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Plenary Lecture Abstracts

Advanced Metaproteomic Approaches to Investigate Diet-host-microbiota Interactions

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Abstract

Metaproteomics is the large-scale identification and quantification of proteins from microbial communities and thus provides direct insight into the phenotypes of microorganisms on the molecular level. Initially metaproteomics was mainly used to assess the "expressed" metabolism and physiology of microbial community members. However, we and others have increased the range of questions that we can address with metaproteomics by developing new approaches that allow us to (1) quantify species biomass contributions to determine community structure, (2) determine in situ carbon sources of community members, and (3) determine the uptake of labeled substrates by community members.

I will briefly discuss some recent advances in my laboratory that further increase the capabilities of metaproteomics. These include approaches for ultra-low input metaproteomics, and for the detection of small proteins and peptides from microbiomes, which will allow detection of critical proteins that are usually lost during sample preparation.

I will present 2 of our recent studies that rely on metaproteomic approaches. In the first study we investigated the effect of different dietary protein sources on the gut microbiota in mice. We found that different sources of dietary protein had major impacts on the composition and function of the gut microbiota and that functional shifts were largely driven by the metabolism of glycan side chains of dietary proteins. Amino acid metabolism also differed significantly between sources of proteins potentially leading to different beneficial or detrimental end products of amino acid degradation. In the second study we used stable isotope fingerprinting with metaproteomics (Protein-SIF) to link different components of the diet to the microbial species in the mouse gut that consume them. We were able to link specific microbial groups to their diet derived substrates. Additionally, we found rapid uptake and rerelease of dietary protein by the mouse host into the gut environment leading to rapid changes in isotope signatures of known host-foraging microorganisms.

*Speaker

Metaproteome Plasticity: Cause or Effect?

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Abstract

Microbial proteomes reveal how microbes sense and adapt to their environment, exhibiting remarkable plasticity at both individual and community levels. This plasticity often manifests as checkerboard distribution patterns. In this talk, I will discuss potential ecological drivers behind such patterns, including niche partitioning, competitive dynamics, and functional redundancy.information levels. Metrics and resulting information will be presented for each of these areas.

*Speaker

Unlocking Marine Microbial Dynamics: Metaproteomic approaches to tackle global pollution

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Abstract

Marine microorganisms are pivotal to oceanic ecosystems and biogeochemical processes but are often overlooked in climate change studies and policy development. Understanding the complex structure and function of microbial communities is crucial for accurately predicting the impacts of global change and pollution. Unlike traditional studies that focus on taxonomic structure and genetic potential, our metaproteomics studies enable us to examine protein regulation, linking genotype to phenotype. This approach deepens our understanding of microbial contributions to biogeochemical cycles, their dynamics, and their responses to multiple stressors.

Over the past decade, our team has developed and applied an environmental metaproteomic workflow to understand the functioning of aquatic microorganisms, from short-term regulation in response to the day/night cycle to long-term exposure to various pollutants across the globe. Our metaproteomics approach has centred on assessing the impact of a vast range of pollutants affecting marine ecosystems globally, including the Deepwater Horizon oil spill in the Gulf of Mexico, UV filters in coastal environments of the Mediterranean and Thailand, and the impact of dyes from the textile industry in India and plastic pollution in Southeast Asia. The diversity of gathered datasets has provided valuable insights into how various pollutants impact microbial dynamics and the resilience of microbial communities across diverse geographical contexts.

After introducing the challenges specific to environmental metaproteomics and the experimental workflow we use, the presentation will highlight the state of the art in ocean dynamics, identify gaps in the literature, and emphasise the novelty of this approach, along with the wealth of data we've collected from numerous studies conducted across different locations and time scales. I will then focus on two critical environmental threats: oil spills and plastic pollution, examining their effects on marine microbial communities and how new molecular approaches can enhance our understanding of microbial roles in pollution mitigation and their resilience to multiple stressors.

In the first case study, we assessed the impact of the chemical dispersant Corexit® EC9500A during the Deepwater Horizon oil spill, the largest in U.S. history, with about 4.9 million barrels of crude oil released into the Gulf of Mexico. Despite the role of Corexit in enhancing oil dispersion by reducing surface tension, its interaction with microbial communities remains controversial. Our metaproteomics approach provides the first molecular evidence that dispersants can intensify stress responses in marine bacteria more significantly than the oil itself within 24 hours. This insight helps refine oil spill models and identify key proteins for potential bioremediation, which could reduce reliance on chemical dispersants and lower clean-up costs.

The second case study explores plastic pollution in different locations, an escalating issue with profound ecological and socioeconomic impacts. Marine microorganisms rapidly colonise plastic surfaces, significantly affecting the fate and risks of these pollutants in various geographical locations. Our research sheds light on microbial dynamics on marine plastic surfaces, noting that different climates influence the activity of key microorganisms such as photosynthetic or hydrocarbonoclastic taxa. By integrating both published and unpublished data, we present the latest advancements and future directions to decipher the functional regulation within the so-called marine plastisphere and the surrounding water column.

