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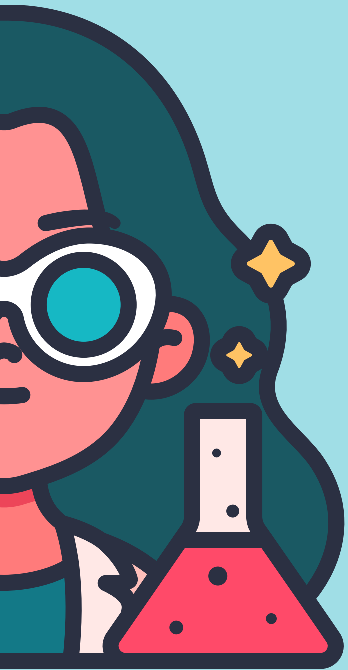

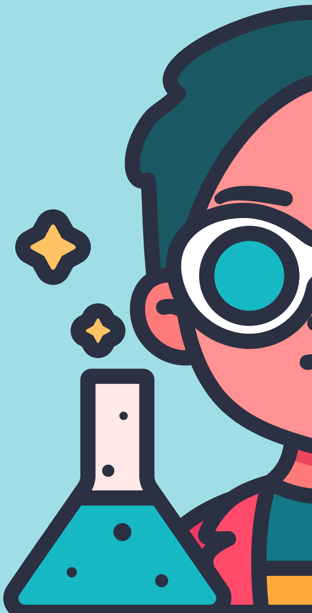

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Anti-inflammatory activity of four saponins isolated from the leaves of Polyscias fruticosa ”Hawaiiana Ming” (Araliaceae)

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Résumé

The Araliaceae family, commonly known as the ginseng family, consists of 41 genera and approximately 1350–1400 species, with most species concentrated in the tropical Southern Hemisphere. The *Polyscias* J. R. Forst. & G. Forst., with around 150 species is the second largest genus in the family, after the pantropical *Schefflera* J. R. Forst. & G. Forst., with around 650 to 900 species. Currently, *Polyscias* is distributed across the South Pacific (from Tahiti to Fiji, New Caledonia, and nearby islands), extending through Australasia, Malesia, the Indian Ocean Basin, and Africa, reaching its westernmost point in São Tomé and Príncipe. Because of its tonic and anti-inflammatory qualities, *Polyscias fruticosa* has been used extensively in traditional Asian medicine. Its roots were used to treat ailments like fever, diarrhea, neuralgia, and rheumatic pains, while its leaves were frequently used to heal digestive issues, lower inflammation, and had antibacterial properties. In order to test the ethnopharmacological claims on anti-inflammation and advance the chemotaxonomy on the genus, a phytochemical study of a *Polyscias* cultivar and hybrid, *Polyscias fruticosa* ”Hawaiiana Ming”, was carried out. This poster presentation details the extraction, separation and purification protocol used to isolate four saponins as well as their structure elucidation with extensive 1D and 2D NMR and HRESIMS experiments. The *in vitro* results regarding their anti-inflammatory activities will be evaluated in macrophages through NADPH oxidase inhibition and cell viability assays.

Mots-Clés: saponins, Araliaceae, Polyscias

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*Intervenant

Effects of agroecological infrastructures on pollinator networks and on the performance of a pollinator-dependent crop: blackcurrant (Noir de Bourgogne).

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Résumé

Pollinators and the pollination services they provide are threatened by intensive agriculture, yet
agriculture itself relies on this pollination service to assure the yields of many flowering crops of economic

importance. Agroecological farming practices offer a solution by simultaneously reducing the pressure on

pollinators and providing habitat resources, such as flowering strips of wild plants, to preserve pollinators and

pollination services to crops. This PhD project will study the efficacy of flowering strips in supporting the

pollinator communities and pollination of a crop of social and economic importance: the cassis (Noir de

Bourgogne). They will investigate how flowering strips affect the structure and function of networks of

pollinator interactions between the crop and wildflowers and if the wild flowers compete for or facilitate the

pollination service to the crop. They will determine the species traits or network properties that support

pollination and performance of the crop and they will discover how these effects are modulated in different
landscape contexts.

Mots-Clés: Pollinators, Flower strip, Interaction network, Blackcurrant, Agroecology

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*Intervenant

Enhancing dendritic cells activation and antigen presentation through NMD inhibition

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Résumé

Immunotherapies are a major tool in the fight against cancer, but its effectiveness remains limited. Nonsense-mediated mRNA decay (NMD), originally described as a degradation mechanism of aberrant mRNA, can also regulate the degradation of normal transcripts. Its activity is regulated during cancer, and patients with NMD escape and immune checkpoints inhibitors treatment have a better prognosis than those without NDM escape. It is therefore important to understand the effects of NMD inhibition on anticancer immunity, and its ability to create immune tumor microenvironment (TME) suitable for immunotherapy action.
We hypothesize that NMD chemical inhibition leads on the one hand to improved immunogenicity of tumor cells, and on the other hand to increased antigen presentation by dendritic cells to T cells. To this end, NMD chemical inhibitors (NMDi) were used to treat immortalized dendritic cell line (DC2.4), and the total RNA of these cells was sequenced (nanopore). A co-culture model with NMDi-treated DC2.4 and T cells was also performed. Finally, NMDi were tested in vivo in mice, in combination with immunotherapy (mRNA vaccination).

We observe that treatment of DC2.4 with NMDi increased the activation of DC2.4. Moreover, antigen presentation by DC2.4 cells is enhanced when treated with NMDi. Finally, tumor growth in a murine melanoma model is slowed when the mRNA vaccine is coupled to NMDi.
Thus, NMDi could enhance the efficacy of mRNA vaccination by potentiating activation of the immune system against the tumor, notably by improving antigenic presentation by dendritic cells to T cells.

Mots-Clés: NMD, cancer, immunotherapy, dendritic cell, antigenic presentation

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*Intervenant

Thèse en cours Évaluation des performances technico-économiques et environnementales de systèmes de culture riches en légumineuses et économes en pesticides

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Résumé

Agriculture faces a number of environmental, economic and social challenges. Reducing the use of pesticides is one of them, as they are being questioned due to their harmful effects on the environment and human health. Diversification of cropping systems appears to be one of the main levers for reducing dependence on chemical inputs. One crop diversification practice consists of introducing minor crops into systems, such as legumes. Indeed, legumes play a major role in improving the protein autonomy of production systems but their adoption rate remains low due to a number of socio-technical lock-in. In this context, the aim of the thesis is to provide scientific knowledge on strategies for integrating legumes into low-input cropping systems, to characterize these systems in terms of management strategy, and to assess the consequences of their adoption in terms of productivity, profitability, reduced environmental impact, and social criteria such as feasibility in terms of work organization. The thésis will address these issues for the field crop sector (including mixed crop-livestock farming), using data produced by the DEPHY farm network and the CA-SYS (INRAE Dijon) and ABY (INRAE Bourges) experimental platforms.

Mots-Clés: Agronomie, Grandes cultures, Diversification des cultures, réseau DEPHY, plateformes expérimentales

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[*] Intervenant

Reappraisal of the Ediacaran-Lower Ordovician stratigraphic framework and of the variscan deformation in the external Maures massif, southwestern Europe.

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Résumé

The PhD thesis is carried out within the framework of the RGF (Geological Framework of France), which is the national program dedicated to understanding the geology of the territory. Its aim is to improve the three-dimensional knowledge of the subsurface and to provide coherent digital information for various applications and societal challenges. The PhD project focuses on the study of Ediacaran to Devonian protoliths from the Maures massif and external crystalline massifs of the Alps, with the aim of understanding their nature, age, and paleogeography. The scientific issue lies in the need to better understand the geodynamic evolution between the margins of Gondwana and Laurussia, from the end of the Pan-African cycle to the Variscan orogeny, considering sedimentary deposits often affected by various tectono-metamorphic events. To tackle these challenges, our approach combines extensive field data acquisition, sampling and laboratory analyses, including U-Pb zircon dating, geochemical investigations, and isotopic tracing. Here, we present the first results from geological mapping and structural analysis carried out during two field campaigns in the outer part of the Maures massif conducted this autumn and winter. Our findings prompt a significant reevaluation of the western sectors of the 1:50,000 scale maps of Hyères and Collobrières. In particular, a new litho-stratigraphic log is proposed, offering fresh insights into the depositional sequences of the north-Gondwanan margin. This revised interpretation challenges previous models of the large-scale architecture by refuting the existence of major anticline and syncline folds. Moreover, our detailed structural analysis has identified two planar fabrics, S1 and S2, which overprint the primary S0 stratification. A preliminary map illustrates these initial results, with sample positions for dating shown on the lithostratigraphic log. The expected ages, ranging from the Cambrian to the Ordovician, may support interpreting the western Maures as a continuous Cambro-Ordovician metasedimentary series.

