatives du phytoplancton face à ces modifications de l'écosystème côtier et de comprendre si ces perturbations sont une conséquence directe de la prolifération de certaines microalgues, des cultures de dinoflagellés toxiques et non toxiques (*Alexandrium minutum* et *Scrippsiella donghaienis*) obtenues par germination, ont été analysés par le biais d'une approche métabolomique.

Alexandrium qui est une microalgue pélagique qui produit justement des efflorescences de plus en plus importantes en nombre et en intensité l'été, est une espèce dont certaines souches sont productrices de toxines paralysantes de la famille des saxitoxines. Ces toxines, lorsqu'elles s'accumulent dans les mollusques bivalves, sont un danger pour la santé humaine puisqu'elles peuvent entrainer le décès des consommateurs par asphyxie.

Alexandrium minutum et *Scrippsiella donghaienis* produisent, au cours de leur cycle de vie, des kystes de résistance, revivifiables qui peuvent s'accumuler dans les sédiments de l'environnement marin côtier durant des centaines d'années. C'est pourquoi dans le cadre du projet MEDINA, il a été proposé que ces deux populations phytoplanctoniques des milieux estuariens aient "enregistré" dans ces cellules préservées sous forme de kystes dans les sédiments anciens, leur adaptation à ces changements de leur écosystème.

Grâce à une approche métabolomique, menée sur une large variété de métabolites produits par des microalgues, dans des conditions de culture données nous présentons les résultats d'une comparaison entre des souches " anciennes " supposées représenter les populations du passé qui auraient conservé des adaptations aux conditions du milieu de leur époque versus des souches "modernes " représentatives des conditions actuelles du milieu côtier.

La conception expérimentale a été réalisée sur la base de cultures discontinues d'Alexandrium minutum et de Scrippsiella donghaienis, non axéniques pour 4 phases de croissance, 2 espèces, 2 âges par écosystème (48 échantillons, n = 3), à partir de sédiments datant de 1986 pour les espèces dites " anciennes " et de souches " modernes " datant de 2006.

Le suivi des différentes cinétiques de croissance des souches anciennes et modernes *d'Alexandrium minutum* et de *Scrippsiella donghaienis* a été analysé par LC-HRMS et un workflow statistique complet a été conçu. Il a servi pour les tests d'hypothèses univariés et la modélisation multivariée par PCA sur une plateforme de chimiométrie, Agilent Mass Profiler Professional (MPP).

Les empreintes métaboliques des différentes étapes ont permis de mettre en évidence la distribution des métabolites communs et / ou spécifiques de l'âge de la culture mais aussi ceux communs et spécifiques aux différentes phases de croissance (exponentielle ou stationnaire et complémentée ou limitée en phosphore).

L'examen des réponses physiologiques et adaptatives obtenues a été comparé selon 2 modèles de workflows: une version " constructeur " citée ci-dessus et un portail collaboratif dédié au traitement des données métabolomiques, à l'analyse et à l'annotation « Workflow4Metabolomics »

P45

‘DEsCriBe’ project: A wide investigation of phytochemical diversity in leaves, roots and seeds of Brassica napus

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Intraspecific phytochemical diversity is a key determinant of crop biotic interactions and a source of plant resistance traits. Breeding efforts for low glucosinolate (GSL) contents in seeds have largely shaped the phytochemical landscape of oilseed rape varieties. The genetic architecture of GSL profile in seeds and leaves has been investigated by many research groups. However, several aspects of the phytochemical intraspecific diversity in vegetative parts of *Brassica napus* still deserve further extensive investigations. DEsCriBe is a multi-team project recently started at IGEPP, aiming at the most possibly exhaustive metabolomic study of specialized compounds in a diversity panel of 250 accessions of *B. napus*, including low and high GSL winter and spring oilseed rape varieties, forage rape, and swedes. The resulting intraspecific chemical diversity will be compared to the one of two core collections of the progenitor species *B. oleracea* and *B. rapa*. The work will focus on endogenous (seeds, roots and leaves) and volatiles secondary compounds, with a specific emphasis on glucosinolates, phenolics and low molecular weight terpenes. The starting point of the project includes the development of analytical procedures dedicated to the assessment of putatively rare compounds that might be specific of some genotype subsets. As a main output, the project will define a phytochemical identity card for every accessions in the panel, a dataset that will support the choice of genotypes in further works on plant biotic interactions. GWAS analysis of phytochemical traits will give genetic indications on the possible impact of recent *B. napus* breeding history on the metabolomics features of vegetative organs. This work will be also a starting point for further investigations on the molecular basis of the biosynthesis and regulation of rare or understudied Brassica-specific specialized metabolites that are absent from Arabidopsis.

P46

Some pitfalls to avoid in marker annotation

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The use of untargeted metabolomics approach is a promising strategy to discover new markers in different disciplines. The workflow of this approach includes in general: sample acquiring; sample preparation and analysis; data processing/variable extraction; statistical analysis, and Identification. Each step of this workflow is crucial for final marker annotation. However, some issues may exist in the identification step of this workflow that could lead to false annotation. Database search is the first step of the specific workflow of metabolites annotation. However, not all isomers that are present in different biological systems are present in some databases (in house and public), which could lead to false annotations.

Identifying the correct isomer require the interpretation of fragmentation spectra. Not only the fragment signal itself is crucial for the identification also the signal intensities are needed for correct identification. We will illustrate these circumstances by two examples found in our last studies.

P47

Evaluation de l'approche métabolomique pour l'étude des interactions chimiques dans le dépérissement de la lavande

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Les cultures de lavande (*Lavandula angustifolia*) et de lavandin (*L. angustifolia X L. lati-folia*) sont, depuis une dizaine d'années, affectées par le phytoplasme du Stolbur (*Candidatus phytoplasma solani*), agent pathogène transmis par la cicadelle (*Hyalesthes obsoletus*). Cet insecte se nourrit de la sève de la lavande transmettant la bactérie de plant infecté en plant sain (Ahmad, 2011). Le déperissement de la lavande a des conséquences économiques importantes sur la filière lavandicole et les moyens de lutte sont actuellement inexistants ou restreints. Par ailleurs, les connaissances autour des interactions chimiques entre la cicadelle, le phytoplasme et la lavande sont encore limitées, c'est pourquoi nous cherchons à mettre en place une approche métabolomique pour étudier les composés organiques non volatiles qui pourraient avoir une influence dans cette interaction tripartite.
La mise est place de l'approche métabolomique a été réalisé à partir d'échantillons de feuille et d'inflorescence de deux variétés de lavande (Maillette et 7713) et de lavandin (Grosso et Abrial) collectés sur le plateau de Sault (Vaucluse, France). Afin d'arrêter le métabolisme de la plante, les échantillons ont été immédiatemment plongés dans de l'azote liquide. Les échantillons ont ensuite été séchés par lyophilisation et réduit à l'état de poudre. Les extractions solide-liquide assistées par ultra-sons ont été réalisées avec un mélange eau-éthanol (50:50, v/v). Après une étape de centrifugation, les extraits ont été analysés en chromatographie liquide grâce à une UPLC-HRMS (XevoG2 QTOF, Waters). Après retraitement (normalisation, alignement,...), les données obtenues sont exploitées à l'aide des analyses statistiques multivariées (ACP, PLSDA) sur la plateforme Workflow4Metabolomics 3.0. Afin d'aboutir, à l'identification de biomarqueurs spécifiquent :

- du caractère sensible ou tolérant d'une variété,
- ou de l'état symptomatique du plant (sain ou infecté).

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P48

Utilisation d'une approche multi-omique pour l'analyse de la qualité du cacao fermenté séché en provenance de Côte d'Ivoire.

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La qualité du cacao est dépendante d'un nombre considérable de facteurs comme : le cultivar, les pratiques de cultures, les méthodes de fermentation, [...]. Même si la plus grande partie du développement aromatique du cacao se fait lors de la torréfaction, une large part des notes florales et fruitées apparait lors des étapes de transformation précédant la torréfaction [1], [2]. En Côte d'Ivoire, le premier producteur mondial avec plus de 30% de la production de cacao, l'entreprise Cemoi, dispose de centres de fermentation qui maîtrisent une partie des paramètres lors de la transformation du cacao. Cependant, il subsiste des variations notables de la qualité des fèves de cacao fermenté séché issues de ces centres.
Le projet SENSOCAO, en partenariat entre Cemoi et le Criobe et initié en mai 2016, a pour objectif de trouver des biomarqueurs de la qualité du cacao en Côte d'Ivoire. Pendant 3 ans, des échantillons provenant de centres de fermentation répartis sur l'ensemble du territoire ivoirien seront collectés afin d'établir les profils aromatiques et chimiques des échantillons.

Les données organoleptiques sont acquises par la dégustation des lots de cacao par un panel d'experts. Pour étudier une large gamme de métabolites, allant de 50 à 1000 Da, une méthode d'extraction et d'analyse de la fraction volatile du cacao par Headspace-SPME-GC-MS a été développée et une méthode d'analyse non-ciblée des molécules non-volatiles par UPLC-Qtof a été optimisée [3].

Nous présenterons le résultat de l'analyse de la récolte intermédiaire 2017 (; 5 lots/centre pour 7 centres). Le prétraitement des données brutes a été réalisé avec XCMS, via la plateforme workflow4metabolomics. La combinaison de plusieurs approches métabolomiques et l'utilisation d'outils de statistiques multivariées a permis de faire émerger des biomarqueurs de la qualité du cacao. Les différences observées seront analysées au regard des facteurs climatiques, agronomiques ou encore des traitements post-récoltes. Par cette approche intégrative, les partenaires proposent un outil innovant permettant de contrôler la qualité du cacao produit afin de répondre au mieux aux exigences du marché.

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P49

HR-MAS NMR-based metabolomics reveals metabolic changes in lung of mice infected with P. aeruginosa consistent with the degree of disease severity, and is a powerful evaluation tool for new treatment.

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Pseudomonas aeruginosa is a critical pathogen for human health, due to increased resistances to antibiotics and to nosocomial infections. There is an urgent need for tools allowing to better understand mechanisms underlying the disease processes and to evaluate new therapeutic strategies with animal models. Here, we used a novel approach, applying high resolution magic angle spinning nuclear magnetic resonance spectroscopy (HRMAS NMR) directly to lung biopsies of mice to better understand the impact of infection on the tissue at a molecular level. Mice were infected with two *P. aeruginosa* strains of different virulence levels. Statistical analysis applied to HRMAS NMR data allowed us to build a multivariate discriminant model to distinguish the lungs' metabolic profiles of mice infected from not. The sensitivity of HRMAS NMR-based metabolomics is enough to appreciate the degree of severity of infection. The metabolic profiles are dominated by some key metabolites which are related respectively to bacterial carbon metabolism (glycerophosphocholine) and to septic hypoxic stress response of host (succinate). This approach could therefore be useful both for the definition of biomarkers of severity of infection and for an earlier characterization of therapeutic efficacy.

P50

Ellagic acid modulates intestinal microbiota and related metabolites production of a Urolithin-Metabotype A donor, at several stage of digestion

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Introduction

Ellagitannins are bioactive polyphenols present in berries (strawberries, raspberries, blackberries), pomegranate, tea, and nuts. They have anti-oxidant, anti-inflammatory, anti-microbial, anti-cancer properties. Pomegranate (Punica granatum L.) is one of the fruit presenting the highest content in polyphenol. Ellagitannins are hydrolysed in ellagic acid in the stomach and proximal intestinal tract and are poorly absorbed. They reach the large intestine where they are metabolized in urolithins by the gut microbiota. Three phenotypes of urolithins (metabo-types)[1] are observed in the gut: ”urolithin metabotype A (UM-A)” produce UroA conjugates, ”B, UM-B” produce UroA, isoUroA and UroB conjugates while for ”metabotype O, UM-O”, these urolithins were not detected. While urolithin production has been associated with the presence of different colonic microbiota communities such as *Gordonibacter* or *Clostridium* species[2], it is well recognized that other unknown bacterial species are involved in the production of the final urolithin metabolites. The aim of this study was to measure the effect of two concentrations of a commercial pomegranate extract at every stage of digestion using the in vitro gastrointestinal model SHIME® in order to evaluate the production of short chain fatty acid, urolithin, succinate, lactate productions, and bacterial composition in each part of the colon using a metagenetic and metabolomic approach.