The outcomes of these recent case studies, widely covered in numerous UK media outlets, demonstrate the power of metaproteomics in uncovering microbial regulation and adaptation, offering new approaches to tackle pollution and multiple stressors. Our objective is to enhance marine microbial ecology using cutting-edge metaproteomic tools, which are crucial for a deeper functional understanding in the context of climate and environmental changes. This will ultimately guide the development of sustainable strategies to effectively mitigate pollution challenges, providing direct evidence that can inform new regulatory policies aimed at protecting marine ecosystems.

*Speaker

Uncovering Microbial Mysteries: Novel Peptide Sequencing to explore Ecosystems Across Time and Space

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Abstract

Despite their important role in maintaining ecosystem health, environmental microbiomes still contain many under sequenced and/or unknown prokaryotes. Importantly, the full extent of their ecological contributions remains difficult to quantify or model. Due to the dynamic nature of marine environments that directly influence marine microbiome taxonomy and function, it is additionally difficult to fully grasp how these microbiomes vary across space and time, and which units of measure of space and time are relevant on ecosystem scales. Peptide-level analysis of marine metaproteomic data helps to resolve some of the complexity around studying such a diverse system. Through the use of the lowest common ancestor approach in assigning peptide identities (e.g., metaGOmics), we have characterized full community functional contributions to marine systems. We have applied this approach to diverse questions, from understanding microbiome impacts on nutrient cycling in the Arctic Ocean, to water quality in a commercial shellfish hatchery, to monitoring the microbiome in order to forecast the initiation of harmful algal blooms. To capture the diversity in taxonomy and function across these systems, we have previously shown that an accurate interpretation of a metaproteome requires a time- and location-specific metagenome. However, with complex microbiomes, such as those found in the environment, a fully sequenced metagenome drastically increases the metaproteomic search space, thereby testing too many hypotheses and negatively impacting the false discovery rate. This realization has led us to explore the application of de novo sequencing tools to create more time- and cost-effective search databases for our metaproteomics pipelines. These developing pipelines, applied to a large time course coinciding with an algal bloom and to a sea ice microbiome from Antarctica, suggest the potential to increase the depth and breadth of metaproteomics results through creation of highly specific databases and potentially obviating the need for costly metagenomes if thresholds for confidence in protein inference and annotation can be met.

*Speaker

Pushing the Envelope of Metaproteomics for More Comprehensive Characterization of the Human Microbiome in Disease

Robert Hettich*[1], Megan Elliott[1], Julia Ortiz-Sunyer[2] Samantha Peters[1], Steven Tavis[1], Jong Kim[1], Tuesday Lowndes[2], Oskar Hickl[2], Cedric Christian Laczny[2], Patrick May[2], Paul Wilmes[2]

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Abstract

Metaproteomics holds great potential for the molecular characterization of the context-dependent metabolic activities of microbiomes but has lagged behind metagenomics and metatranscriptomics in terms of active deployment for general microbiome research. Although untargeted global metaproteomic measurements reveal intricate molecular details of the functional dynamics of microbiome changes, the depth of proteome measurement and subsequent metabolic pathway insights have been somewhat limited. Efforts to address these issues have spawned vigorous research in the metaproteomic community and has driven the formation and activities of organized groups such as the IMS.

A collaborative project between the University of Luxembourg and Oak Ridge National Lab focuses on the deployment of a multi-omics approach to characterize a fairly large cohort study of the human microbiome in the context of health vs. Rheumatoid Arthritis or Parkinson's Disease. To date, we have completed ~360 deep metaproteome measurements and database searches (with matched metagenomes), yielding tens of thousands of protein identifications. This systematic and expansive dataset provided the perfect opportunity for us to explore three potential areas to deepen the information content present in metaproteomes: 1) better detection and identification of small open reading frames (smORFs), which are usually missed or ignored in most measurements, 2) more expansive evaluation of differential proteins of unknown function (PUFs), and 3) expansion into new high-performance mass spectrometry platforms. These areas have the potential to greatly enhance the depth and quality of metaproteome characterization and thereby potentially propel metaproteomics into the realm of previously inaccessible information levels. Metrics and resulting information will be presented for each of these areas.