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*Intervenant

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Mots-Clés: Variscan belt, Maures massif, Alps, Protoliths, U, Pb dating, Paleogeography

Detection of Mucorales antigen in bronchoalveolar lavage samples using a newly developed lateral-flow device

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Résumé

Background: Mucormycosis is a severe emerging invasive fungal disease caused by fungi in the order *Mucorales.* Mortality rate remains high at approximately 50%. Rapid diagnosis and prompt initiation of targeted treatment is associated with improved prognosis. Gold standard diagnosis procedures have poor sensitivity and long turnaround time. *Mucorales* PCR in blood and respiratory samples has improved diagnosis, but this technique is not widely available due to high cost and the need for specialist equipment. A murine monoclonal antibody, binding to an extracellular polysaccharide (EPS) antigen secreted by all *Mucorales* fungi has been recently developed and integrated into a lateral-flow device (TG11-LFD). The aim of this study was to establish the clinical performance of TG11-LFD on bronchoalveolar lavage fluids (BAL) for the diagnosis of mucormycosis.

Methods: Thirteen BAL samples from 13 patients with mucormycosis, all of which tested positive for *Mucorales* qPCR (*Mucor/Rhizopus* (n=5), *Lichtheimia* (n=2), *Rhizomucor* (n=5) and *Cunninghamella* (n=1)) were used to assess the TG11-LFD. We also selected 49 BAL from 25 patients with other invasive fungal infections (IFI) (aspergillosis, *Pneumocystis* infection, candidiasis, and possible IFI) and from 20 patients without IFI, for use as negative controls. The intensities of the test and control lines were recorded using a Cube reader. The diagnostic performance was assessed by analyzing the Receiver Operating Characteristics (ROC) curve with the Jamovi software package (version 2.6.13).

Results: The area under the curve of the ROC curve was 0,739. Using a threshold value positivity £531 a.u, the TG11-LFD test has a sensitivity and specificity of 76.92% and 75.51%, respectively, a positive predictive value (PPV) of 45.45%, and a negative predictive value (NPV) of 92.5%.

Conclusion: In this study, we evaluated the performance of TG11-LFD on clinical samples for the first time and demonstrated its significant potential for enhancing the rapid detection of mucormycosis. Combining antigen detection with qPCR, as successfully applied in the diagnosis of aspergillosis, is likely to yield the most reliable diagnostic approach.

Mots-Clés: Mucormycosis, Mucorales, Diagnosis, Antigen detection, Lateral Flow Device

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*Intervenant

Context, objective, and approach of my PhD: adaptation of pea (Pisum sativum L.) to the impacts of climate change in France

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Résumé

My PhD focuses on the adaptation of pea (*Pisum sativum* L.) to the effects of climate change in France, particularly to abiotic stresses such as water deficits and extreme temperatures. To address this issue, I use crop modeling as a decision-support tool to identify effective adaptation strategies. The objective of my work is to assess the impacts of climate change on pea cultivation, to identify genotypes adapted to new French climatic conditions, and to propose optimized technical management strategies based on agro-pedo-climatic situations. I use the Azodyn-Pea dynamic cultivation model. I first evaluate the model in its initial version using sensitivity analyses and comparisons between simulations and observed data. I then improve the model by integrating new formalisms that describe genotypic responses to abiotic stresses. I re-evaluate the enriched model to validate its performance. Finally, I conduct prospective simulations to identify the best "genotype x environment x management practices" combinations under current and future French climates.

Mots-Clés: Crop modeling, Pea, Legumes, Azodyn, Pea, STICS

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*Intervenant

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Petrophysical changes and fluid flow during dehydration reactions.

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Résumé

A wide range of fluids flow through the Earth's shallowest (1 - 10^2 m) to deepest (1 - 10^3 km) layers. Geological heterogeneities resulting from rock properties (mineralogical composition, petrophysical properties, and mechanical behaviour) and structures (brittle as fractures and faults, and ductile as folds and shear zones) constrain fluid migration and allow fluid pressure to reach dramatically high values under specific conditions. These so-called fluid overpressures play a key role in many geological processes, such as the formation of mineralized veins and ore deposits, partial melting of hot crustal and mantle rocks, and for controlling kinetics of mineralogical reactions; in particular, high fluid pressures are known for altering rock mechanical properties, promoting failure, and in some cases triggering earthquakes.

At depth, as pressures and temperatures rise, some rocks undergo a mineralogical reaction called dehydration, releasing amounts of water comparable to the volumes of oceans over a million years and modifying rock hydraulic properties, inducing an important part of the worldwide seismicity.

Numerical modelling of fluid pressure diffusion is used here, along with laboratory experiments and field studies, to understand the generation and maintenance processes of fluid overpressures during dehydration reactions, and the interplay between dehydrating systems, seismic cycles and fluid flow in the lithosphere. As mathematical and numerical challenges merge from the highly non-linear, coupled and discontinuous equation system, validation methods used to verify the produced models are also presented here.

Mots-Clés: dehydration reactions, rock mechanics, fluid flow, earthquakes, diffusion equation, finite differences methods

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*Intervenant

Microscopic and Spectroscopic exploration of brewer's spent grain fermentation by Aspergillus oryzae for kojic acid synthesis.

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Résumé

Kojic acid is a secondary metabolite produced by many fungi, especially *Aspergillus*, during the fermentation of certain Asian foods. It takes its name from koji, the rice fermented by *Aspergillus oryzae* used in sake production, from which it was first isolated by Saito in 1907 (Saeedi, 2019). It is a well-established active ingredient in cosmetics for its depigmenting and UV-protective properties. It is primarily used to treat hyperpigmentation disorders such as dark spots, melasma and acne scars. Its mechanism of action relies mainly on inhibiting tyrosinase, the enzyme that catalyzes melanin synthesis, by chelating the copper ion at the enzyme's active site. In addition to its depigmenting effects, kojic acid exhibits antioxidant and antimicrobial activities, making it a valuable component in skin-care formulations (Chib, 2023).

Meanwhile, the food and beverage industry generates large amounts of under-utilized by-products such as brewer's spent grain (BSG), the residual malt grains left after beer production. BSG are depleted in fermentable sugars and on average consist of about 70% lignocellulosic carbohydrates (cellulose, hemicellulose, arabinoxylan, lignin), 20% protein, 10% lipids, and trace minerals and amino acids (Mussatto, 2006; Mitri, 2022).

In this study, we propose to evaluate the potential of BSG as a feedstock for active cosmetic ingredients via fermentation. We selected *Aspergillus oryzae* for its cellulolytic activity and its ability to synthesize kojic acid. By combining metabolic analyses with scanning fluorescence microscopy and infrared vibrational spectroscopic, we aim to determine how *A. oryzae* colonizes and degrades BSG to produce kojic acid. Kojic acid concentration will be measured using the Bentley colorimetric assay at 500 nm, which quantifies the complex formed between kojic acid and ferric chloride. Scanning fluorescence microscopy, using Calcofluor-White staining, will reveal the spatial distribution of *A. oryzae* on BSG. Finally, Optical Photothermal Infrared (O-PTIR) microspectroscopy will be applied to assess the fermentation state of the grains by comparing the IR spectra of BSG and *A. oryzae* to those of pure reference powders.

This project is part of a larger effort to develop a sustainable and innovative approach to producing high-value molecules by valorizing agro-industrial wastes through fermentation.

Mots-Clés: Kojic acid, fermentation, microscopy, cosmetics, brewer's spent grain, waste valorization.

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*Intervenant

RELATIONSHIP BETWEEN ASTRINGENCY PERCEPTION AND TONGUE TACTILE SENSITIVITY

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Résumé

Astringency is often described as a tactile sensation in the literature. However, the relationship between the astringency perception (AP) and the tongue tactile sensitivity has been little studied. This study aims to understand if the AP triggered by tannic acid is related to the tactile sensitivity. Astringency thresholds were estimated using the Best Estimate Threshold method (n=193), and this test was carried out twice for 78 of them, corresponding to the extremes of the starting population. Twenty-seven volunteers were selected according to the repeatability of their AP threshold measurements, two groups were identified: one group with high AP (median=0.04 g/L, n=12) and another with low AP (median=0.41 g/L, n=13). For these subjects, the salivary flow at rest was measured, and three different aspects of tactile sensitivity were quantified: sensitivity to light touch (one-point pressure test), spatial accuracy (two-points discrimination test), and roughness sensitivity (paper coupons of varying grit sizes). The two astringency sensitivity groups differed from each other only in the light touch sensitivity test. No significant differences were found for the other two tests. These results suggest that individual sensitivity to astringency cannot be attributed to general tactile sensitivity, but rather to a specific type of tactile input involving slowly adapting mechanoreceptors. More specifically, they point toward the involvement of SAI and SAII afferents, associated respectively with Merkel cell complexes and Ruffini endings.

Mots-Clés: Astringency sensitivity, tongue tactile sensitivity, food

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*Intervenant

ILC3 Cells as Direct Modulators of CD8 T Cells

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Résumé

The Innate Lymphoid cells, discovered about ten years ago, are still under investigation to understand their role in diseases and homeostatic conditions. As members of the ILC family, ILC3s play a crucial role in regulating immune homeostasis and defensive responses at barrier interfaces, notably in the gut, lungs, and skin. Historically recognized for their ability to produce IL-17 and IL-22 cytokines in response to pathogenic signals, ILC3s are involved in maintaining epithelial barrier integrity, producing antimicrobial peptides, and recruiting immune cells such as neutrophils. However, their rarity, the absence of specific markers, and the difficulty in studying their direct cellular interactions have long limited our understanding of their functions.