Material and methods

Pomegranate extract was a commercial food supplement. The Simulator of Human Intestinal Microbial Ecosystem (SHIME®) consists of six double-jacketed vessels simulating the stomach, small intestine and three colons regions (ascending (AC), transverse (TC) and descending (DC) colons)[3]. The colon unit was inoculated with a fecal sample from a healthy adult volunteer (age 40, male) and the experiments were performed three times corresponding to three individual runs. For each run, an initial two-week stabilization was followed by a treatment period of one week for each tested concentrations of pomegranate (C2 = 1300 mg and C4 = 2600 mg per day).

This experimental phase was followed by a washing-out period of one week before the next treatment. Samples were collected and analyzed before and at the end of each treatment. Samples were collected three times a week in triplicate, at regular hours and immediately stored at -20°C before processing to analyses. Samples dedicated to SCFA, urolithins, lactate and succinate analysis were centrifuged and the supernatant was filtered-sterilized (Millipore,

0.22 µM filters) before freezing at -20°C for NMR analysis. The pellet was kept at -20°C for metagenetic analysis. Finally, a ^{1}H-NMR-based metabolomics analysis was applied to the fecal samples at each stage of the SHIME.

Results

After one week of treatment with C2 and C4, the measured concentrations of total SCFA, acetate, propionate, butyrate showed a trend toward a decrease in SCFA concentration in AC. In other colons (TC and DC), no significant changes were observed for any of the tested concentrations. L-lactate, D-lactate and succinate showed also a trend toward a decrease in each part of the colon. Highest urolithins production is carried out in DC2. Uro A, C and D, precursors of Uro A, are detected in DC compartment. An increase of global biodiversity is observed after the treatment and several changes were observed in the microbiota with the two tested concentrations. A significant decrease of *Parabacteroides, Porphyromonadaceae*, *Allobaculum* was observed while an increase of *Faecalibacterium*, unclassified *Firmicutes*, *Blautia* and *Roseburia* were observed after treatment with C2. After treatment with C4, a significant decrease of several OTU corresponding to *Prevotella* was observed while a significant increase of several OTU corresponding to *Bacteroides* was observed. The main Prevotella OTU that has been identified as decreasing was the *Prevotella FJ685435*. The main Bacteroides species that has been identified as increasing was Bacteroides thetaiotaomicron. Finally, metabolomics analysis of spectral data revealed a good separation between samples taken from different stages of digestion progression. Furthermore, different concentrations of pomegranate did significantly alters the colon metabolome. This analysis led to the identification of relevant changes of some metabolites levels (i.e. decreased ornithine, lysine and propionate in DC samples with pomegranate compared to DC samples without pomegranate)

Discussion

A decrease in production of SCFA in AC had already been described for UMA and UMB profile after one week of pomegranate extract administration, probably due to a toxic effect of pomegranate extract on intestinal microbiota. Urolithin production has been associated with the presence of different colonic microbiota communities such as *Gordonibacter* or *Clostridium* species. No *Gordonibacte*r were detected in our samples but a significant increase in population of *Faecalibacterium*, *Firmicutes*, *Blautia* and *Roseburia* has been observed, suggesting indeed that other bacteria could be able to metabolize EA into UA.

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NMR Metabolomic analysis as a tool to identify antiplasmodial compounds in Poupartia borbonica extracts.

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Malaria remains a global burden, responsible for 429 000 deaths registered in 2015.(*1*) Despite the existence of several antimalarial compounds, the increasing multidrug resistance is still the bottle neck preventing their efficacy and sustainability. The research of new compounds is, therefore, necessary. Since more than 60 % of antiparasitic drugs discovered between 1981 and 2014 have natural origins (*2*), plants remain potential sources of new and interesting therapeutic agents. In this study, *Poupartia borbonica* was selected, an endemic plant from the Mascarene Islands, based on a previous screening. Antimalarial cyclohexenone derivatives were isolated.(*3*) Nuclear magnetic resonance-based metabolomic analysis was performed to evaluate different extracts (MeOH; MeOH/H2O/Chloroform; MeOH/H2O) from different parts of the plant (leaves and bark). The aim of the study was to highlight a correlation between the 1H NMR signals of the cyclohexenones derivatives and the activity of the extract. The fingerprints of the extracts can predict the presence or the absence of anti-plasmodial compounds and could allow a large reduction in the number of in vitro tests performed in order to detect new molecules.

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P52

Endometriosis: a deep insight into the pathology through metabolomics

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Endometriosis is defined as the presence of endometrial tissue outside the uterine cavity, affecting approximately 10% of women in reproductive age, and mostly associated with infertility and/or pelvic pain[1,2]. The current lack of an efficient non-invasive diagnosis method affects the delay between the first symptoms and the identification of the pathology[1]. Therefore, the discovery of a combination of biomarkers measurable in biofluids is essential for a more efficient treatment of patients. Metabolomics appears to be an innovative and powerful tool to obtain a deep insight into this disease.

In this study, we applied a NMR-based metabolomics analysis on urine and serum samples obtained from 90 patients with endometriosis (”endometriotic”) and from 98 normal patients (”controls”). The analysis of sera spectral data did not allow the distinction between the metabolic profiles of endometriotic and control patients. However, the analysis of the urine samples revealed a clear separation between”endometriotic” and ”control” groups. This distinction is already observable at an early stage of the disease. This analysis led to the identification of relevant changes in some metabolites levels. These changes in the metabolic profile could be linked to some biochemical pathways that seem to be affected by endometriosis. These results could help the comprehension of the occurrence and the evolution of this pathology.

This study demonstrates that, despite the complexity of this disease, metabolomics could be a valuable tool to explore endometriosis. The results obtained by the analysis of urine samples could hold the key to finally unravel endometrial clinical biomarkers.

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Metabolic adaptation of various human leukemic cell lines in different culture conditions

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Background: Acute Myeloid Leukemias (AMLs) are a set of malignant proliferations leading to an accumulation of blasts in the bone marrow and the blood. Their prognosis is pejorative due to their molecular complexity and the multiple pathways implicated in leukemogenesis. Roughly half of AML displays chromosomal abnormalities (translocations, inversions, etc.) and the other half is cytogenetically normal with one or several mutations. Despite the considerable progress in understanding the molecular bases of AML, we need to go further in the characterization of leukemic cells. Metabolites, which are end products of metabolism, could provide important clue for that aim. Indeed, it is well known that metabolism in cancer cells is strongly modified compared to normal cells. One of these mechanisms is the metabolic reprogramming of aerobic glycolysis described in the 1920s by Otto Heinrich Warburg. To decipher metabolic pathways that could play a role in leukemia progression we performed a metabolic characterization of leukemic cells using an HRMAS NMR method that allows analyzing intact cells from patients. **Objectives**: The objective was to compare the metabolic profiles of leukemic cells in normal condition of culture and in deprivation conditions during 48h, in order to observe their behavior under metabolic stress, and finally after refilling them with classic medium during 48h.

Methods:

Cell culture: Four human leukemic cell lines, KG1, K562, HEL and HL60, with different cytological and molecular characteristics have been studied. The four cell lines were cultured in the different conditions previously described. At 2h, 4h, 24h and 48h, using flow cytometry, we have performed cell cycle and apoptosis evaluation. In parallel, 3 to 5 million of cells were pelleted, washed and then introduced into inserts for HRMAS NMR.

NMR acquisition: For the HRMAS NMR experiment, we used high-resolution 1H-NMR at 500 MHz. MAS spin rate was set at 4000 Hz and sample temperature at 277K. A CPMG pulse sequence was used to minimize lipid contribution.

Data processing: All the HRMAS NMR spectra were corrected for phase and baseline distortions and the spectral region were divided into buckets with equal width of 0.001 ppm using the NMRprocflow open source software. Then buckets were transferred to the SIMCA R V14.1 software for multivariate statistics (PCA and OPLS-DA), in order to find metabolites that are over or under expressed in the different cell lines and depending on the experimental conditions. For that aim, we have performed pairwise comparisons, because we do not have control cell line.

Results: We have firstly shown that each cell line has a specific metabolic profile at basal state. Globally, HL60 is characterized by an increase of glutamate and proline compared to other cell lines, KG1 by an increase of phosphocholine, HEL by an increase of myoinositol and K562 by an increase of creatine/phosphocreatine. When cultured in serum-free medium, we observe that all cell lines have a tendency to recover their basal metabolic profile, more or less depending on the cell line.
This indicates that all the cell lines develop a rapid metabolic adaptation (2h) which allow them to continue to proliferate despite the lack of nutrients. Currently, other stress conditions are tested for these four cell lines, notably iron depletion. Also, these tests are coupled with cell cycle and apoptosis tests too.

Conclusion: Our preliminary results shows that human leukemic cell lines with different origins, and representing four different subtypes of acute myeloid leukemia, display different metabolic fingerprint, in the basal conditions of culture. Moreover, we showed that under stress condition leukemic cells could benefit of a metabolic reprogramming that allowed them to maintain proliferation.

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Validation of global quantitative analysis of tryptophan metabolites in mice using LC-HRMS

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Tryptophan (Trp) is an essential amino acid that plays a key role in protein synthesis and acts as a precursor to many biological substances. Disturbances in the catabolism of Trp are associated with many diseases. The exhaustive and quantitative monitoring of these metabolites would allow a better understanding of the pathophysiology of these diseases while proposing a diagnosis and therapeutic follow-up based on a multiplexed approach. We have validated a method for quantifying tryptophan catabolites by liquid chromatography coupled with tandem mass spectrometry (LC-MS / MS). These developments were carried out on four types of mouse samples to obtain a global view of this metabolism at the animal scale. The detection limits for all metabolites are between 0.15 and 100 000 nmol/L and the dynamic ranges of calibration curves have been adjusted to allow quantification of endogenous levels. The matrix effects matrix was evaluated using labeled internal standards. The matrix effects are on average between 62 and 97%, inaccuracy < 8% and accuracy between 5 and 8.4%. Our method was finally used to determine the concentrations of these 20 metabolites in five different compartments in mouse such as the digestive tract, serum, and urine. The results obtained first make it possible to have reference values for the concentrations of each metabolite in these compartments and also to better understand the exchanges between these compartments.

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Etude des variations chimiques des racines de tomates (Solanum lycopersicum) à différents stades de croissance grâce à la mise en place d'une approche métabolomique.

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Pour faire face aux ravageurs (insecte, micro-organisme, nématode...) les plantes utilisent diverses stratégies de défenses dont la biosynthèse de molécules qui peuvent avoir un impact répulsif, voire toxique sur ces ravageurs. La tomate (Solanum lycopersicum), par exemple, accumule une diversité de métabolites secondaires incluant des composés phénoliques, des phenolamides et des glycoalcaloïdes. Ainsi dans ce projet, des plants de tomate ont été soumis à différentes combinaisons de ravageurs (de 0 à 4) afin d'évaluer leur impact sur les défenses chimiques de la plante. Pour étudier ces variations moléculaires une approche métabolomique a donc été mise en place. Ce travail a été réalisé sur les racines et les feuilles de tomate, soumises ou non, à différentes combinaisons de ravageurs (Helicoverpa armigera, Macrosiphum euphorbiae, Oidium neolycopersici, Meloidogyne incognita). Pour chaque modalité, 9 répétitions ont été menées. Deux lots de plants témoins ont été mis en place, un lot récolté 27jours après la culture, et un autre récolté le 49ème jour après la mise en culture. Pour mettre au point la stratégie analytique et statistique nous nous sommes focalisés dans un premier temps sur l'identification des composés qui varient dans les racines saines (conditions contrôles), aux deux stades de développement.

Compte tenu des familles moléculaires ciblées et de la faible quantité de matériel végétal disponible pour chaque échantillon une méthode d'extraction dans un mélange méthanol/eau assistée par ultrasons a été développée.
Les empreintes métabolomiques des extraits de racines ont été obtenues grâce à un couplage UHPLC-HRMS-ESI-QTof. Une méthode rapide permettant d'éluer une large gamme de co-posés et d'analyser dans un temps raisonnable le grand nombre d'échantillons a été développée. Les données générées ont ensuite été introduites sur la plateforme workflow4metabolomics pour réaliser le pré-processing des données (XCMS). Enfin, les analyses statistiques non supervisées (ACP, ACH) et supervisées (PLS-DA) ainsi que les expériences de spectrométrie de masse en tandem (MS2) ont permis d'identifier les différences métaboliques dans les racines saines de tomate à différents stades de développement. La méthodologie mise au point est actuellement appliquée à la détermination des composés impliqués dans les interactions plante-ravageurs de la tomate.

Recherche de biomarqueurs précoces d'anévrismes, par approche métabonomique, et évaluation des bénéfices d'un implant modulateur de flux multicouche.

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L'anévrisme aortique abdominal est une atteinte vasculaire commune et silencieuse. Afin de trouver de nouveaux biomarqueurs potentiels de cette pathologie, connaître plus en profondeur les mécanismes sous-jacents et évaluer les bénéfices possible d'un nouveau concept de stent, nous avons mené une étude métabonomique basée sur la RMN du proton chez le rat. Cette étude se base sur différents biofluides (urine, sang et extraits cellulaires) afin de détecter des signes précoces de cette pathologie à partir de changements spectraux de ces biofluides. Un profile métabolomique spécifique est ainsi obtenu à partir de ces biofluides et peut dès lors être utilisé comme empreinte d'une pathologie.
Dans cette étude, la performance d'un nouvel implant Modulateur de Flux Multicouche (MFM, Cardiatis, Belgique) sera évaluée. Ce stent n'exclut pas le sac anévrismal de la circulation sanguine mais provoque, de par son maillage, une modulation du flux sanguin turbulent en flux laminaire au sein de cette excroissance. Ce changement de flux provoque un arrêt de la croissance de l'anévrisme et mène à la formation d'un thrombus organisé au sein de l'anévrisme et, à partir des cellules progénitrices circulantes, comble entièrement la cavité.

En plus d'évaluer la pathologie ainsi que les bénéfices possibles de cet implant, l'approche métabolomique permettra de donner de nouvelle piste sur les mécanismes sous-jacents à partir de l'analyse d'extraits cellulaires provenant du dit anévrisme

Cette étude est basée sur la collecte d'échantillons humains et sur un modèle d'anévrisme chez le rat. Pour ce modèle, la souche de rat SHR (spontanément hypertendu) est utilisée pour développer chirurgicalement un anévrisme aortique abdominal. Cela est possible via le traitement périphérique de l'artère à l'aide d'une gaze imbibée de CaCl2 0,5 M pour une durée de 15 minute. Ce traitement provoque une apoptose des cellules musculaires lisses, de l'inflammation, une réaction artériosclérotique et, après 4 semaines, un anévrisme à la localisation désirée.
Dans un premier temps, c'est l'hypertension en elle-même qui a été étudiée chez cette souche de rat spontanément hypertendus afin d'en déterminer la signature spectrale urinaire spécifique de cette atteinte. Elle met en évidence des changements au niveau du cycle de Krebs, du TMAO, de la taurine, de l'allantoïne et de la créatinine. Les données complètes seront discutées plus en détails sur le poster.

Etude par approche métabonomique et histopathologique d'une surconsommation en saccharose chez la souris

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Etude par approche métabonomique et histopathologique d'une surconsommation en saccharose chez la souris.
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Introduction.
La consommation excessive d'aliments hypercaloriques (graisses saturées, et sucres) est bien connue pour induire des altérations métaboliques complexes (syndrome métabolique, obésité, diabète, NAFLD: Non alcoholic fatty liver disease, NASH: Non alcoholic steatohepatitis).

La surconsommation de saccharose seul (donc de glucose et de fructose) pourrait aussi induire des altérations métaboliques et histopathologiques similaires dans l'organisme. Nous avons utilisé un modèle expérimental murin afin d'évaluer les changements métabonomiques des urines par l'étude en 1 H NMR et les altérations histophatologiques hépatiques survenant chez des souris SWISS soumises pendant 4 semaines à un régime purifié contenant 71.5% en saccharose et 5% d'huile de maïs.

Matériels et Méthodes.
12 souris Swiss femelles, âgées de 4 semaines, réparties en groupe équivalent, sont nourries ad libitum en cages individuelles, soit par le régime témoin industriel Carfil contenant 39% de 'sucres et amidon' et 10% de 'matières grasses', soit par le régime purifié contenant 71.5% de saccharose. Les souris sont placées 24h en cages métaboliques individuelles, 1 jour par semaine pendant 4 semaines. Les urines sont recueillies dans un récipient réfrigéré en présence d'azide de sodium. Après centrifugation, les urines sont stockées à -80°c. Enfin les souris et les aliments sont pesés durant toute la durée de l'étude. Les échantillons d'urine préparés dans un tampon phosphate PH 7.4, en présence de TSP 1mM, sont passés sur un spectrométre Bruker Avance 500MHZ. Les analyses multivariées sont effectuées à l'aide du logiciel Simca P+. Enfin les échantillons de foie sont préparés en fin d'étude pour les examens histopathologiques en microscopie optique et électronique.

Résultats.
On observe que sur les poids des souris nourries sur le régime purifié (saccharose) augmentent faiblement (+7% en moyenne), par rapport à ceux des témoins (Carfil), bien que la prise alimentaire soit isocalorique dans les deux groupes.
Les analyses multivariées montrent que les souris exposées au saccharose se séparent nettement dès le premier jour de mise sous saccharose, en raison d'une augmentation de l'excrétion urinaire de saccharose, fructose, indoxyl sulfate et d'une diminution de l'excrétion urinaire d'allantoïne, de citrate, de cis aconitate, de céto glutarate, de taurine, et de TMAO.

Enfin, bien que les prises alimentaires soient isocaloriques, une stéatose, sans signe majeur d'inflammation, est observée par les études histopathologiques, chez tous les animaux exposés au saccharose

Discussion.

Lorsque le saccharose alimentaire est ingéré en excès, les activités disaccharidases des cellules de l'intestin grêle sont débordées et une faible part de ce saccharose non hydrolysé passe dans le sang avec l'excès de fructose et de glucose. Non métabolisé, le saccharose est excrété par les urines. Le fructose, chez les rongeurs, sert rapidement à la synthèse de triglycérides qui sont stockées dans le foie. Une fructosurie est néanmoins observée. Tous les autres métabolismes dépendants de la glycolyse et du cycle de Krebs (le glucose étant aussi en excès) sont déprimés.

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Analyse métabolomique par RMN 1H de sérum lors de la prise d'un petit déjeuner standardisé : Influence des habitudes alimentaires

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Introduction : Les mécanismes métaboliques en lien avec la nutrition-santé restent peu explorés jusqu'ici. Leur élucidation est cruciale pour mieux comprendre la survenue des maladies et améliorer les stratégies de prévention.
Le but de l'étude Nutrivasc réalisée par l'équipe de recherche en épidémiologie nutritionnelle (EREN) était d'étudier les relations entre la qualité nutritionnelle de l'alimentation évaluée par les recommandations du Programme National Nutrition Santé (PNNS) et des facteurs de risques cardiovasculaires. Nous avons utilisé des échantillons de sérum collectés lors de cette étude pour en faire l'analyse du métabolome. L'utilisation de la métabolomique dans le domaine de l'épidémiologie est une approche récente et alternative permettant d'explorer ces relations afin de découvrir de nouveaux biomarqueurs.

Objectifs: 1/Comparer les profils métaboliques RMN des sérums des individus ayant un mauvais comportement alimentaire (score bas PNNS) de ceux qui respectent les recommandations nutritionnelles (score PNNS élevé). 2/Identifier les biomarqueurs durant la période postprandiale dans les deux groupes.

Matériels et méthodes : 49 individus appartenant au 1er et 4ème quartile des scores PNNS ont été sélectionnés. Lors de l'étude, des prélèvements sanguins ont été réalisés à jeun (T0), puis 1, 2, et 3 heures (T+1h, T+2h et T+3h) après la prise d'un petit déjeuner standardisé. Les échantillons ont été analysés par résonance magnétique nucléaire (RMN) du proton 1H. Les spectres acquis sur un spectromètre RMN Bruker à 500 MHz en utilisant la séquence CPMG ont été soumis à des analyses statistiques multivariées (ACP, OPLS).

Résultats : Les métabolites responsables de la principale source de variabilité ont été identifiés. En prenant en compte l'ensemble de la population aucune discrimination n'a été possible entre le 1er et le 4ème quartile par ACP et OPLS. Cependant une discrimination des échantillons, suivant le temps de prélèvement, a été observée. Les métabolites discriminants ont été identifiés par comparaison avec la base de donnée HMDB (www.hmdb.ca) et l'analyse des spectres RMN 2D (*J*-resolved, TOSCY, HSQC).

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Identification of coffee leaves by the comparison between two NIR spectrometers

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An abundant literature relative to coffee beans (green or roasted) chemical description is available, but few studies have been devoted to coffee leaves. Given the fact that coffee leaves are used for food and medicinal consumption, it was of interest to develop a rapid screening method in order to identify coffee leaves taxa (species). Investigation by Near Infrared Spectroscopy (NIRS) was performed on nine *Coffea* taxa leaves harvested over a one-year period in a tropical greenhouse of the Botanic Garden Meise (Belgium). The only process after leaves harvesting was an effective drying and a homogeneous leaf grinding. For the identification of these taxa, three sources of variability were considered in the spectral data namely: taxon, aging leaves stage and harvest period. NIRS combined with an appropriate statistical analysis such as soft independent modelling by class analogy (SIMCA) allowed to discriminate the spectral profiles across taxon, aging stage (mature and senescence coffee leaves) and harvest period. The results of benchtop data permitted to point out that it was possible (i) to classify the different taxa, (ii) to identify their aging stage and (iii) to identify the harvest period for the mature stage with a minimal correct classification rate of 100 %, 90 % and 80 %, respectively. The investigation with a hand held NIR equipment gave the same classification rate results with respect to the aging stage identification (90%) but the ability to discriminate different taxa (93%) and harvest period (50%) was less powerful compared to the benchtop equipment. The developed models could be used in quality control in order to verify the identity of the harvested leaves or to identify adequate harvest time. Comparison of the data obtained by NIR using a hand held and a benchtop apparatus were pointed out.

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MR Metabolomics Investigation of the Effect of Adipocytes on Breast Cancer Cellular Metabolism

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HER2+ breast cancer is a particularly invasive type of breast cancer and responds poorly to chemotherapy. Although targeted therapies such as trastuzumab revolutioned the management of the disease, resistance to these therapies stands as major therapeutic issue and remains to be understood at the molecular level. Interestingly, adipose cells have been shown to act on HER2+ cells to decrease their responsiveness to trastuzumab.