Recent studies have demonstrated that ILC3s play a key role in regulating T cell–dependent immune responses through antigen presentation. Indeed, these ILC3s are able of processing and presenting antigen to CD4 T cells via MHC class II molecules, leading to either activation or anergy of CD4 T cells depending on the environment, as well as the inducible expression of costimulatory molecules by ILC3s. Their role in modulating CD8 T cells, however, remains unexplored.

Based on preliminary data obtained in the laboratory, this project aims to explore the relationship between ILC3s and T cells and assess its implications in antitumor immunity. The team has already observed that ILC3 activation after chemotherapy promotes CXCL10 production and the recruitment of CD8 T cells to the tumor site. Moreover, the absence of ILC3s significantly reduced the antitumor efficacy of the treatment. These results suggest an essential and previously unrecognized role of ILC3s in directly modulating the cytotoxic response. We have already observed that ILC3s can trigger CD8 T cell activation in an antigen-dependent manner.

We will use complementary *in vitro* and *in vivo* approaches, in both mice and humans, to: (1) characterize the molecular mechanisms and stimuli required for ILC3 activation and their cross-presentation capacity; (2) analyze the functional consequences of this interaction on the phenotype and activity of CD8 T cells; and (3) study the impact of this relationship on the antitumor response and the organization of intratumoral lymphoid structures.

This project thus aims to uncover a novel immune circuit with significant implications for the development of innovative therapies in immuno-oncology.

Mots-Clés: Innate Lymphoid Cells (ILC), CD8 T cells, Adaptive immune response, cancer

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*Intervenant

Impact of environmental stresses on the production of membrane vesicles by Oenococcus oeni.

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Résumé

Membrane vesicles (MVs) are increasingly recognized as important mediators of stress responses in bacteria, including Gram-positive species, where their role remains poorly explored (1). These membrane-derived structures are involved in various adaptive functions, such as the transport of stress-related proteins, communication molecules, or enzymes that help bacteria cope with hostile environments (2). In the context of winemaking, *Oenococcus oeni,* a key lactic acid bacterium involved in malolactic fermentation (MLF), must survive and remain active under extreme environmental conditions encountered in wine, including high acidity, elevated ethanol content, and limited nutrient availability (3). While classical mechanisms like chaperone protein activation, pH homeostasis, and membrane fluidity regulation have been well documented, the potential involvement of MVs in *O. oeni*'s stress response has yet to be characterized. Investigating these MVs in *O. oeni* could thus reveal novel aspects of its physiology and contribute to the development of more robust enological strains.

In the present work, MVs were isolated via ultracentrifugation from (i) a standard *O. oeni* culture or (ii) stressed cultures subjected to 13% ethanol, pH 3 or mitomycin C (1 μg/mL) to enhance the MVs production. Transmission electron microscopy (TEM) observations revealed structures consistent with membrane vesicles. Analyses of MVs from stressed cultures are ongoing and will include both quantitative and qualitative characterization and comparison to MVs content from standart cultures.

These findings provide initial evidence of *O. oeni*'s ability to produce MVs. Future steps will focus on assessing how stress influences MV quantity and composition. In the longer term, functional assays, such as supplementing stressed *O. oeni* cultures with purified MVs, will help to determine whether these vesicles contribute to bacterial survival and stress adaptation. This research may ultimately lead to new strategies to enhance the robustness of malolactic starters and ensure successful MLF under challenging winemaking conditions.

(1) Abe *et al.*, 2021 Environ. Microbiol 23, 2632–2647

(2) Toyofuku *et al.*, 2023 Nat Rev Microbiol 21, 415–430

(3) Bartowsky et Borneman, 2011 Appl. Microbiol. Biotechnol. 92, 441-447

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*Intervenant

Mots-Clés: Membrane vesicles, Oenococcus Oeni, wine, stress response

Metabarcoding of intestinal parasites for a paleoepidemiological approach. Application to the Saint-Seurin necropolis in Bordeaux.

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Résumé

The detection of parasites infecting individuals is a direct testimony of the level of hygiene and habits of the affected people. When applied to ancient contexts, this research, called paleoparasitology, allows for a better understanding of past health conditions and behaviours, while complementing archaeological data. This discipline lies at the intersection of palaeoecological sciences, anthropology, parasitology, and co-evolutionary sciences.

The new metabarcoding approach, combined with microscopy will allow us to analyse more efficiently the samples from the Saint-Seurin necropolis in Bordeaux. This late-antique necropolis was in use during a mortality crisis of unknown origin. The use of metabarcoding on paleoparasitological analysis will help with paleo-epidemiological interpretations especially on the impact of the parasitic presence during the mortality crisis as well as the state of health of the population before, during and after the epidemic episode. This technique uses the Next Generation Sequencing (NGS) technology and biomolecular markers specific to parasites to identify every parasite species present inside the sample at the same time.

The first results in microscopy, show the presence of Ascarides, a frequent morphotype in the late antique gallo-roman population. This indicates a lack of hygiene associated with orofecal contaminations and these samples are yet to be analysed in metabarcoding.

Mots-Clés: Genetics, Anthropology, Paleoparasitology, Paleoecology, Parasitology

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*Intervenant

Granzyme A in extracellular vesicles produced by T lymphoma affects anti-tumoral immune cells in the tumor microenvironment

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Résumé

Extracellular vesicles (EVs) are biological nanoparticles secreted by various types of cells. Depending on the donor cell composition, they carry a specific cargo that is delivered to recipient cells, influencing their fate. Through their EV production capacity, tumor cells can modulate their microenvironment (TME), including immune cells, often in the context of promoting tumor progression. Lymphoma is a type of hematopoietic cancer originating in lymph nodes, developing from lymphocytes or their precursors. Non-Hodgkin (NH) T-lymphomas comprise a small part, accounting for less than 10% of cases. Their rarity, combined with their aggressiveness, makes them a challenging subject of study.

To model the TME *ex vivo*, we used a Transwell system coated with endothelial cells. Immune cells isolated from the spleens of mice developing T or B lymphoma (EL4 or A20) were placed into the upper compartment of the Transwell. Using ultracentrifugation and Nanoparticle tracking analysis, we isolated EVs from EL4 and A20 cells, labeled them with a lipid-intercalating fluorescent dye (PKH67), and placed them into the lower compartment. We demonstrated that EL4-EVs more readily target immune cell populations, whereas A20-EVs show minimal targeting of these cells. Most immune subtypes analyzed by flow cytometry are affected by EL4-EVs, leading to their exhaustion and subsequent premature death. Thus, we believe that T-cell lymphoma EVs may carry a specific cargo that impacts immune cell viability in the TME, which may explain the cancer aggressiveness. Proteomic analysis, by HPLC-MS-MS, showed an expression of markers linked with T-cell signalization, as well as the overexpression of Granzyme A (GzmA) in EL4-EVs. We generated a loss of GzmA in EL4 cells and EVs via stable expression of a shRNA following lentiviral infection (shGzmA),

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validated by a capillary-based protein analysis. Furthermore, shGzmA EVs lost their ability to affect immune cell viability *in vitro*, compared to control shREN EVs. *In vivo*, the tumor growth of EL4 shGzmA cells is reduced compared to shREN. GzmA silencing leads to an increased immune infiltration of Cd8+ and Cd4+ T-cells inside the TME as opposed to fewer tumor-associated macrophages (TAMs), coupled with a shift toward the anti-tumoral M1 phenotype.

Our study highlights the role of EL4-derived EVs in modulating the TME and negatively impacting immune cell viability via the transfer of tumoral GzmA. These findings underscore the functional impact of EV cargo in lymphoma progression and suggest that targeting tumoral EV-associated molecules like GzmA could offer new therapeutic avenues in aggressive T-cell lymphoma.

Mots-Clés: Cancer, lymphoma, extracellular vesicles, granzyme A, immune cells

Genetic determinism of legumes resistance to bruchids and trade-off between resistance and overall performance

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Résumé

Thanks to their protein-rich seeds, legumes species such as pea (*Pisum sativum*), lentil (*Lens culinaris*) and faba bean (*Vicia faba*) provide an interesting alternative to meat. However, bruchids (*Bruchus* sp., Coleoptera) cause detrimental damage by affecting seed quality required for human consumption. Because of the limited effectiveness of chemical treatments and previously observed variability of seed infestation among genotypes, genetic leverage seems a promising solution to combat this pest. Understanding this genotypic variation is the aim of my PhD work, financed by SPECIFICS project which promotes the use of legumes in cultural systems and reduction of pesticides. The objective of the PhD work is to (i) identify genomic regions associated with resistance to bruchids in three legume species, (ii) study the conservation of these genomic regions among species and (iii) evaluate trade-offs between resistance and other phenotypical traits. First of all, genome wide association studies (GWAS) will be performed on pea, lentil and faba bean collections to detect quantitative trait loci (QTL), i.e. genomic regions, associated with resistance to bruchids. Then, greenhouse and field trials will be carried out to validate the effect of some of those QTL using near isogenic lines having favourable or unfavourable alleles at the studied loci. In a second part, identified QTL within each species will be compared to the genome of the other species using a graph knowledge database, OrthoLegKB, to unveil potential conservation mechanisms. Transcriptomic and metabolomic data will help identifying candidate gene within detected QTL and give clues for resistance mechanisms. Finally, co-localisation of QTL will be studied to determine if genomic regions associated with resistance to bruchids can control other traits, through pleiotropy or linkage. The goal of this work is to characterise legume resistance to bruchids at the genetic and molecular level. This is a prerequisite to breeding programs. Identifying genes involved in this trait will enable breeders to improve their breeding programs and speed up the development of resistant varieties. This can lead to a reduction in pesticides use while preserving high seed quality.