Here, we present a Nuclear Magnetic Resonance (NMR) metabolomic investigation, carried out on cell culture supernatants, aiming at the identification of metabolic fingerprints associated with the effect of adipose-secreted factors on HER2+ cancer cells. Samples were prepared by incubating BT474 cells (HER2+ cells) with either their reference culture medium, adipose cells culture media, or adipocytes and preadipocytes conditioned media. Cell cultures were conducted in the presence or absence of several drugs modifying the cellular metabolism. In total, 32 different conditions were investigated in triplicates, with exposure to 7 different drug molecules tested, for a total of 254 samples of cell culture supernatants analysed by 1H NMR at 800.14 MHz. 49 Metabolites were identified in these culture media and quantified in all samples (ChenomX NMR Suite) to evaluate their production or consumption for each group. Metabolic signatures were then obtained by comparing the different groups of samples. Multivariate statistical analysis shows a good discrimination between adipose-conditioned media and supernatants of BT474 incubated in those media. Metabolites related to the discrimination include mainly amino acids as well as carboxylic acids and carbohydrates. Comparison of the different metabolic profiles shows that BT474 cells have a strong metabolism in adipose-conditioned media.

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Differentiation of coffee species through untargeted-profiling studies using LC-HRMS in negative ion mode

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Differentiation of coffee species through untargeted-profiling studies using LC-HRMS in negative ion mode

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Good diet is directly linked to the chemical food composition. Thus, it is essential to mon-itor its composition by analytical techniques such as LC-HRMS. Because of their content of caffeine, chlorogenic acids and others polyphenols, coffee seeds are used as beverage but also in cosmetic and food industries. In this study we focused on comparisons of *Coffea* species having botanical, genomic, or consumed interest by performing an untargeted LC-MS analysis in negative mode.

We focused especially on coffee leaves because leaves infusions are consumed essentially for medical purposes [1,2,3]. We performed metabolomics studies to get more information about the metabolites biosynthetic pathways and by this fact to improve the composition of future supplements. Particularly, we analysed leaves in order to identify several key metabolites negatively charged for the discrimination between species. All species studied grew around 10 years in the tropical greenhouses of Botanic Garden Meise (Belgium) with the same environmental and edaphic conditions. In a previous study, leaves were also analysed by LC-HRMS but in positive mode, and a comprehensive statistical workflow was designed. It served for univariate hypothesis testing and multivariate modelling by PCA and partial PLS-DA on the Workflow4Metabolomics infrastructure [4]. This strategy permitted to investigate the metabolomics data and their relation with botanic information [5]. In the present study, the identification of the main metabolites in negative mode for the discrimination between species was characterized showing us how it was possible to discriminate *Coffea* species and subspecies based on their metabolomics profiles. Among the identified metabolites, several as chlorogenic acids or (epi)catechin derivates were highly discriminant.

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1H-NMR metabolomics of TIEG1 KO muscle mice

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Introduction: TIEG1 (TGFb inducible early gene-1) est un facteur de transcription à doigt de zinc de la famille des " Kruppel-like factor " (KLF10). L'invalidation du gène TIEG1 entraine des modifications des propriétés mécaniques pour les muscles lent (soleus) et rapide (EDL : extensor digitorum longus), des changements de texture (Kammoun et al. Muscle and Nerve. 2017) ainsi qu'une hypertrophie et une hyperplasie des fibres musculaires (Kammoun et al. PlosOne. 2016). Afin de mieux comprendre le rôle du gène TIEG1 sur le métabolisme des muscles lent (soleus) et rapide (EDL), nous avons utilisé une approche métabolomique par RMN du 1H.

Matériels et Méthodes : Trois pools de 5 muscles (soleus ou EDL) WT (wild type) et TIEG1 KO ont été lyophilisés avant d'être extraits par un mélange MeOH/CHCl3/H2O ratio 1 :1 :1 (Wu et al. Analytical Biochemistry. 2008). La phase polaire contenant les métabolites est récupérée, évaporée avant d'être analysée en RMN. Les échantillons sont reconstitués dans un tampon phosphate deutéré (pH=7.4). Les spectres 1H sont réalisés sur un spectromètre Bruker 600MHz cryosonde. Après post-processing, les spectres sont découpés en 58 variables spectrales avec AMIX (Bruker Biospin, Kalsruhe, Germany) et les intensités spectrales sont normalisées à l'aire totale du spectre pour une analyse statistique multivariée (SIMCA-P+, Umetrics, Umea, Sweden).

Résultats : L'analyse en composante principale (PCA) obtenue avec les données 1H-NMR provenant des soleus WT, soleus KO, EDL WT et EDL KO montre clairement une différence de profil spectral entre EDL et soleus sur la première composante (PC1=49.7%) et une différence entre génotype sur la deuxième composante (PC2=24%). Pour explorer la différence génotypique, une PLS-DA a été réalisée pour chaque muscle, mettant en évidence une différence de profil spectral.

Conclusion: Cette étude a montré des dérégulations métaboliques engendrées par l'inhibition du gène TIEG1 aussi bien dans le muscle lent (fibres oxydatives) que dans le muscle rapide (fibres glycolytiques).

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Metabolism adaptations of variegated scallop, Mimachlamys varia, facing emersion conditions during rhythmic tidal phases

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The variegated scallop (*Mimachlamys varia*) is a filter feeder bivalve of the *Pectinidae* fam-ily encountered in marine regions of the Atlantic coast. Their wide geographical distribution and their ability to bioaccumulate pollutants make them useful organisms for the biomonitoring of marine pollution. Present up to 100 m deep, they also can live in intertidal areas and can thus endure emersion conditions during alternating tidal phases. In order to use *Mimachlamys varia* as sentinel species in pollution biomonitoring and have normalized sampling methods, it is important to characterize their metabolism and its response to emersion conditions experienced during tidal cycles. Indeed, even if scallops live in the lowest part of the intertidal zone, they may be exposed to up to 2 hours of aerial situation and have to face hypoxia and dessication. The objective of this study was to characterize the metabolites present in the gills of *M. varia* and to highlight the eventual metabolic and energetic pathway shifts observed during alternation of emersion/immersion tidal cycles.

After acclimatization of a pool of individuals in mesocosms recreating *in situ* conditions, metabolites composition was followed, after sampling scallop gills during 3 days through 6 tidal cycles performed as 10h of immersion and 2h of emersion. Sampling was performed before the end of each immersion and emersion phases.

UHPLC/QTOF-MS and APGC-ToF-MS analyses were performed to study metabolite composition of samples. They showed that a large number of metabolites presented significant relative abundance variations between immersion and emersion phases. Among identified metabolites, compounds typical of anaerobic metabolism (such as malate, succinate, citrate and carnitine-conjugated metabolites) accumulated at low tide compared with immersion condition, showing a clear shift in energetic metabolism. This study brings the first evidence of metabolic pathway shifts of variegated scallop exposed to the environmental pressure of tidal hypoxia. It also provides a first step of normalized sampling method to assess the health of this aquatic organism.

P64

Metabolomics confirms a specific ecology of Agrobacterium fabrum conferred by its specific genes during bacterial-plant interactions

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Agrobacterium is a bacterial genus composed of many species capable of establishing commensal interactions in plant rhizosphere even if it is mainly known to be phytopathogenic by inducing crown gall, a disease characterized by the formation of tumors on many plant species. Remarkably, field investigations showed that several species of agrobacteria generally co-exist in the same biotopes (soil, rhizosphere or tumors). To explain the occurrence and persistency of this biodiversity, we hypothesize that different species must have particular ecological niches allowing them to evade from competitions with their closest relatives, and the adaptations to these particular niches are encoded by ”species specific” genes.
Indeed, comparative genomic studies showed the occurrence of particular genes specific to each *Agrobacterium* species. In the case of *A. fabrum*, these genes are mainly clustered into genomic regions called ”specific regions” encoding functional units whom annotations predicted they are related to metabolism or transport of plant compounds (sugars, phenolic compounds...). This led us to assume a specific ecology of *A. fabrum* in tight relation with its lifestyle with plants. Thus, in order to verify this prediction, the present study aims at verifying the involvement of *A. fabrum* specific genes on plants at both commensal and pathogenic levels (roots and tumors). For this purpose, we compared the wild-type strain and the specific regions deletion mutants for their effect on 1) the bacterial fitness in planta and 2) the plant metabolome using a metabolomic approach.

Competition tests between wild-type and deleted mutants strains showed that specific regions significantly increased *A. fabrum* fitness in rhizospheres or tumors. By the same token, root and tumor extracts were analyzed by UHPLC-UV / DAD-MS QTOF to compare secondary metabolites profiles. Descriptive statistics show differences between the plants profiles (WT vs mutants) but also distinguished discriminating metabolites among the treatments showing a modification of metabolites following bacterial inoculation.
Taken together, the present results show that *A. fabrum*-specific regions are involved both in the bacterial fitness during commensal and pathogenic lifestyles, and in a complex molecular dialogue between bacteria and plants. This not only confirms our hypothesis but also strongly suggests that adaptations to lifestyles with plants could be finely tuned. The continuation of this study will allow us to precise the individual role of specific regions for a better understanding of the specific ecology of *A. fabrum*.

P65

Input of multidimensional phenotyping in the metabolic syndrome stratification

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Metabolic syndrome (MetS) is defined by a cluster of cardio-metabolic factors including obesity, hypertension, dysglycemia, and dyslipidemia. It affects a growing number of persons, in particular older adults often suffering from multiple chronic diseases, and its prevalence is now a public health challenge. In the context of personalized medicine/nutrition, new tools are necessary to bring additional knowledge about MetS etiology, better stratify populations and customise strategies for prevention.
A nested case-control study on MetS was designed within the Quebec Longitudinal Study on Nutrition and Successful Aging (NuAge). It includes 61 cases and 62 controls of similar age (68-82 y.o.), selected among the 853 men. Both targeted and untargeted metabolomic/lipidomic approaches, available within the MetaboHUB French infrastructure [1], will be performed on serum samples collected at recruitment 2003-2005 (T1) and three years later (T4). Data analysis will be performed using reproducible online Galaxy workflows [2].

The metabolomic/lipidomic data will be processed to identify specific signatures of MetS and its components, and study their stability over time. Then, these data will be analysed for evaluation of a molecular reclassification of the MetS phenotype. Finally, they will be integrated with phenotypic and detailed nutritional data available to better characterize sub-phenotypes.
The approach developed here will open a door for a more comprehensive understanding of the metabolic phenotype resulting from the complex interplay between intrinsic and extrinsic factors. Thus, this project will allow an improved description of MetS associated characteristics and will offer new tools for better patient stratification in elderly populations.

P66

Absolute quantification of metabolites in serum-containing samples using coaxial NMR tubes

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Absolute quantification of metabolites is required in order to have a comprehensive overview and understanding of the metabolic changes occurring in a living organism. This metabolites quantification can be done by the analysis of protons in nuclear magnetic resonance (NMR) spectroscopy. Typically, concentrations of metabolites are calculated by considering the concentration of one reference compound in addition to the signals integration, number of protons of both metabolite and reference compound. The 3-(Trimethylsilyl)propionic acid (TMSP) is one reference compound commonly used for NMR calibration and it can be used also for quantification purpose.

In the context of cell culture, serum is usually added to the medium as it is required to efficient cell proliferation since it brings the growth factors in cell media. Proteins in the added serum cause distortion of the spectra baseline but specific sequence like the Carr-Purcell-Meiboom-Gill sequence (CPMG) experiment allows removing the contribution of proteins in the proton NMR spectra. Nevertheless, TMSP is binding to the serum proteins. Such a sequence can also suppress TMSP signal and is thus disallowing accurate quantification.