Mots-Clés: Genetic, legumes, Bruchus, seed

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Omega-3 polyunsaturated fatty acids prevent enteric nerve system injury in high-fat diet induced obesity

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Résumé

Obesity, which is the main cause of several diseases including intestinal neuropathy, has steadily increased in the last century and becomes a significant health concern around the world. Our previous studies have shown the benefits of omega-3 polyunsaturated fatty acids (PUFAs) against obesity and associated metabolic disorders and endotoxemia. Numerous studies have also reported that omega-3 PUFAs could alleviate neuron damages. While the function of omega-3 PUFAs against enteric nerve system injury during high-fat induced obesity is still unknown.

We established high-fat diet (HFD) induced mice model basing on fat-1 male mice and their wild-type (WT) littermates. Body weight, oral glucose tolerance, whole gut transit time (WGTT) and gut permeability were assessed before sacrifice. As expected, after 9 weeks of HFD, wild-type mice developed obesity, associated metabolic disorders and delayed WGTT, but not fat-1 mice. Innovatively, we used dynamic full-field optical coherence tomography (D-FFOCT) imaging to detect the distribution and cellular activity within colonic enteric nerve system (ENS). We evidenced that HFD-fed WT mice suffered from significant ENS injury. Indeed, active cells area/ENS area is decreased by 70% compared to control-fed WT mice when HFD-fed fat-1 mice are partially protected. This protection was confirmed by qPCR results focused on markers of glial cells and neurons as GFAP and PGP9.5 respectively. Moreover, we found that colonic TLR9 (a lipopolysaccharide receptor expressed by enteric neurons) mRNA expression and cleaved-caspase 11 (marker of pyroptosis) protein level are up-regulated in HFD-fed WT mice when they remain similar to control-fed animals

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in fat-1 mice.

Our results evidence for the first time that endogenously synthesized omega3 PUFAs in fat-1 mice play an important role in preventing HFD-induced ENS injury by regulating TLR9/pyroptosis axis, providing a new therapeutic target against enteric neuropathy occurred in obesity. Moreover, our results support D-FFOCT to be a good way to detect ENS changes, which could be a relevant method for examining enteric neuropathy in clinical patients.

Mots-Clés: Omega, 3 polyunsaturated fatty acids, Dietary obesity, Enteric nerve system

Study of the mechanisms of immune regulation through the analysis of LysM-RLK (LYK) Receptors in Grapevine

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Résumé

Plant diseases pose a serious threat to agricultural yields. A major challenge is the development of effective methods to protect plants against pathogens. Plants possess an innate immune system capable of detecting pathogens and triggering defense responses to prevent infection. Pattern recognition receptors (PRRs) play a key role in the perception of biotic attacks through the detection of microbe-associated molecular patterns (MAMPs) or damage-associated molecular patterns (DAMPs), and in the regulation of immune responses. In grapevine (*Vitis vinifera*), previous research has identified 16 Lysin motif receptor-like kinases (LysM-RLKs, also called LYKs) and has highlighted the roles of VvLYK1-1, VvLYK5-1, and VvLYK6 in the recognition and signaling of chitin-a major component of fungal cell walls. However, several VvLYKs, such as the three members of the VvLYK3 subfamily, remain poorly characterized. Notably, VvLYK3-1 is downregulated during interaction with the fungal pathogen *Botrytis cinerea*, suggesting a potential role as a negative regulator of immunity. In this context, our objectives are to: i) identify the ligand(s) of the VvLYK3-1 receptor; ii) modify receptor abundance through a functional genomics approach using CRISPR/Cas9 genome editing on *Vitis vinifera*; and iii) study the spatio-temporal regulation of VvLYKs suspected to act as negative regulators of immunity through membrane trafficking (*e.g.*, endocytosis). The later will be studied using grapevine cells expressing VvLYKs fused to fluorescent proteins and their subcellular fate alone or in complex (*e.g.*, VvLYK6 with its co-receptor VvLYK1-1) following contact with MAMPs and DAMPs.

Initial results suggest that VvLYK3-1 may be involved in the perception of oligogalacturonides with a degree of polymerization (DP) of 10–15, which are pectocellulosic wall degradation products and recognized as DAMPs, and in the activation of the MAPK signaling cascade. However, its exact role in immune regulation remains unclear. Furthermore, VvLYK6, a receptor for chitin DP6 (a MAMP), appears to dissociate from its co-receptor VvLYK1-1 after ligand perception, and is subsequently internalized through an endocytosis mechanism while VvLYK1-1 seems to remain at the plasma membrane.

These studies will provide insights into the underlying mechanisms that regulate cellular immune responses and may reveal new targets for breeding or engineering grapevine lines with enhanced tolerance to fungal pathogens.

Mots-Clés: Vitis vinifera, LysM RLK, VvLYKs, endocytosis, MAMP, DAMP, plant immunity

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Validation of two markerless motion capture systems (OpenCap and Ochy) for kinematic analysis of running

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Résumé

Running is one of the most widely practiced physical activities but also implies a high risk of injury. In this context, assessing running biomechanics is essential for both injury prevention and return-to-sport decision-making (1). Since traditional technologies, such as optoelectronic camera are expensive, time-consuming, and limited to controlled laboratory environments, markerless systems, such as OpenCap and Ochy, have been developed to assess biomechanics in both clinical and athletic contexts. Although OpenCap has already been validated for biomechanical assessment of walking and squatting (2), it has never been validated for running. Furthermore, Ochy, a single-camera system designed specifically for running analysis, has yet to be validated in scientific literature. This study aims to determine the validity and reliability of OpenCap and Ochy for assessing kinematics and spatiotemporal parameters of running.

Twenty recreational runners (18–35 y.o.) will be recruited for the study. Participants will perform two experimental sessions involving treadmill running at preferred speed, as well as ± 20% of that speed. During each session, 3D trajectories of 19 anatomical markers as well as joint angles of the hip, knee, and ankle will be simultaneously recorded using all three systems. From these data, joint angles maximum and joint angle at initial contact and toe-off will be analysed as well as stride parameters (flight and contact time, stride length and frequency). Correlation coefficients, root mean square error (RMSE) and mean absolute error (MAE) will be used to determine the validity of Ochy and OpenCap relative to the optoelectronic system. Furthermore, reliability will be assessed using intraclass correlation coefficients (ICC) between sessions.

Eight participants have already completed the experiment, and data analysis is in progress and expected to be completed by the end of May. We hypothesize that both OpenCap and Ochy will show correlations with the reference system, although larger errors are expected in the frontal and transverse planes, as well as at higher running speeds. Validating these systems would represent a significant step toward broader clinical and field-based use of motion capture in running biomechanics.

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Mots-Clés: Reliability, Biomechanics, Gait analysis, Treadmill running, Injury prevention

Characterization of membrane vesicles produced by the intestinal bacterium Faecalibacterium duncaniae

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Résumé

The field of probiotics has evolved in alongside with advances in microbiological and biomedical research, leading to the emergence of *next-generation probiotics* (NGPs). These microorganisms, often isolated from the human gut microbiota, exhibit enhanced potential for the treatment of specific diseases, such as Crohn's disease. Among them, *Faecalibacterium duncaniae*, an extremely oxygen-sensitive bacterium, is particularly notable for its potent anti-inflammatory properties (1). Its extreme sensitivity to oxygen poses major challenges for its large-scale production and therapeutic application, as maintaining strict anaerobic conditions throughout the industrial process is technically demanding. In light of these limitations, current research is increasingly focused on the bioactive components released by such bacteria, particularly during their metabolic activity. One promising alternative involves membrane vesicles (MVs) which are nanoscale extracellular structures ranging from 20 to 400 nm in diameter. These vesicles play diverse biological roles, including intercellular communication and modulation of the host immune system. As such, they represent an interesting alternative to live probiotic administration and hold significant promise as postbiotic agents for alleviating the symptoms of inflammatory bowel diseases (2).

Following the isolation and characterization of these vesicles, we evaluated their potential bioactivity by assessing both their anti-biofilm activity against the pathogenic strain *Salmonella Enteritidis* and their anti-inflammatory properties. Our results demonstrate that *F. duncaniae* membrane vesicles exhibit dose-dependent effects, comparable to those observed with *Lacticaseibacillus casei* (3). These findings indicate that *F. duncaniae*-derived products may serve as effective agents for limiting pathogen colonization, with potential applications in gut health and industrial bioprotection.