Thus, we are here evaluating the used of coaxial NMR tubes for the quantification of metabolites in samples containing serum. These tubes allow the separation of internal standard and the samples in different volumes. Some adjustments are required to perform quantification with this type of tube. Accuracy, precision, limits of quantification and detection of this method are here presented.

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Using NMR metabolomics to unravel the pathways underlying the host-microbiota crosstalk in cancer cachexia

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Cachexia is a multifactorial syndrome which results from the interaction of several patholog-ical processes. It mainly leads to muscle atrophy and fat mass loss and affects especially patients with cancer. The gut microbiota has recently be pinpointed as a promising therapeutic target for cancer cachexia. Indeed, previous studies demonstrated that, in a mouse model of leukemia and cachexia, nutritional intervention targeting the gut microbiota has the potential to decrease cancer progression, inflammation, muscle atrophy and fat mass loss.
In this context, this research project aims to investigate new metabolic pathways potentially involved in the host-microbiota interactions in cancer-associated cachexia. Next-generation sequencing and 1H-NMR metabolomics are used to characterize both the microbial ecosystem and the host metabolism of cachectic mice (colon carcinoma 26 model). First results obtained by 1H-NMR metabolomics of liver samples and next-generation sequencing of cecal content demonstrate an important alteration of liver metabolites as well as profound microbial changes in cachectic mice.
Following up on this work, we will perform 1H-NMR metabolomics of cecal content, to further characterize the functions of the microbial ecosystem, as well as of blood samples, to get deeper insights in the host metabolism. In addition, through model and network buildings, we will pinpoint key microbes and related metabolites potentially involved in the microbiota-host relationship in cancer cachexia. Finally, the relevance of such biological pathways will be validated in vivo.

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Zika and Dengue dual inhibitor research : digging into chemical diversity of tropical Euphorbiaceae

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Dengue (DENV) and Zika (ZIKV) are arthropod-borne viruses associated with severe morbidity and responsible for massive epidemics. Euphorbiaceae family is characterized by the production of various diterpenoid skeletons endowed with a wide range of biological activities. [1] In this study, 311 extracts from endemic Euphorbiaceae species of New Caledonia and French Guiana were screened for their inhibitory effects on ZIKV and DENV NS5 RNA-dependent RNA-polymerase (NS5 RdRp). [2] Based on the molecular networking approach, [3] we focus on the identification of natural dual inhibitors of DENV and ZIKV NS5 RdRp. The screening was performed using a newly developed fluorescent assay. To target original bioactive scaffolds, the biological activities were mapped on the global molecular network generated from LC-MS2 data. We present here the bio- and MS-guided purification of several Zika and Dengue dual inhiitors from one New Caledonian Euphorbiaceae species.

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P69

Metabolomics approach to determine biocontrol products' resilience time and preharvest intervals

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To meet both farmers and consumers' expectations, and the EU Directive 128/2009, French and Spanish governments want less use of conventional pesticides and promote the use of plant protection products from natural sources, the biopesticides. Although the biopesticide market recorded a growth rate of around +15% per year, there is a need of technical efficacy studies and ecotoxicological profile references.
Based in Catalogne and Roussillon, the PALVIP project (local Mediterranean crops' alternative protection) associates universities and technical structures to evaluate new biocontrol products developed by the small local businesses partners. The purpose of PALVIP is 1) to characterize biocontrol products adapted to local Mediterranean cultivations (wine, fruits and vegetables growing), 2) to give advices to the farmers about the use of these products, and 3) to enhance the development of biocontrol industry and sustainable agriculture.

To reach that goal, the biopesticides selected in the project are studying according to their efficiency through field experimentation, their effect on plants and their environmental impact. In a first stage the UPVD (University of Perpignan Via Domitia) will contribute to the part of the project regarding the evaluation of biocontrol products' environmental impact. To date, the half-life, t 1/2, was often used to study the fate of pesticides in environmental matrices. However, this value gives restricted information as it doesn't give any information regarding the formation of by-products and the effect on biodiversity.
Moreover, biopesticides are usually really complex mixtures and yield chromatograms that are extremely difficult to interpret which precludes the t 1/2 from being considered as a viable tool. Consequently, an innovative approach based on metabolomics (LC-MS), the Environmental Metabolic Footprinting (EMF), was recently developed in the laboratory (Patil et al, 2016; Salvia et al, 2017).
On one hand, the EMF gives rise to a new integrative proxy, the resilience that corresponds to the time needed for the compound dissipation and its effects on the matrix. It has the potential to evaluate all the post-application effects of the biopesticide.
On the other hand, the EMF can be used to determine the preharvest interval (PHI) that corresponds to the time needed to have no residue difference between the treated sample and the control.

Different and complex matrices from fields (fruits, leaves and soils) will be studied. Optimizations will be done in completely controlled environment (climatic chamber). Then, on the basis of these optimizations, samples from the field experimentations will be analyzed in order to determine the effects of the different treatments through the evaluation of the resilience time and the PHI in particularly.

Key words: biocontrol compounds, environmental impact, LC-MS, metabolomics, resilience time

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P70

Real-time metabolism assessment using NMR analyses: application to the optimization of energy usage in mammalian hybrid Cell-free protein synthesis systems

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Nuclear Magnetic Resonance (NMR) is well validated in the context of metabolomics studies as a powerful metabolic phenotyping platform that can contribute to biological investigations. Real-time NMR is a technical approach developed to study composition-evolving solutions by providing series of spectra that are followed over time, from which the sample composition and evolution can be characterized. As NMR can detect, identify and quantify numerous metabolites in a non-destructive manner and under physiological conditions (pH, temperature), it is uniquely suited to assess complex biological metabolic reactions: applied to fresh cell lysates (200 uL, obtained from culture cells, biological specimens or biopsies), real-time NMR allows to quantify substrates consumption (and metabolic pathways downside products evolution), after the addition of specific substrates to re-activate metabolic activities.
We illustrate how real-time NMR investigation of metabolites kinetics can deliver a detailed picture of the energetic metabolism for hybrid cell-free protein synthesis (CFPS) systems composed of rabbit reticulocyte lysates (RRL) ribosome-free supernatant complemented with ribosomes from different mammalian cell-types. A counterintuitive strategy, based on reducing the ribosomal fraction in RRL, is rationalized using a real-time NMR metabolomics investigation. We show that persistent ribosome-associated metabolic activity consuming ATP is a major obstacle for maximal protein yield, and reveal the potential of real-time NMR for optimization of CFPS systems.(1)

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P71

Development and comparison of Data Dependent Acquisition (DDA) and Data Independent Acquisition (DIA) LC-MS/MS workflows for untargeted metabolomics

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Due to its ability to detect hundreds of metabolites in biological matrices, high resolution electrospray ionization mass spectrometry (HRMS) coupled to liquid chromatography is one of the most widely used analytical techniques in metabolomics. Typical untargeted HRMS-based-metabolomics workflows rely essentially on ”full-scan-only” analyses performed using LC-HRMS from which metabolites of interest are first deduced. Those hits are then tentatively identified by tandem mass spectrometry following multiple injections into the LC–MS/MS system using targeted MS/MS workflows. Such procedure is highly tedious as well as time- and sample-consuming, and relies essentially on analyst manual data analysis. Thanks to recent technological advances in mass spectrometry (e.g., in terms of acquisition speed), new acquisition workflows combining MS and MS/MS acquisitions at increased mass resolving power can be implemented. Data-dependent acquisition (DDA), in which the MS instrument switches from full-scan MS mode to MS/MS mode when an eluting peak rises above a predefined threshold, can be employed. DDA workflows need to be thoroughly developed and optimized to avoid a loss of data in the MS mode when MS/MS data are being acquired at high resolution with potentially poor duty cycles, thus often making DDA less than ideal for fast analysis and narrow, rapidly eluting, peaks. Alternatively, Data independent acquisition (DIA) allows fragmenting all the compounds present in a given mass range. Although fragment ions can be theoretically collected for every precursor detected, DIA workflows result in highly complex MS/MS spectra that require highly efficient proper post-acquisition data processing software tools.

We will report the development and careful optimization of DDA and DIA workflows in a metabolomics context. These two methods were developed on an Orbitrap Fusion instrument using a set of 50 standard compounds analyzed under LC-MS conditions in solvent and biological media. Head-to-head evaluation of the analytical performances of those two workflows has been conducted and will be reported.

P72

Transcriptomique et métabolomique : deux approches complémentaires pour mieux comprendre les mécanismes d'interaction entre le maïs et la bactérie phytostimulatrice Azospirillum lipoferum CRT1

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Les bactéries rhizosphériques stimulatrices de croissance (PGPR) du genre *Azospirillum* sont utilisées commercialement pour leur capacité à stimuler la croissance et à augmenter le rendement des céréales via une relation associative complexe et peu comprise. Des analyses du contenu phytochimique des racines, feuilles et sève ascendante de plantules de deux cultivars de maïs (FuturiXX et Seiddi) non-inoculées et inoculées avec la souche commerciale PGPR *A. lipoferum* CRT1 ont été réalisées par GC-MS (Rozier et *al*, 2016). Elles ont suggéré pour la première fois l'importance de la communication racine-feuille et des sucres simples dans l'augmentation de croissance et du potentiel de conversion photochimique de jeunes plantules par *A. lipoferum* CRT1. Des analyses transcriptomiques effectuées en parallèle ont révélé un impact modéré au niveau des racines et des modifications de nombreux nœuds régulateurs des processus biologiques cellulaires des feuilles, dont ceux contrôlés par les auxines et l'acide abscissique. Les analyses 'omics' réalisées dans ce travail ont de même montré la réponse cultivar-spécifique des plantules à l'inoculation avec la PGPR.

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Role of the Sterol Regulatory Element-Binding Protein 1 (SREBP1) in melanoma cancer metabolism : an in vitro model using a 1H-NMR based approach

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INTRODUCTION :
Sterol regulatory element-binding protein 1 (SREBP1) is a transcription factor involved in fatty acid biosynthesis and regulation. In order to promote cell proliferation and invasion, cancer cells are able to modify their own metabolism and stimulate *de novo* lipogenesis from glucose and glutamine, making the SREBP1 transcription factor a key element in cancer development and survival. In the specific case of BRAFV600E mutated melanoma, the overstimulation of the BRAF / MERK / ERK pathway has a positive effect on the SREBP1 transcription factor and consequently *de novo* lipogenesis. BRAF inhibition is used as a therapy in melanoma, but rapidly evolves in therapy resistance with no effect on the SREBP1 transcription factor level. This study focuses on melanoma cells metabolism using an *in vitro* model in order to find potential solutions in the resistance occurence, and also target the SREBP1 transcription factor as a tentative treatment of resistant melanoma cells.

MATERIAL AND METHODS :
The 451-Lu human melanoma was the main cell line used in this study, declined in four cell conditions:
- the 451-Lu cells which are therapy responsive
- the 451-Lu R cells which are resistant to the BRAF inhibition
- the 451-Lu R SREBP +/- resistant cells in which SREBP1 is partially downregulated
- the 451-Lu R SREBP -/- resistant cells where SREBP1 is totally downregulated

Both intracellular (MOH-H2O-CCL3 extracts) and extracellular compartments were analysed using a 1H-NMR Bruker Advance 500 MHz spectrometer. NMR samples were prepared with D2O, phosphate buffer and TSP as a reference. NMR spectra were processed with 3 softwares (MestRenova®, Excel®, SIMCA P+®) to identify descriptors discriminant for groups separation. Finally, discriminant metabolites were identified using CHENOMX® and in-house tables.

RESULTS AND DISCUSSION :
The partial downregulation of SREBP1 in therapy resistant cells switches the metabolism of these cells towards a more sensitive form, by acting directly on glycolysis. On the other hand, the total downregulation of SREBP1 disrupts the glycolytic pathway, with an activation of the polyol pathway and a decrease in the other final products (lactate, myo-inositol, alanine). In addition, the total downregulation also modifies the tumor microenvironment, with a decrease in lactate release and consequently a decrease in acidosis, and with a decrease in glucose consumption supporting the idea that these cells are less active metabolically speaking. Finally, two metabolites (succinate, glutamate) tend to show that other pathways depending on membrane proteins are altered and need to be investigated using other approaches.
This study also gives potential solutions in the resistance understanding. First, the resistance seems to be based on a switch of the glycolytic pathway, with an increase of lactate and a decrease in myo-inositol. Secondly, glycine level is increased and suggesting that cells produce more GSH and are better prepared to counteract cellular stress.

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New insight in Metabolomics based study of Age Related Macular Degeneration (AMD): Lipoprotein profiles and subclass analysis.

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Age-related macular degeneration (AMD) is the leading cause of blindness among the elderly population in developed countries. 90% of all vision loss due to AMD result from the exudative form of this pathology, which is characterized by choroidal neovascularization (CNV). Currently, diagnosis of AMD relies on ophthalmologic exams and treatments of the exudative form are based on the use of anti-angiogenic drug targeting vascular endothelial growth factors (VEGF). Despite these advances, several clinical challenges have to be overcome, among these the choice of adapted therapeutic treatments and the identification of biomarkers as an indicator of patient stratification, disease progression and treatment responses are essential. For these reasons we decide to study AMD using an innovative translational metabolomics approach.
Here, NMR-based metabolomics was applied on AMD patients and on a laser-induced murine choroidal neovascularization experimental model(1). The human cohort included (i) bleeder AMD patients that are under treatment, (ii) non-bleeder AMD patients and (iii) Healthy volunteers.

NMR analysis on collected sera allow the differentiation between control and AMD patients and between laser-induced and control mice group. Moreover, the same discriminating spectral zones have been identified in human and mouse model, leading to the emergence of different putative biomarkers. Among these, lipoprotein profile is of particular interest since some studies associated high density lipoprotein cholesterol and oxidized low density lipoprotein with early AMD (2;3) and para-inflammatory process inducing pathologic AMD respectively(4).

Our primary data shows similar evolution of the whole lipoprotein profile trough the different groups on human and mice studies. These data suggest that investigate lipoprotein profile could be a turning point in the comprehension of the pathologic processes that occurs during the apparition and/or the development of pathologic CNV process.

In order to better understand the relationships between lipoproteins profile and AMD and/or CNV progression and status, we develop a methodology that allows the separation and the isolation of lipoproteins from blood samples and the NMR measurements of proper lipoprotein subclasses.

The work is still in progress and the creation of a deconvolution algorithm that enable the quantification of distinct lipoprotein subclass in NMR spectra collected from AMD patient and from the CNV mouse model get closer.

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P75

Connections between metabolic diversity and neutral genetic diversity. The case of a wild tree of African tropical forests

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To understand how evolutionary forces interact to shape phenotypic variations observed among natural populations, it is essential to elucidate the relationship between the diversity of adaptive phenotypic traits and the diversity of neutral genetic markers. A population with a high diversity of adaptive phenotypic traits is expected to better face a range of selective pressures (Ouborg et al 2010).
Microsatellites are repeated DNA sequences dispersed in the nuclear genome which variation is *a priori* not related to any survival, growth or reproductive parameters that are neutral with respect to adaptation of individuals to their environment.

A nice example of plant adaptive traits is highlighted by the metabolome present in a plant organ at a certain time. As metabolites are the result of a complex network of gene expression, protein interactions and other regulatory processes, they are therefore closer to the phenotype than mRNA transcripts (transcriptome) or proteins (proteome) alone (Wu, 2018; Arbona et al. 2013). Secondary metabolites with complex molecular structures are also expected to show a supraspecific phylogenetic signature (Wink 2003).

In the present study, we aimed at comparing the untargeted metabolic diversity to the neutral genetic diversity of wild tropical *Erythrophleum* trees. The objective was to test whether populations with high neutral genetic diversity also have a large diversity of metabolites which is expected to be related to a higher adaptive potential in the natural environment. The diversity of the metabolome can be considered as the ultimate expression of regulatory processes, influenced both by the genome and the environment, and this metabolic diversity is here studied as a proxy of adaptive traits. Neutral genetic markers (microsatellites) and metabolites (investigated using liquid chromatography coupled with a high resolution mass spectrometer) were assessed in individuals from 5 genetically distinct *Erythrophleum* tree populations (Gabon, Republic of the Congo, Cameroon) growing in a common garden in Cameroon.
The kernel PCA method (Mariette et al. 2017) was used to integrate metabolomics and microsatellite data. Kernel enables to take into account data heterogeneity. Once individuals were discriminated according to the modalities of the biological factor of interest like species, important features within each block were determined using permutatio

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Phytochemical analysis by NMR-based metabolomics approach in amaryllidaceae plants

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Keywords: Biomolecules, Metabolomics, NMR, Amaryllidaceae, Alkaloids.

Summary:

Natural substances represent a strong potential for drug discovery and NMR-based metabolomics are powerful technique [1-2].

Our work focuses on the NMR-based metabolomics study of species belonging to the family Amaryllidaceae.

In our laboratory we studied extensively the biosynthesis of the Amaryllidaceae alkaloids [3-4]. Our models are *Leucojum aestivum, L. aestivum 'Gravetye Giant'*, *Narcissus tazetta*, *Galanthus woronowii* and *G. elwesii* from different geographical origins.

The metabolome profiles were established both from *in vivo* bulbs and *in vitro* bulblets of these plants.

These plants have been chosen for their ability to synthesize high value-added molecules such as alkaloids, for example galanthamine and lycorine. We performed one-dimensional and two-dimensional NMR analysis of these species.

Qualitative and quantitative assessments of primary and secondary metabolites were determined by direct comparisons of recorded spectra.

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P77

Étude des composés organiques volatils (COVs) impliqués dans le dépérissement de la lavande

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Lavande (*Lavandula angustifolia Miller*) et lavandin (*Lavandula x intermedia*) sont des plantes à parfum, aromatiques et médicinales (PPAM) emblématiques de la Provence et largement exploitées pour leurs huiles essentielles en parfumerie, cosmétique et aromathérapie (1). Or, depuis une quinzaine d'années, ces cultures sont touchées par le dépérissement de la lavande qui provoque un important déclin des lavanderaies. Le dépérissement de la lavande est principalement dû au phytoplasme du Stolbur (*Candidatus phytoplasma solani*), une bactérie sans paroi transmise par un insecte piqueur-suceur, la cicadelle (*Hyalesthes obsoletus*) (2). Les moyens de lutte pour limiter l'étendu du dépérissement sont, à ce jour, assez réduits car il est impossible d'utiliser des antibiotiques ou des insecticides sur ce type de culture.
Dans ce projet, nous proposons d'étudier les interactions chimiques entre le phytoplasme, la cicadelle vectrice, la lavande et le lavandin afin de mieux connaitre les défenses de la plante et de proposer des moyens de lutte alternatifs. Une partie de ce projet porte donc sur l'étude des composés organiques volatils (COVs), molécules souvent associées au recrutement de prédateurs par les plantes infectées (3). L'étude de ces COVs nécessite la mise en place d'outils analytiques sensibles capables d'extraire et de détecter des molécules à l'état de trace dans un environnement chimique complexe. Dans le cadre de cette étude, notre choix s'est porté sur la microextraction sur phase solide (SPME) pour caractériser les COVs émis et stockés par les parties racinaires et l'extraction en espace de tête dynamique (DHS) pour les COVs émis par les parties aériennes (4). Les composés piégés ont été thermodésorbés et ensuite analysés par un système de chromatographie en phase gazeuse couplé à la spectrométrie de masse ((ATD-)GC-MS). Afin de mettre en évidence des différences d'émissions entre plants sains/infectés et entre variétés sensibles/tolérantes, le traitement des données a été réalisée à l'aide de logiciels tels que XCMS pour l'alignement des données chromatographiques et le logiciel R (package mixOmics) pour les analyses discriminantes (PLS-DA) (5).

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P78

A preliminary study of gut microbiota and energy metabolism relationship in obese dogs under different prebiotic and protein content diets

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Obesity and insulin resistance are major issues in pets meaning that nutritional strategies have to be developed, for example promoting greater protein and fibre intake. The study aimed to evaluate effects of dietary protein level and prebiotic supplementation on glucose homeostasis and relationships between microbiota and the metabolism of obese dogs. Six obese Beagle dogs received a diet containing 25.6 or 36.9% crude protein, with or without 1% short-chain fructo-oligosaccharide (scFOS) or oligofructose (OF), in a Latin-square design study comprising 5-week periods. Fasting insulin and glucose were measured at the end of each period. Faecal and blood samples were also collected for metabolomics and metagenomics. A multi-block analysis was performed to build a correlations network in order to identify relationships between faecal microbiota, metabolome, and phenotypic variables. The diets did not significantly affect glucose homeostasis, but scFOS increased faecal butyrate content, faecal microbiota richness and significantly modified the faecal profile metabolome. The correlations network highlighted that bile acids and certain amino-acids were related to insulin resistance state and that specific genus gathered in a metavariable had a high number of links with phenotypic and metabolomic parameters. It also suggested faecal aminoadipate and hippurate as potential markers of insulin resistance state.

P79

MineMS^2: Mining MS/MS libraries for common fragmentation patterns

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Metabolite structural elucidation is a major challenge in metabolomics. Tandem mass spectrometry (with collisional activation) is a powerful method to obtain reproducible fragmentation fingerprints from ionized compounds and has allowed, as such, the generation by metabolomics laboratories and platforms of libraries of product ion spectra of standard compounds occurring in biological matrices. Unsupervised analysis of such spectrum collections is of high interest to cluster collisional spectra and to gain insight on structural information for unknown compounds. Classical approaches (such as GNPS; Wang et *al*., 2014) rely on distances between product ion spectra. As a result, similarities resulting from shared substructures may be overlooked. Very recently, a strategy based on text mining has been described to extract shared motifs within collisional spectra (Mass2LDA; van der Hooft et *al*., 2016). In this method, however, fragments in a spectra are considered as words occurring in a text. In contrast, the concepts of fragmentation tree or graph (as used by the top performing prediction methods) are powerful models of fragmentation processes and would facilitate the interpretation of the patterns as chemical substructures.
We have therefore developed a new workflow to extract and visualize recurrent chemical patterns using a frequent subgraph mining approach (FSM). We applied our workflow to two spectrum datasets: 1) a library of 500 spectra from endogenous and exogenous compounds of interest in human biofluids, and 2) a spectrum collection from the pathogenic fungus Penicillium verrucosum. We show that meaningful fragmentation patterns can be extracted in a few minutes and linked to structural similarities.

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P80

LC-MS Separation and absolute quantification of new synthetic branched fatty acyl esters of hydroxyl-fatty acids in plasma.