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Mots-Clés: Membrane Vesicles, Faecalibacterium duncaniae, Probiotics, Postbiotics, Biofilm

Rôles des protéines du Nucléo-Adhésome au cours de la Transition Epithélio-Mésenchymateuse

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Résumé

Au sein d'une tumeur solide, les cellules cancéreuses sont soumises à de nombreux stress (hypoxie, manque de nutriments, cytokines pro inflammatoires, etc). En réponse à certains de ces stimuli extracellulaires, les cellules cancéreuses peuvent subir une transformation appelée la Transition Epithélio-Mésenchymateuse (TEM). La TEM est un phénomène progressif et réversible au cours duquel la cellule passe d'un phénotype épithélial vers un phénotype mésenchymateux. Cette transition s'accompagne de la perte des caractéristiques épithéliales (adhérence cellule-cellule, polarité apico-basale...) et de l'acquisition de caractéristiques mésenchymateuses (individualisation, migration, invasion...). L'adhérence cellulaire est complètement modifiée au cours de la TEM.
En 2022, Byron et collaborateurs ont identifié un groupe de protéines principalement décrites comme impliquées dans l'adhérence cellulaire qui peuvent être retrouvées dans le noyau et dont certaines y ont une fonction de régulateurs de l'expression génique. Ils ont défini ce groupe de protéines comme le Nucléo-Adhésome.

Or, de récents travaux au sein de notre laboratoire ont montré que les protéines HIC-5 et CRP2, toutes deux membres du Nucléo-Adhésome, peuvent migrer vers le noyau au cours de la TEM, interagir avec des enzymes de l'épigénétique et participer à la régulation de gènes liés à la TEM (données non publiées).
Les objectifs de ma thèse sont d'étudier les rôles nucléaires de ces protéines au cours de la TEM en i) découvrant leurs partenaires protéiques au cours de la TEM par Bio-ID, ii) étudiant si leurs fonctions sur la transcription sont bien dues à leur localisation nucléaire. Enfin, nous tenterons de confirmer ces résultats grâce à des validations fonctionnelles (migration, invasion).

Mots-Clés: cancer, TEM, épigénétique

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EFFECTS OF HERBICIDES SPOT-SPRAYING ON WEED MANAGEMENT

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Résumé

Introduction

Conventional chemical weed control, applied uniformly across fields, leads to economic and environmental losses by not exclusively targeting weed-infested areas. This practice results in excessive herbicide consumption and negatively impacts agroecosystem biodiversity. For perennial weeds like thistle (*Cirsium arvense*), with heterogeneous distribution, localized spraying offers a promising alternative. This method uses advanced detection technologies to precisely target infested areas, potentially reducing herbicide volumes, optimizing water use, and limiting negative environmental impacts (Parven et al., 2025). While some studies highlight its benefits, trials are few and not consistently repeated over time (Anne et al., 2024; Leise, 2024).

This PhD aims to evaluate the effects of localized spraying compared to broadcast spraying across various soil and climatic conditions and cropping systems in farmers' fields.

The main objective is to compare weed control efficiency, herbicide savings, and crop yield performance between broadcast and spot applications.

Particular attention will be paid to weed flora dynamic to determine if reduced inputs impact weed communities over time. Beyond comparisons, this research intends to provide insights into low-input cropping systems. Identifying problematic weeds and assessing localized spraying over multiple years will help integrate this technique into sustainable management. It could complement agroecological practices to reduce reliance on chemical herbicides.

Materials and methods

Trials are conducted over three years at four distinct sites in France (Guer (56), Grignon (78), Somsois (51), Dijon (21)), covering various crops (sunflower, grain and silage maize, sugar beet, winter wheat) and soil and climate conditions, in collaboration with agricultural

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Results and conclusions

Statistical analysis of the first year's results shows a significant reduction in weed density after treatment, but the first data are variable and there is no significant difference between the weed control efficiencies of the two spraying methods.
These three years of study will enable us to evaluate weed control efficiency, untreated area, and yield. Integrating biodiversity indicators and contextual data will refine the understanding of factors influencing treatment effectiveness and help design innovative and resilient farming systems, optimizing chemical input use and reinforcing agroecological levers.

Mots-Clés: Precision agriculture, weed community analysis, field trials, herbicide savings.

Understanding the effect of environmental parameters on the survival and growth of Clostridium perfringens: Application to the development of decontamination processes in slaughterhouses

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Résumé

Clostridium perfringens is an anaerobic pathogenic bacterium with the ability to form spores, involved in food poisoning and commonly found in slaughterhouse environments where it can contaminate meat. The survival of *C. perfringens* in slaughterhouses can be influenced by environmental parameters such as relative air humidity (RAH). The aim of this study was to understand the effect of this parameter on bacterial survival and to evaluate the effectiveness of an innovative decontamination process. Effects of environmental parameters on bacterial pathogens in slaughterhouses remain poorly documented. To fill this gap, measurement campaigns were carried out in several cattle, pig and poultry slaughterhouses to characterise the RAH and assess its potential impact on the persistence of *C. perfringens*. Afterwards, the survival of vegetative cells and spores was studied at different RAHs (11%, 43%, 75% and 100%) under aerobic and anaerobic conditions, in order to distinguish between the hydric effects of dehydration and those of oxidation. In addition, the effectiveness of photo-oxidative treatments based on ultra-high intensity blue light was evaluated on partially or completely dehydrated samples. The results showed that dehydration caused more than 99% of vegetative cells to die within the first day, regardless of the presence of oxygen, suggesting a lethal effect of hydric origin. Analyses using epifluorescence microscopy and flow cytometry showed that dehydration and rehydration altered membrane integrity, contributing to cell inactivation. On the other hand, spores proved to be much more resistant, with about 10% survival after two months of stress, without any significant effect of dehydration. Photo-oxidative treatments resulted in marked inactivation of spores and vegetative cells, with efficacy strongly influenced by the degree of prior dehydration and the duration of light exposure (up to 99% inactivation for spores dried at 11% RAH after 10 min exposure). This work highlights the critical role of RAH in the inactivation of *C. perfringens*. While vegetative cells appear to be particularly fragile, spores remain a major obstacle. Photo-oxidation is emerging as a promising strategy for decontamination in industrial environments. Further research is being carried out to gain a better understanding of the inactivation mechanisms involved.

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Mots-Clés: Clostridium perfringens, Slaughterhouse, Relative air humidity, Decontamination

Phytase activity during Lactic Acid Fermentation : Kinetic and Impact on Protein Solubilty

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Résumé

Aim: Legume proteins concentrates are interesting ingredients for formulating of plant-based food products with high proteins contents and good structural properties. However, antinutritional compounds such as phytic acid can bind to proteins, reducing their solubility and functionality. This study focuses on the phytase activity of lactic acid bacteria during fermentation of fava bean protein extract, and its impact on protein solubility throughout the acidification process.
Method: Soluble protein extracts were obtained from a 10% (w/w) from a fava bean protein concentrate dispersion at pH 7.5 and was used as media for simulated acidifications and fermentations. The solubility of phytic acid and protein through acidification was determined with lactic acid (0.1M or 1M) for a pH range of 7.5-4.5. Fermentations were carried out by using *Limosilactobacillus fermentum* CIP 102980 for 48h et 37○C. Samples were collected during the fermentation to evaluate the acidification and growth kinetics, phytic acid consumption and proteolytic activity. Protein solubility was determined before and after fermentation.

Results: The solubility of proteins was greatly lowered during acidification with lactic acid with a maximum between pH 6 and 5.5. The phytic acid was mainly soluble until the pH reached 4.5. Indeed, the phytic acid / proteins ratio (w/w) continuously increased in the supernatant from 0.068 at pH 7.5 to 0.194 at pH 5 but dropped to 0.159 for pH 4.5. Additionally, a two steps acidification was observed during fermentation: first, the pH decreased from 7.5 to 6.2 within the first five hours, followed by a static phase with no further pH decrease up to _˜20h. Then, the pH slowly decreased again from 6.2 to 5 over the subsequent ten hours. No further evolution of pH was observed after 30h.Phytase activity, proteolytic activity and the solubility of fermented proteins were analysed and compared to the results of acidification.
Conclusion: Phytic acid seems to be mainly in the soluble fraction during acidification from pH 7.5 to 5. During fermentation, the pH was not lowered below 5, allowing high phytic acid solubility and phytase activity. This was confirmed by the reduction of _˜7% of phytic acid content after 48h of fermentation.

Mots-Clés: Fermentation, Protéines végétale, Solubilité, Acide phytique, Phytase

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Role of autophagy proteins in the immunosuppressive and pro-tumoral functions of HSP70 and HSP110 in colorectal cancer

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Résumé

Heat shock proteins (HSPs) 70 and 110 are frequently overexpressed in cancers and contribute to tumor progression through multiple functions. These proteins can be released by cancer cells into the tumor microenvironment (TME), where they can interact with immune cells.
One aim of my PhD project is to understand the mechanisms underlying the immunosuppressive functions of HSP70 and HSP110, focusing on those linked to conventional and non-canonical activities of autophagy proteins. We will determine whether targeting autophagy proteins could modulate the action of HSP70/HSP110 on immune cells infiltrating solid tumors. We will thus identify potential new therapeutic approaches that could increase the capacity of HSP70 and HSP110 inhibitors currently developed in the laboratory to sensitize so-called "MSS" (Microsatellite Stability) colorectal tumors to anti-cancer immunotherapies.