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A new class of bioactive lipids has recently been reported (Yore et al.,*Cell*, 2014). This family of branched fatty acyl esters of hydroxyl-fatty acids, named FAHFAs, consists of a large number of regioisomers formed by numerous combinations of fatty acids (FA) and hydroxyl-fatty acids (HFA). These FAHFA were detected in mammalian adipose tissue, in blood plasma but also in rodent and human foods. While saturated fatty acids are usually associated with negative impacts on human health, the regioisomers 5-PAHSA and 9-PAHSA, two branched FAHFAs comprised of palmitic acid (PA) and hydroxystearic acid (HSA) with the ester bond located at position 5 and 9 respectively, exert a positive systemic effect on glucose metabolism. The synthetic 9-PAHSA also showed anti-inflammatory effects (Yore et al.,*Cell*, 2014). The function of these molecules are not known yet but it seems already very important to be able to quantify them in biological matrix.
The aim of this work is to develop a LC-MS method to quantify this new family of lipid in human plasma. To develop such a method we need some pure standards, few of them are already commercial but some family were unavailable so chemists developed new strategies to obtain them(*Balas, OPC, 2016*). We improved the extraction and the SPE pre-concentration of FAHFA to optimized yield of extraction and matrix effect. The main effort was done to find new chromatographic conditions to accelerate the separation, and to improve the sensitivity of the quantification with a derivatization of the carboxylic function of the FAhFA. This optimization will be present with the first result obtained with plasma and adipose tissus.

P81

Étude des effets métaboliques hépatiques induits par l'exposition à l'acide perfluorooctanoïque (PFOA) par des approches de métabolomiques combinées.

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La toxicité fortement suspectée des composés perfluorés, et plus particulièrement celle de l'acide perfluorooctanoïque (PFOA), un des représentants principaux de cette famille, pose aujourd'hui des questions majeures de santé publique. Plusieurs études suggèrent que le PFOA aurait des effets hépatotoxiques et cancérigènes, mais des données plus complètes sur ses effets métaboliques sont nécessaires pour la caractérisation des dangers associés à cette molécule.
L'objectif de nos travaux de recherche est d'explorer l'ensemble des voies métaboliques hépatiques altérées lors de l'exposition à différentes doses de PFOA. Dans ce but, nous avons choisi de développer des approches de métabolomique globale qui visent à identifier les métabolites dont les concentrations varient en fonction de la dose d'exposition en PFOA et de la durée d'exposition. Pour cela, nous avons choisi de travailler sur un modèle cellulaire hépatique humain : la lignée cellulaire HepaRG. Après 15 jours de différenciation en hépatocytes matures, les cellules HepaRG sont exposées pendant 24h (exposition aigüe) ou 7j (exposition sub-chronique) à 3 concentrations de PFOA (10 µM, 0.1 µM et 0.001 µM). Les concentrations utilisées ont été choisies de manière à refléter les concentrations plasmatiques de PFOA mesurées dans la population générale et dans des sous-populations très exposées (personnes vivant à proximité d'usines de fabrication utilisant ce composé ou travaillant dans ces usines).

Les milieux extracellulaires de chaque condition ont été récupérés afin d'observer les métabolites sécrétés et absorbés par les cellules. Les milieux intra-cellulaires ont été prélevés de manière à obtenir des résultats reproductibles pour les différentes conditions d'expositions au PFOA. Pour cela, le métabolisme cellulaire doit être stoppé et les cellules doivent être récupérées rapidement. Cela permet d'éviter un maximum de modifications non physiologiques pouvant apparaître lors d'une exposition à un stress et ainsi limiter la variabilité entre les échantillons. Pour cela nous avons utilisé un protocole dit de " Quenching " (Giuseppe Martano et al. *2014 Fast sampling method for mammalian cell metabolic analyses using liquid chromatography–mass spectrometr*).

Les milieux intracellulaires ont été analysés par LC-MS en quantification absolue avec l'utilisation de standards marqués au 13C pour les métabolites centraux et acides aminés (environ 80 métabolites quantifiés). Les métabolites du milieu extracellulaire ont été analysés par RMN (600 MHz) en quantification absolue à l'aide d'un standard interne, et par LC-MS avec des standards d'acides aminés marqués au 13C. Cela nous permettra ainsi de générer un grand nombre de données complémentaires, nous permettant de voir les effets d'une exposition au PFOA au niveau intra et extracellulaires. Ces données seront ensuite analysées dans le contexte du réseau métabolique par des approches de modélisation afin d'identifier les voies métaboliques affectées par une exposition au PFO

P82

NMR metabolomic signatures reveal predictive plasma metabolites associated with long-term risk of developing prostate cancer

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Background:
Combination of metabolomics and epidemiological approaches opens new perspectives for ground-breaking discoveries. The aim of the present study was to investigate whether plasma untargeted metabolomic profiles, established from a simple blood draw from healthy men, could contribute to predict the risk of developing prostate cancer within the following decade and to better understand the etiology of this complex disease.

Methods:
A prospective nested case-control study was set up in the SU.VI.MAX cohort,including 162 prostate cancer cases diagnosed during a 13y follow-up, and 162 matched controls. Untargeted NMR metabolomic profiles were established from baseline pre-diagnosis plasma samples using NOESY 1D and CPMG NMR sequences from 500 MHz NMR spectrometer Bruker Avance III. Multivariable conditional logistic regression models were computed for each individual NMR variable.

Results:
Men characterized by higher fasting plasma levels of valine, glutamine,creatine/albumin lysyl, tyrosine, phenylalanine, histidine, albumin, 3-methylhistidine and lower plasma levels of urea, CH2 lipoproteins, methionine and citrate had a higher risk of developing prostate cancer. The metabolite the most associated with prostate cancer risk was histidine (OR=1.46 [1.12-1.88], p=0.004).

Conclusion:
This study highlighted associations between baseline NMR plasma metabolomic signatures and long-term prostate cancer risk. These results provide interesting insights to better understand complex mechanisms involved in prostate carcinogenesis. If replicated in independent cohort studies, they may contribute to develop screening strategies for the identification of at-risk men for prostate cancer well before symptoms appear.
Trial registration: SU.VI.MAX, clinicaltrials.gov NCT00272428. Registered 3 January 2006
Funding:
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P83

Une approche métabolomique multiplateforme identifie des marqueurs associés aux émissions de méthane dans le lait de vaches laitières

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Le méthane, puissant gaz à effet de serre (GES), est produit dans le rumen des bovins par la fermentation microbienne anaérobie des aliments. Cette production est également responsable d'une perte d'énergie pour l'animal représentant 6 % à 8 % de l'apport alimentaire. Pour ces raisons, plusieurs travaux de recherches sont entrepris pour réduire ces émissions, en jouant sur la composition des aliments ou sur l'utilisation d'additifs alimentaires. Actuellement, la mesure des émissions de méthane se fait par différentes techniques, qui présentent des inconvénients, notamment le coût ou encore la difficulté d'application à grande échelle sur le terrain. De ce fait, de nombreuses recherches sont menées pour trouver des alternatives de mesure indirecte du méthane nommées "proxy". Les acides gras du lait ont été particulièrement étudiés pour établir des équations de prédictions du méthane. Cependant, ces équations ont un caractère prédictif limité (Negussie et al., 2017). Des métabolites associés aux émissions de méthane pourraient aider à améliorer les équations de prédiction des émissions de méthane ou être utilisés indépendamment comme proxy.
L'objectif de mon travail de thèse est de rechercher de nouveaux marqueurs de la production de méthane par une approche métabolomique multiplateforme dans le lait. Ces échantillons analysés, sont issus d'une expérimentation animale utilisant vingt-cinq vaches laitières primipares Holsteins séparées en deux groupes suivant l'ajout ou non d'un composé anti-méthane spécifique dans l'alimentation.
Les émissions de méthane chez les animaux alimentés avec le composé anti-méthane ont été réduites de 23 %. L'analyse des profils métaboliques du lait en RMN et en LC-MS combinées avec l'analyse statistique multivariée a permis de séparer les deux groupes d'animaux. Parmi les variables responsables de la séparation, seize métabolites appartenant à différentes familles ont été identifiés. L'analyse des réseaux métaboliques impliquant ces métabolites-marqueurs a montré que les voies de la valine, leucine, isoleucine et le métabolisme du glutamate sont particulièrement affectées.

Multi-informative molecular network to explore the chemodiversity of the endophytic communities from a long-lived rainforest organism: Astrocaryum sciophilum

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Endophytic microorganisms (principally fungi and bacteria) are an endosymbiotic group of microorganisms colonising the living internal tissues of plants without symptoms, during a variable period of their life. They represent an under-explored field of natural products research and possibly a promising source of new agents of biological, pharmaceutical and ecological interests (1). This work is part of the European project SECIL (Study of Endophyte Communities In a Leaf) which proposes to investigate the microbial crosstalks in plant leaf endophytes searching for innovative antibacterial agents, accompanied by comprehensive taxonomical, genetic and biological study of each strain (http://secil.obs-banyuls.fr/). Our objective was to simultaneously explore the chemodiversity and bioactive potential within a unique collection of 102 endophytic strains isolated from the leaves of *Astrocaryum sciophilum*. This palm tree from the primary forest in French Guiana is known for its longevity, and its very diverse and unusual fungal and bacterial communities (2). The pure strains were cultivated, extracted by organic solvents and submitted to UHPLC-HRMS/MS chemical profiling and antibacterial screening. Chemical data were then mined with innovative and multi informative molecular networking (MN) on the full collection, by overlaying different information (taxonomy, bioactivity). This method allowed the organisation of 604'000 MS2 spectra into 3'185 clusters containing 17'770 nodes. The taxonomical information and the antimicrobial bioactivity screening results were associated as metadata. The resulting MN helped to have a first overview of the chemo-diversity and chemo-taxonomy of our set of data. By annotation against experimental and *In Silico* spectral NPs databases (3), we could identify molecular families previously described in some of the species of the collection, as N-acyl homoserine lactones or enniatins. The exploration of the MN via this bioactivity mapping allowed to highlight clusters containing nodes from bioactive extracts (4). The approach permitted to putatively detect unknown compounds and analogues of compounds that are likely to be bioactive and the data generated will be used to target their isolation. The results demonstrate coherence between taxonomy, bioactivity and chemical composition and show that the MN of all strains holds well-structured information and provides a rich map to explore molecular diversity, chemotaxonomy and bioactive potential in complex microbial communities

P85

Omega-3 PUFAs and isoprostanoids regulate inflammation in microglial cells

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Obesity is an energy balance disorder that increases the susceptibility of developing metabolic diseases and that is characterized by a state of chronic low-grade inflammation. Thus, obesity is as an atypical form of inflammation induced primarily by the accumulation of fatty acids in tissues (liver, adipose tissue, muscle) altering metabolic regulation. This type of inflammation is not limited to peripheral tissues as it extends to the CNS, leading to the development of neuroinflammation. Microglia, the resident immune cells of the brain, represent a novel way to target neuroinflammation in order to potentially mitigate obesity and its health consequences. In this context, we investigated the impact of PUFAs in microglial cells on markers of inflammation and oxidation. Based on the close link between inflammation and oxidative stress, we focused specifically on non-enzymatic lipid oxidative products.

Our studies could be divided into two parts:

- First, in primary microglia cultures under inflammatory conditions induced by LPS, we determined by liquid chromatography-tandem mass spectrometry (LC/MS/MS) the qualitative and quantitative profiles of isoprostanoids in cells and media, on the basis that these metabolites constitute excellent biomarkers of oxidative stress and exhibit also a wide range of bioactivities.

- Then, under similar conditions, we evaluated the putative protective effects of PUFAs and some of their oxidized metabolites on LPS-induced inflammatory responses. To this end, we quantified by qPCR and ELISA the expression and secretion of pro-inflammatory cytokines (IL-1b, IL-6, TNFa, MCP-1).

To our knowledge, we demonstrate for the first time that LPS increased the production of oxidized metabolites of EPA and DHA in primary microglial cells. Our results also show that EPA, DHA and oxidized metabolites decrease the expression and secretion of pro-inflammatory cytokines.

P86

Stearoyl-CoA Desaturase-1 (SCD1) drives cancer malignancy via lipid desaturation.

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Targeting the metabolic pathways of cancer is actually the most contemporary topic of drug discovery. The unique and high metabolic requirement of cancer cells to sustain proliferation and survival pave the way for innovative therapeutic intervention. In this regard, we have contributed to the emergence of new facet of cancer metabolism in cancer adaptation and evasion to therapeutics. We previously show that tumor adaptation to angiogenesis inhibitors relies on a metabolic reprogramming towards a glycolysis and lactate production during targeted therapy (RTKI) and *de novo* lipogenesis after treatment cessation associated with tumor aggressiveness. The concept of targeting lipid metabolism to improve efficacy of targeted therapy has been validated by sequential targeting VEGF pathway and FASN inhibition in cancers. Whereas FASN is recognized as an important target in the development of anticancer drug for many types of human cancers, its complete inhibition has showed poor pharmacokinetics with heavy side effects, highlighting the need for the identification of other therapeutic targets that inhibit lipid metabolism. Since enhanced fatty acids (FAs) synthesis was detected after tumor hypoxia and re-oxygenation (sunitinib and sorafenib withdrawal) we applied lipidomic approaches based on LC-MS and used mass spectrometry imaging (MSI) for lipids on tumor sections. Differential isobaric lipid distribution and composition were detected by MSI, between treated and untreated tumors, at different time points after tumor re-oxygenation. Lipidomics analysis with internal controls of 380 lipid species revealed an increase in mono- and poly-unsaturated FAs (MUFA and PUFA) in the most aggressive tumors. Interestingly, SCD1, a key Stearoyl-Coa Desaturase enzyme that inserts double bonds into acyl-CoA chains was found to be up-regulated (microarray analysis) after tumor re-oxygenation and correlates with increased aggressiveness of lipogenic tumors. Furthermore, these unsaturated FAs are mainly engaged in PC and PE, the main known components of lipid rafts in cells. SCD1 expression was also validated in human breast cancer samples and shows a marked increase in both primary tumors and metastases.

P87

Fatty Acid Binding Protein 4 enhances cancer cell survival and tumor relapse during hypoxic and metabolic stress

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Anti-angiogenic treatment generates hypoxic and metabolic stress that is detrimental to cancer cell survival. Previously we have shown that tumor adaptation to harsh tumor microenvironment results in the acquisition of aggressive phenotype. During this process de novo lipogenesis was exacerbated and lipid accumulation within tumors was enhanced after tumor re-oxygenation. Here we investigated the role of lipid accumulation and mobilization within tumor microenvironment to sustain angiogenesis and tumor aggressiveness.

By using mass spectrometry analysis of tumors, we were able to find targets related to lipid metabolism including FASN and FABP4. FASN expression was investigated in our previous work (Sounni et al. 2014). Here we analyzed FABP4. The protein was overexpressed in tumors not only during the hypoxia but also after tumor oxygenation. Further immunohistochemistry analysis revealed that FABP4 upregulation was not found in cancer cells but in tumor endothelial cells (TECs). Interestingly, quantification of FAPB4 positive TECs show a significant increase in treated tumors when compared to control tumors. A close distribution of FABP4+ TECs around adipocytes was found in hypoxic tumors treated with anti-angiogenic and after re-oxygenation and relapse, whereas no FABP4+ TECs were found around adipocytes. Upregulation of FABP4 by hypoxia or sunitinib treatment was confirmed in vitro in HUVEC but not in lymphatic endothelial cells (LECs) or in cancer cells. The specific induction of FABP4 in TECs suggests a role in TECs survival during hypoxia through lipid transport and proliferation/sprouting after re-oxygenation and tumor regrowth. To investigate the biological significance of FABP4 in tumors, pharmacological inhibition of FABP4 in vivo showed a strong delay in tumor growth and reduced relapse after tumor re-oxygenation after treatment cessation. Tumors subjected to hypoxic and metabolic stress by anti-angiogenic treatment and re-oxygenation after treatment cessation were analyzed for their lipid storage. By immunofluorence detection of specific marker of lipid droplets adipophilin (Perilipin-II) known to be regulated by hypoxia, we found that hypoxic tumors have increased staining when compared to control tumors or after re-oxygenation. Double immunostaining for adipophilin with epithelial cells or macrophage markers, CK7 and F4/80 respectively, reveals increased lipid droplets in cancer cells. After tumor re-oxygenation adipophilin expression is reduced but the level of perilipin-I, the marker of mature adipocytes was up-regulated. The increase in lipid uptake by cancer cells was also reproduced in vitro by using BODIPY 493/503 on cells cultured under hypoxia or treated with sunitinib that induces a pseudo hypoxic state by stabilizing HIF1-α.

Our data show that during metabolic and hypoxic stress imposed by anti-angiogenic therapy, tumor cells increase lipid uptake form TME and TECs up-regulate FABP4 expression. After tumor re-oxygenation, cancer cells decrease their lipid uptake, increase de novo synthesis of lipids and increase in mature adipocytes and FABP4+ TECs. The preferential localization of FABP4+ TECs around adipocytes suggests a close interaction of ECs and adipocytes for lipid exchange and point to the key role of FABP4 in lipid accumulation and transport within tumor compartments. All together, our data demonstrate the key role FABP4 in cancer progression and the potential of targeting FABP4 for the abrogation of TECs adaptation to metabolic and hypoxic stress of anti-angiogenic therapy.

P88

Benefits of SWATH® Acquisition over Traditional DDA for HighRes Untargeted Metabolomics Applications

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SWATH® Acquisition Improves Metabolite Coverage over Traditional DDA for Untargeted Metabolomics

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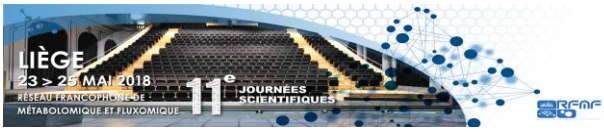
SWATH® acquisition, a data independent acquisition (DIA) work ow is well adopted in quantitative discovery proteomics, but not commonly used in discovery metabolomics. SWATH® acquisition combines the benefits of quantitation at the MS2-level of targeted MRM- based workflows with MS2- level based untargeted identification for metabolite identification of DDA workflows with the comprehensive nature of the MSMSall workflow. Because of the comprehensive, non-stochastic nature of the fragmentation in SWATH® acquisition, more fragmentation and thus structural information of the analytes compared to the DDA approach is achievable. Reproducibility and coverage is lower for DDA approaches compared to DIA workflows. Here we describe the improvements in metabolite coverage using SWATH® acquisition without sacrificing quantitation. Results obtained demonstrated a significant improvement of metabolites identified at the MS2 level by using SWATH® with variable windows in comparison with fixed windows in all analyzed matrices. We compared the ID rate from SWATH® acquisition to standard DDA. Here we were able to identify up to 45% more metabolites from the spectral library by SWATH® acquisition using 30 variable windows than by DDA. More confident MS2 based identifications then lead to more quantifiable metabolites in a metabolite expression experiment, which at the end allows better understanding of the biology. Spiked experiments into matrix samples of heavy labeled metabolites highlighted ten times higher sensitivity (signal-to-noise) using the MS2 ion to quantitate versus the traditional MS1 approach thus demonstrating the specificity nature of SWATH® acquisition to more traditional data dependent approaches.



P89

UVPD as a unique fragmentation tool for complete structure determination and substructure identification of small molecules

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Plant and fungus secondary metabolites are involved in many functions such as pigmentation, UV filtration, and defense mechanisms. These polyphenolic antioxidants have been incorporated for thousands of years in Eastern medicine but have yet to be utilized in Western therapeutics despite their phenomenal record in providing health benefits.

Here we investigate the molecular structures of several phenylpropanoids using various fragmentation techniques (CID, HCD and UVPD) available on an Orbitrap TM Fusion TM Lumos TM Tribrid TM mass spectrometer modified with a 213 nm UV-photodissociation (UVPD) source and a higher mass resolving power capability (1,000,000 at m/z 200). We performed CID, HCD and UVPD fragmentation experiments on these molecules for structural investigation. The data acquired from the different fragmentation techniques were used to reveal the molecular structures of each flavonoid using mzCloud and Xcalibur Qual Browser.

The structural diversity of conjugated flavanoids is extremely large, with the total number of combinations of conjugate sugars on different cores and their linking positions being unknown. We investigate here the fragmentation behavior of several flavonoids and their conjugates to determine diagnostic fragmentation using CID, HCD, and UVPD. When subjected to UVPD, flavone and flavanone both produce unique fragment ions from radical fragmentation channels not observed in either CID or HCD. A detailed UVPD study of luteolin and several of its mono glycosides showed that fragmentation of the core structure could be obtained concurrently with the losses of the glycosides typically observed by other fragmentation techniques. UVPD also provided unique fragmentation channels in both low and high mass range, since UVPD does not suffer the same low mass cut-off observed with CID. These results reveal that UVPD could be a powerful technique for the structural elucidation of conjugated flavonoid structures.

P90

Advantage of High Resolution Accurate Mass Spectrometry for Metabolite Identification in Untargeted Metabolomics Studies

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High resolution accurate mass (HRAM) spectrometry has become the analytical technique of choice for untargeted metabolomics studies. HRAM allows accurate mass assignments, resolving near mass isobaric species from complex mixtures, thus enabling confident compound identification and quantitation. Here, we aimed to investigate the effect of mass resolving power on metabolite identifications. Human plasma (NIST SRM 1950) was analyzed at different mass spectral resolutions ranging from 15,000 to 240,000 with a Q Exactive HF hybrid quadrupole-Orbitrap mass spectrometer operated separately in both positive and negative mode. The data were analyzed using Compound Discoverer 2.1 for metabolite identification. Increased resolving power was extremely useful in defining isotopic distribution and determining elemental composition. Feature detection improved with increasing resolution, yielding the best results at resolving power of 60,000-120,000. Metabolite identification was performed with Compound Discoverer 2.1 searching against mzCloud and Chemspider. Highly confident metabolite identications were obtained with mzCloud, which is a curated high-resolution, accurate mass spectral database. Compound identifications improved with increasing resolution but at a lesser extent than that of feature detection. Our findings suggest that mass spectral resolution higher than 60,000 is essential for obtaining greater metabolome coverage.

P91

High Resolution OrbitrapTM Mass Spectrometry for the Analysis of Deuterium-Labeled Lipids in E. Coli.

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Mass spectrometry (MS) is a powerful analytical tool to advance our knowledge of lipid structures and their biosynthesis. However, lipid flux analysis has been challenging due to isobaric overlap of deuterium labels with the prevalent natural abundance of 13C. Orbitrap technology has now advanced to allow the resolving power required to differentiate the natural 13C isotopes and label-derived deuterium on a scale compatible with high pressure liquid chromatography. Here we demonstrate the efficacy of ultra-high resolution LC-MS/MS for analysis of deuterated glycerophospholipids in E.coli lipid extract. The extracts were separated by reverse-phase chromatography using an AccucoreTM C30 column and subjected for MS/MS analysis. Data were acquired in data-dependent mode on a Thermo Scientific™ Orbitrap Fusion Lumos TM mass spectrometer operated at a resolution of 120, 000 to 500, 000 (FWHM at m/z 200). Analysis of phosphatidylglycerol and cardiolipin molecular species showed that ultra-high resolution is a prerequisite for successful baseline separation of deuterium and 13C isotopes and therefore for accurate analysis of labeled lipid species. These results highlight the potential of ultra-high resolution MS and stable isotope labelling in establishing a more comprehensive view of lipid metabolism in vivo.

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