To this end, murine macrophages and dendritic cells were cultured in the presence of conditioned media derived from Wild Type (WT) and HSP70/110 Knock Out (KO) or short hairpin RNA (sh) HSP70/110 murine colorectal cancer cells. Immune cells were then subjected to LC3 lipidation analysis by Western Blot and measurement of immune mediator expression by RT-qPCR.

LC3 lipidation, a hallmark of both conventional autophagy and Conjugation of Atg8 to Single Membranes (CASM), increases in immune cells exposed to conditioned media from WT cancer cells. We tested the contribution of extracellular HSP70/110 using conditioned media from HSP70/110 Knock Out (KO) or shHSP70/110 cancer cells and observed similar results suggesting that cancer cell-derived extracellular factors distinct from HSP70/110 stimulate LC3 lipidation. Furthermore, conditioned media from WT and HSP70/110 KO cancer cells similarly modulate the expression of genes encoding the pro-inflammatory molecules Il-1β, IL6, TNF and PGE2 by dendritic cells.

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My data suggest that the immunomodulatory action of extracellular HSP70/110 on innate immune cells is independent of autophagy and CASM. We will confirm this using primary cells which could be more representative of tumor-infiltrating immune cells. We will also test whether autophagy and CASM could contribute to the release of HSP70/110 by cancer cells into their microenvironment and thus participate in extracellular HSP70/110 immunosuppressive and tumor-promoting functions.

In conclusion, my PhD work will determine whether modulating autophagy and/or CASM is a viable strategy to reduce the immunosuppressive and tumor-promoting functions of extracellular HSP70 and HSP110. Additionally, the project will offer a broader characterization of how these HSPs influence immune cell behavior in the TME, including their impact on T lymphocytes.

Mots-Clés: HSPs, Autophagy, Cancer, Immunusuppression

Metabolism of plasmalogens in macrophages in the context of atherosclerosis

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Résumé

Introduction

In France, cardiovascular diseases-particularly atherosclerosis-are the leading cause of mortality. Monocytes and macrophages play central roles in the pathophysiology of atherosclerosis and are involved in all stages of atheroma plaque development. The plaque microenvironment modulates macrophage activation, which is associated with profound changes in cellular metabolism. These metabolic shifts include increased biosynthesis and incorporation of fatty acids (FAs) into phospholipids, allowing macrophages to dynamically regulate their phospholipid composition. This composition is critical for the innate immune response, as it influences phagocytosis, Toll-like receptor (TLR) signaling, and the production of lipid mediators. Moreover, membrane lipid composition plays a key role in cell death processes such as apoptosis and ferroptosis.

Plasmalogens are a specific class of phospholipids characterized by an alkyl bond and a plasmenyl group at the sn-1 position, and a polyunsaturated fatty acid (PUFA) at the sn-2 position. These features confer unique properties to plasmalogens in modulating membrane structure and susceptibility to lipid peroxidation. Two key enzymes involved in plasmalogen metabolism have recently been identified: TMEM189, which catalyzes the formation of the plasmenyl bond, and TMEM164, which facilitates PUFA incorporation at the sn-2 position.

Materials and Methods

Using CRISPR-Cas9 technology, we targeted these two enzymes in macrophages to assess their effects on lipid composition, macrophage activation, and susceptibility to ferroptosis or oxysterol-induced apoptosis.

Results

Enzyme inactivation had a direct impact on macrophage lipid composition, a phenomenon not previously described in the literature. Modulating plasmalogen levels by inactivating Tmem164 and Tmem189 altered cell morphology and membrane fluidity. These changes suggest that key macrophage functions such as phagocytosis and danger sensing may be affected.

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Conclusions
Our initial findings underscore the significant influence of TMEM164 and TMEM189 on plasmalogen content and multiple macrophage functions. Further studies are needed to clarify the underlying mechanisms, particularly in an integrated model of atherosclerosis with targeted enzyme deficiencies.

Mots-Clés: Plasmalogens, macrophages, inflammation, atherosclerosis

Effects of an intense auditory stimulus on corticospinal tract excitability

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Résumé

Introduction

It is now clearly demonstrated that a muscle strengthening training (MST) is accompanied by muscle structure adaptations, while the early increases in force production are primarily related to neural adaptations. While this phenomenon is well established, the neural mechanisms underlying the increase in force are not clear. Our hypothesis, underpinned by the results obtained in primates, is that MST-induced neural adaptations are essentially related to mechanisms involving the reticulospinal (RS) tract rather than the corticospinal (CS) pathway. The RS tract can be indirectly activated by an intense auditory stimulus (> 110dB) coupled with electrical or magnetic stimulations at different levels of the nervous system. One parameter that will influence the modulation of muscle responses is the interstimulus interval (ISI). The aim of this study is to determine the ISI that induces the strongest modulation of evoked responses at different levels of the nervous system.

Methods

The results presented here were obtained on 8 participants who took part in 3 experimental sessions, in which MEPs (transcranial magnetic stimulation-induced motor evoked potential), TMEPs (thoracic motor evoked potential elicited by electrical stimulation) and H-reflexes (potential induced by peripheral nerve stimulation) were conditioned by either, an intense auditory stimulus of 115 dB (AS), a sound of 70 dB (S) or a visual stimulus without any sound (V). Responses were evoked during submaximal contraction at an intensity corresponding to 10% of maximal muscle response (Mmax). Five ISIs were tested: 10, 50, 75, 100 and 125ms. The peak-to-peak amplitudes of the responses were normalized to Mmax.

Results

MEP/Mmax ratios were not significantly affected by any stimulus (p=0,298). A significant effect of the type of conditioning was observed for H/Mmax, indicating that this ratio was significantly higher for AS compared to V (p=0,030). Furthermore, a significant conditioning x ISI interaction was found for TMEP/Mmax, showing that at the 75 and 100-ms ISIs, this ratio was significantly higher for AS than S (p< 0,05 and p = 0,018 for 75 and 100ms) and than V (p< 0,05 and p=0,007, for 75 and 100 ms, respectively).

Conclusion

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This study demonstrates that facilitation of motoneuron and spinal excitability can be induced by an intense auditory stimulus. However, no modulation of corticospinal excitability was observed. These results that would be reinforced by increasing the sample size, will allow us to evaluate, in future studies, the contribution of CS and RS pathways to motor activation.

Mots-Clés: Electrophysiology, Motor evoked potentials, Corticospinal excitability

A formulation rich in resveratrol and omega-3 fatty acids improves the efficacy of radiotherapy in colorectal cancer and modulates angiogenesis, DNA damage and cell death

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Résumé

Radiotherapy is a major cancer treatment that involves delivering ionizing radiation to tumors, damaging tumor cell DNA, which leads to proliferation arrest, cell death and immune cell recruitment into tumors. However, radiotherapy is responsible for side effects on healthy tissue, and its efficacy is limited, among other things, by cancer cell resistance. A key challenge in oncology is to identify new treatments that enhance tumor radiosensitivity. In collaboration with the Centre Georges François Leclerc, we are evaluating the radiosensitizing potential of Resvega® (RGA, Laboratoires Théa), a dietary supplement rich in omega-3 fatty acids and resveratrol (a molecule found in grapevines); both known for anti-tumor and radiosensitizing effects. Already used clinically for ocular pathologies due to its anti-angiogenic properties, this supplement may also benefit colorectal cancer (CRC), where angiogenesis supports tumor growth and radiotherapy resistance.
In this study, C57BL/6 mice were subcutaneously injected with CRC MC38 cells and subsequently received daily treatments of RGA (15 or 30 mg/kg) either alone or combined with radiotherapy (3 doses of 8 Gray). *In vitro* experiments were carried out on murine CRC cell lines (MC38 and CT26), exposed to radiotherapy doses (2 or 5 Gray) and/or treated with RGA.

When administered as a monotherapy, RGA led to a reduction in tumor growth in CRC-bearing mice. Its combination with radiotherapy resulted in an even greater therapeutic effect. *In vitro* experiments in murine CRC cells revealed that RGA, when combined with radiotherapy, exhibited a more pronounced radiosensitizing effect compared to omega-3 fatty acids or resveratrol alone. These observations led us to investigate the underlying mechanisms which could explain this radiosensitizing effect. Analysis of signaling pathways triggered by RGA revealed that RGA decreases the secretion of vascular endothelial growth factor A (VEGF-A) and inhibits the formation of vascular pseudotubes by human endothelial cells

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(HUVEC), suggesting an antiangiogenic action. In addition, RGA promotes DNA breaks and/or limits their repair in cancer cells while also slowing tumor cell proliferation by inducing senescence and increasing cell death by apoptosis and ferroptosis.

RGA is currently being studied *in vitro* and *in vivo* to evaluate its effectiveness compared to its individual components and to explore its mechanisms of action in both cancer cells and the tumor microenvironment. Given its preclinical efficacy and its current clinical use, clinical trials combining RGA and radiotherapy are expected to develop rapidly, in CRC but also in other cancers resistant to conventional therapies, opening new therapeutic strategies.

Mots-Clés: Colorectal Cancer, Radiotherapy, Therapeutic Adjuvants, Fatty Acids, Polyphenols

Study of the CD2-CD58 costimulation pathway to optimise new anti-cancer therapies

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Résumé

CD58 is an adhesion molecule expressed on the surface of antigen-presenting cells (APC). CD58 can bind to CD2, which is its costimulatory ligand. CD2 is an adhesion molecule expressed in particular by mature T lymphocytes and NK cells leading to their activation. CD2 is also strongly involved in the formation of the immunological synapse. CD2-CD58 binding induces a rearrangement of molecules within the immunological synapse, which amplifies the signal linked to the TCR. CD2-CD58 binding also leads to signal 2 after binding between the TCR and the MHC-peptide complex. Several types of cancer show CD58 deficiency, especially melanoma and ovarian cancer. Mutation or loss of CD58 is linked to immune resistance and escape. In the treatment of solid cancers, bispecific or trispecific antibodies, or even TCE (T-cell Engagers) have been developed in recent years. TCEs have the advantage of being smaller than antibodies, enabling them to penetrate more deeply into the tumor. They are also designed to recognize more targets, making them more specific and affine. This work aims to test several TCEs with or without an aCD2 domain in CD58-deficient tumors. Enabling the restoration of CD2/CD58 signal in tumors with low CD58 expression could increase the efficacy of T cell engager (TCE) or immunotherapies.
Immune and tumor cells were isolated from the ascites of patients suffering from gynecological cancers. A CD45+ cell bank was established to carry out cocultures of immune cells and CD58-deficient tumor cells. The functionality, activation, and cytotoxicity of the immune cells cultured were used to measure the efficacy of different TCE constructs.

The results obtained suggest that stimulation of T lymphocytes via their CD2 domain in addition to activation via their CD3 and CD28 domains leads to stronger activation of these lymphocytes. In addition, these lymphocytes appear to be more functional. Furthermore, the results obtained from the coculture experiments show that adding an aCD2 domain to the TCE construct provides a real advantage in CD58-deficient tumors. TCE with an aCD2 domain resulted in stronger activation and greater functionality of T lymphocytes cocultured with CD58-deficient tumor cells. The addition of the aCD2 domain to the TCE construct also resulted in improved cytotoxicity against CD58-tumor cells.

Adding an aCD2 domain to new immunotherapies such as TCEs offers a real advantage in CD58-deficient tumors. Stimulation of T lymphocytes via their CD2 domain makes them more active, more functional, and more cytotoxic.

Mots-Clés: Cancer, Immunotherapy, TCE, T Cell Engagers, CD58

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*Intervenant

Metabolic explorations in depression associated insulin resistance: an experimental study using a chronic unpredictable mild stress model of depression in mice

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Résumé

- **1. Introduction and background**

The association between insulin resistance and depression has been widely reported, but the mechanisms involved remain incompletely understood. The endocannabinoid system (ECS) appears to be a plausible candidate, as alterations in this system have been described in depression, but also in the dysregulation associated with metabolic syndrome. Sodium glucose transporter 2 (SGLT-2) inhibitors also appear to have an effect on cardiometabolic health and mental health.

- **2. Study objectives**

Our study objectives are to develop of a mouse model associating depressive-like symptoms and insulin resistance (through Chronic Unpredictable Mild Stress or CUMS), and to comparatively assess peripheral and central ECS activity between mice exposed to chronic unpredictable mild stress, and controls mice.

- **3. Methods**

We use ten weeks (70 days) old C57BL/6J mice (from 20 to 25 g), and define two groups according to CUMS exposure (28 males and 28 females in the CUMS group, 23 males and females in the control group). Mice are subjected to CUMS for six weeks, which involve the random application of numerous minor-intensity stressors. Weight is monitored weekly and behavioral validation tests (sucrose preference test, splash test, and forced swim test) are performed at the end of the procedure. In CUMS and control mice, we evaluate glucose and

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insulin tolerance, biochemical metabolic parameters, and circulating endocannabinoid levels. After euthanasia, samples are collected (liver, adipose tissue, kidney, brain) to study insulin signaling and ECS receptor (CB1R) activity.

- **4. Preliminary results**

We have currently enrolled 18 CUMS mice (7 males and 11 females) and 18 controls (9 males and females). So far, we have found no differences between CUMS males and controls in glucose tolerance, insulin tolerance, and blood levels of insulin, leptin, ghrelin, plasminogen activator inhibitor-1 (PAI-1), and resistin. However, compared to controls, CUMS females displayed small glucose intolerance, a marked insulin resistance associated with higher circulating levels of insulin, PAI-1, and resistin.

- **5. Conclusion and outlook** Our preliminary results, which must be interpreted with caution due to the small sample size, tend to show higher levels of insulin resistance in CUMS females but not in males. A third cohort is underway, and complementary analyses (GIP, GLP-1, glucagon, circulating cannabinoids, CB1R expression, insulin signaling) are planned. Once our mouse model linking depressive-like symptoms to insulin resistance is validated, we will test the efficacy of ECS modulators and SGLT-2 inhibitors in this model.

Mots-Clés: Depression, Chronic Unpredictable Mild Strees, Insulin resistance, Endocannbinoid system.

Better understanding of off-notes issues in plant-protein-based dessert

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Résumé

The individual demand for protein has been growing significantly all over the world since the 19th century. Since then, not only has the amount of protein consumed per person per day increased, but the protein intake profile has also changed significantly. Most notably, between the 19th and 20th centuries, the consumption of livestock-originated-products grew significantly faster than any other protein source. At the same time, the world's population has been increasing, growing from a mere 1 billion to more than 8 billion in 2025, with forecasts predicting around 9.7 billion people by 2050. The combination of this increase in average per capita protein consumption and the rapid rise in global population, has highlighted the necessity of reducing animal protein consumption to address the sustainability issues of our current production and consumption model, with the added benefit of potential health improvements.
Among plants, legumes are a great protein source, also containing dietary fiber and oligo-elements. Conveniently, they are easy to grow, require a fraction of the water needed to raise cattle, and are capable of fixing atmospheric nitrogen, which they then release through multiple mechanisms thus reducing the need for fertilizing agents. Despite these nutritional and environmental advantages, their consumption remains low in France due to the off-notes associated with them, namely a beany-like aroma and bitter taste.

The AlInoVeg project aims to develop plant-based products using pea or faba bean as the primary protein source. As part of this project, this thesis work focuses on gaining a better understanding of the off-notes present in a custard based on Pea Protein Isolate (PPI).

To achieve this objective, the thesis project includes the following steps:

1. SAFE extraction on PPI to review its volatile profile using GC-MS analysis and identification of active odorants with GC-O.

2. Sensory analysis (QDA) on a model unflavoured cream dessert with a trained panel.

3. Liking test with an untrained panel of approximately 100 participants.

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4. Recombination study to identify key odorants.

5. Development of masking strategies targeting key odorants, including both odor and taste to explore both intra- and inter-modal interactions.

6. Evaluation of the relative efficiency of these strategies on products developed by our industrial partners.
The research investigates perceptual interactions that can take place within complex volatile mixtures and their possible cross-modal effects (e.g. enhancement or reduction of bitterness), to support the development of effective masking strategies tailored to this type of plant-based dessert.

Mots-Clés: Plant, protein, off, notes, sensory perception

Assessment of sugar beet vinasse to improve trace element phytoextraction by Poplar (Populus maximowiczii x Populus nigra) from polluted soil

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Résumé

Soil is essential to all life forms on earth, as it is vital to produce plant biomass, which is necessary for animal and human nutrition. However, more than 60% of the world's soil is degraded today, 41% of which is attributable to human activity (ADEME 2020 ; IPBES 2018). Today, one of the major sources of pollution is industrial pollution. The pollutants most often found in soils are trace elements (TE), including zinc (Zn) (European Environment Agency 2023). It is essential to all forms of life, but in excess, it becomes toxic and has an impact on the health of all organisms (Bartzatt 2017). Phytoextraction can be used to limit soil pollution. One of the most commonly used plant species is the poplar, as it can establish easily on marginal lands, in different climatic conditions, and is fast-growing (Castiglione et al. 2009 ; Ciadamidaro et al. 2019). Extraction efficiency can be improved by adding amendments that modify the mobility and bioavailability of the ET.
The first part of this study was focused on selecting an amendment that would improve the mobility and bioavailability of Zn in polluted soil. Five amendments were applied to the soil, including sulphur, activated carbon, compost, sugar beet vinasse, and citric acid. The pH of the pore waters and their Zn concentration were monitored over twelve weeks. The second part of this study was focused on evaluating the potential of sugar beet vinasse to improve the phytoextraction of poplar. The vinasse was applied to pots, each containing a poplar cutting, in one or two applications. The pH and Zn concentration of the pore water and soil were monitored, as well as stem size and chlorophyll content.

The first experiment identified sugar beet vinasse as the amendment that mobilises Zn on the same scale as sulphur, which rapidly becomes toxic to plants (20 mM.kg-1). The second experiment is currently showing that sugar beet vinasse still mobilises Zn, especially in the presence of plants. In addition, the second application increases the mobilisation of Zn, at

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least in the short term. However, it also shows that vinasse is not inoffensive for poplars, as its application slows down their growth and causes foliar damage.

Current results suggest that sugar beet vinasse is a good candidate for increasing the mobility and bioavailability of Zn in soil. Further analyses are currently underway to determine how to improve the phytoextraction of poplar.

Mots-Clés: Phytoextraction, Trace elements, Populus sp., Amendment, Pollution

sEV-PD-L1: a promising new circulating biomarker to monitor duodeno-pancreatic neuroendrocrine tumors (DPNET) in PRODIGE 31-REMINET cohort

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Résumé

There is a critical need for reliable biomarkers enable to monitor neuroendocrine tumors and refine therapeutic strategies. Chromogranin A (CgA) and other biomarkers used in current clinical practice lack specificity, making computed tomography (CT) and magnetic resonance imaging (MRI) the primary methods for disease monitoring. However, those methods can miss small pancreatic or gastrointestinal lesions and can be costly. New markers must be explored, among which immunosuppressive molecules the like of PD-L1 and HSP70. Our study concentrates on markers found in liquid biopsies obtained through simple blood collection.
In that prospect, we conducted a retrospective multicentric clinical study to determine whether the levels of PD-L1 and HSP70 in sEV (sEV-PD-L1 and sEV-HSP70) isolated form patients' plasma could predict disease progression. Patients form the REMINET clinical studies were treated by placebo or lanreotide.

Plasma samples were drawn at various stages of the disease, allowing to linked results to disease progression. Plasma-EVs where isolated using a precipitation kit. EVs markers were tested by western-blotting to control the isolation. Size and concertation were measured by nanoparticle tracking and transmission electronic microscopy. ELISA assays were performed on isolated EVs and patient's plasma to quantify immunosuppressive molecules.

HSP70 and PD-L1 were identified in DPNET-patient's peripheral blood, as well as sEVs isolated form said blood samples. We show that molecules circulating into the blood or isolated from EVs were of different origins. Eventual linked between the variation of those molecules and disease progression were tested, revealing a significant correlation between levels of sEV-PD-L1 and disease progression in patients treated with lanreotide.

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Circulating sEVs were analyzed and proved to be pertinent in the monitoring of disease progression in DPNET patients. A first promising circulating molecular marker was identified, strongly correlated to cancer progression. Further studies are needed to confirmed sensitivity and specificity of our marker and its potential use as a predictive marker of disease progression in DPNET. Those findings could also contribute to the advancement of rapid and non-invasive diagnostic tests in DPNET.

Mots-Clés: extracellular vesicles, circulating biomarker, neuroendocrine tumor, PD, L1

Effects of drought on growth and bioaccumulation of trace metals by a poplar hybrid (Populus nigra × P. maximowiczii) on contaminated soil

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Résumé

In France, more than 4,100 polluted sites have been identified. With the implementation of the "zero net artificialisation" law, pressure to reuse industrial wastelands is high, and their rehabilitation has become an environmental and societal priority. Less costly and less environmentally damaging than soil extraction, nature-based solutions such as phytoextraction (extraction of contaminants by plants) are the subject of numerous projects. This study is part of the Horizon Europe project EDAPHOS, which aims to restore polluted soils and ensure that the processes developed can be integrated into a commercial offer. The aim is to determine the effect of drought, a recurring constraint linked to climate change, on the capacity of young poplar cuttings to grow and accumulate trace metals (TEs) such as lead (Pb), cadmium (Cd) and zinc (Zn). Poplar hybrid cuttings (Populus nigra × P. maximowiczii) selected for their growth and accumulation capacities were grown under controlled conditions on soil from the Alliaire parc (Vieux Charmont, Doubs, France),former industrial wasteland used as one of the project's workshop sites, contaminated with lead, cadmium and zinc, for 62 days under normal soil hydration conditions or drought conditions. The results obtained show that drought, although visible on certain parameters such as stomatal conductance and poplar biomass, does not appear to have had any effect on cadmium and lead accumulation (respectively 1 and 0. 7 μg/g respectively). On the other hand, it seems that the drought experienced by the poplars reduced the bioaccumulation of zinc (620 μg/g for the drought and 707μg/g for the controls). The effect of this drought on the bioaccumulation of trace metals will therefore be discussed.

Mots-Clés: Phytoremediation, trace element, poplar, drought

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*Intervenant

Development of a tool to obtain peptides responsible for oxidative stability from grape must

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Résumé

Introduction

The oxidative stability of wines refers to their intrinsic ability to preserve organoleptic qualities during aging and bottle storage. When compromised, it can lead to aroma degradation. A significant contribution of peptidic nitro-sulfur compounds (containing nitrogen and sulfur atoms) to the antioxidant metabolome has led to the hypothesis that this metabolome is defined during grape ripening and extracted from the must during pressing.

These peptides are obtained from grape juice through a two-step process. The first step involves the purification of major grape-derived proteins, primarily thaumatin-like proteins and chitinases. These proteins are then enzymatically digested under optimized experimental conditions (temperature, buffer, time). Various biochemical techniques are applied to assess the efficiency of each step, both qualitatively and quantitatively.

Materials and Methods

Grape juice was centrifuged prior to purification. Protein purification began with a chromatoflash pre-purification step using silica cartridges, Followed by fast protein liquid chromatography (FPLC) with a cation exchange resin to further purify and isolate proteins from other compounds. The purified proteins were then concentrated using a SpeedVac evaporator.

Protein digestion was performed using pepsin and Aspergillopepsin I under two different conditions: incubation at 37◦C for 24 hours in an oven, and incubation in an oil bath at 70◦C for 40–60 minutes.

The efficiency of each step was evaluated using gel electrophoresis for qualitative analysis, and size-exclusion chromatography (SEC) coupled with multi-angle light scattering (MALS) for quantitative analysis.

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Results

Each purification step successfully concentrated and isolated proteins from the must, as indicated by intense bands in gel electrophoresis and strong signals in SEC-MALS. These results confirm successful protein purification.

Following digestion, based on previous results, the bands and signals corresponding to these proteins decreased or disappeared, indicating progressive protein breakdown and peptide formation.

Conclusion

The development of this method has enabled the extraction of peptides from grape juice proteins and the establishment of a reference peptide pool. The next phase will involve the characterization of these peptides using various analytical techniques to determine whether they contain the compounds responsible for oxidative stability.

Mots-Clés: purification, enzymatic digestion, biochemistry, oxidative stability

State of the art of natural hydrogen: A path toward a new sustainable energy in Bourgogne Franche-Comté.

The modern world is highly dependant of energy for industry, agriculture, transportation, residential life, mainly in the form of electric and thermal energy. These energies are mostly produced by fossil resources which release greenhouse gases. To acting against global warming, we need to developpe numerous renewable energies and restrict gas emissions.

Hydrogen is one of the promising candidates which emerged to meet this demand as it can be formed continuously through various natural processes. Hydrogen use is also attractive due to its lack of greenhouse gas emissions when burned, low operational cost and the absence of post-operation treatment. Since the discovery of natural hydrogen gas field in Bourakébougou, Mali, in 2012, numerous research projects have been launched to better understand hydrogen generation, transport and storage through geological settings. These studies have shown that most natural hydrogen is produced through water-rocks interaction processes such as serpentinization (up to 0.25 mol/kg) and ferrous iron alteration (up to 0.038 mol/kg). However, other mechanisms are also of interest for achieving viable hydrogen production including water radiolysis, rocks crushing, thermal alteration of organic matter and the outgassing of primitive hydrogen.

The Bourgogne Franche-Comté region presents an interesting opportunity for exploring natural hydrogen by its geological heterogeneity. Geology of the region is composed by old crystalline rocks in the Morvan, stemming from the Pangea formation and controlled by depth fault. Post-orogenic extension has led to the creation of intracontinental sedimentary basins rich in organic matter. During Jurassic, continental drift created an ocean and led the deposition of marine sediments (carbonate and marls). During Oligocene, after a major marine regression, an extensional phase led to the expansion of a rift and the formation of the Bressan grabben. During Miocene, the recovery of alpine compression has succeeded to the formation of the Jura Mountains.

To establish the regional hydrogen potential, a bibliographic approach is needed to understand hydrogen system and to apply this knowledge to the regional geology. By this, we can edit a regional map to reference geological complexes that are likely to produce hydrogen, call a favourability map. To complete this first step, soil and subsurface gas emission will be measured throughout the region to identify a few promising sites, with sufficient hydrogen flow for its use.

My PhD aim (1) to understand the processes of hydrogen formation and storage in Bourgogne Franche-Comté and (2) propose a common methodology for exploring hydrogen in different geological systems.

State of the art of natural hydrogen: A path toward a new sustainable energy in Bourgogne Franche-Comté.

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Résumé

The modern world is highly dependant of energy for industry, agriculture, transportation, residential life, mainly in the form of electric and thermal energy. These energies are mostly produced by fossil resources which release greenhouse gases. To acting against global warming, we need to developpe numerous renewable energies and restrict gas emissions.
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*Intervenant

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Mots-Clés: Natural Hydrogen, Regionnal geology, Sustainable Energy