*Speaker

Minisymposium Abstracts

Assessment of DIA methods for metaproteomics - Challenges and opportunities

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Abstract

Metaproteomics has historically relied on data-dependent acquisition (DDA) methods for proteins identification and quantification and suffered some inherent limitations such as lack of depth, difficulties with the control of the false discovery rate and low reproducibility across runs. Recently, the metaproteomic field has started to employ data-independent acquisition (DIA) methods with the hope to mitigate these DDA limitations. However, DIA methods also come with their own drawbacks such as the very high complexity of the spectra and heavy computational burden which limits the usability of DIA for metaproteomics and users have tried different approaches to circumvent those issues.
Recently, we have produced a ground truth metaproteomic dataset with known gene expression differences between multiple conditions and levels of challenge for proteins identification and quantification. The dataset comprised several mixes, acquired in quadruplicate using DDA methods, and consisted of a complex matrix to which multiple different species were added at different ratios from different growing conditions.
Here, we have re-acquired this dataset with a DIA method and have used this new acquisition to assess different approaches applied recently in metaproteomics. I will present these methods, their advantages and limitations, and discuss the usability of DIA methods for complex metaproteomic data.

Keywords: DIA, Bioinformatics, Statistics

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FASTA Lake: Multi-Omics Framework Reveals Host-Microbe Dynamics in Acute Decompensation of Cirrhosis

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Abstract

Acute decompensation (AD) of cirrhosis marks the transition from compensated to decompensated disease and is characterized by sudden complications, including bacterial infection, hepatic encephalopathy, and ascites. This condition involves moderate systemic inflammation with a 90-day mortality rate of 10%. When AD progresses to acute-on-chronic liver failure (ACLF)-characterized by multi-organ failure and heightened inflammation-the 90-day mortality rate escalates dramatically to 53.7%. Microbial interactions along the gut-liver axis are hypothesized to drive this progression.
To explore these host-microbe interactions, we employed a mass spectrometry-based multi-omics approach, allowing analysis across evolutionary kingdoms. Our "FASTA Lake" method uses sample-specific metagenome-derived FASTA files when available and supplements these with metaproteomics-derived FASTA files for samples lacking metagenome data. High-quality de novo peptide identifications were used to filter sample-specific databases, which were later merged to create a consolidated, study-specific FASTA Lake. This allows for longitudinal analyses by retaining proteins identified in samples from later visits, maximizing protein sequence coverage. Cross-study protein sequence headers facilitate robust protein-based comparisons in a multi-omics setting.

This novel metaproteomics framework captures functional profiles from both microbial and host perspectives, employing EggNOG for annotated sequences and DeepFRI for functional predictions of unannotated sequences. Our approach leverages multi-omics synergy to uncover mechanisms underpinning cirrhosis progression, differentiating AD from ACLF. This now allows us to provide mechanistic insights underpinned by genotypic data.

*Speaker
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Keywords: Multi, Omics, Liver, Gut, Liver, Axis

An ultra-sensitive Metaproteomic solution (uMetaP) redefines the "dark" microbiome, reaches single-bacterium resolution and discovers Host-Microbiota interactions during metabolic injury in-vivo.

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Abstract

Metaproteomics (MetaP) has emerged as a powerful tool for investigating host-microbiome functional interactions. However, classical MetaP methods are limited when analysing the complex peptide landscape and the vast dynamic range presented in microbiome samples. Novel solutions significantly increasing sensitivity, profiling depth, throughput, and quantification accuracy are urgently needed.
Our previous work introduced the benefits of DIA-PASEF and deep neural network-based analysis. We now present uMetaP, an ultra-sensitive metaproteomic solution combining cutting-edge mass spectrometry technologies and a novel machine-learning-based de novo module (NovoMP). NovoMP uses a de novo algorithm uniquely trained in the four dimensions of PASEF data and incorporates a multi-tier quality filtering pipeline offering peptide confidence similar to the ones obtained by classical database search engines.

Querying the mouse gut microbiome, uMetaP identified 210,000 microbial peptides and 119,000 protein groups, with 32,400 and 48,000 enabled by NovoMP, respectively. Reflecting improved sensitivity, uMetaP detected an average of 200 microbial and 76 host protein groups at an ultra-low sample amount of 10 pg. It also achieved single-bacterium resolution (500 femtograms) with exceptional quantification precision and accuracy.

Taxonomically, uMetaP annotated 825 species (367 enabled by NovoMP) and redefined the "dark" metaproteome by improving 5,000 times the previous lower limits of detection and quantification. Functionally, uMetaP enables the study of thousands of functional pathways covering 24 COG categories.

Using unique conditional transgenic and gnotobiotic mice models of intestinal metabolic injury, uMetaP paralleled and extended metagenomic findings underlying the disease phenotype. Moreover, we found functional meaningful perturbations in the host and microbiota, which provided a list of potential protein targets for disease intervention.
From deciphering the interplay of billions of microorganisms with the host to exploring microbial heterogeneity, uMetaP will open new avenues for our understanding of the microbial world and its connection to health and disease.

*Speaker

Keywords: Ultra, sensitivity, denovo, "dark", metaproteome, single, bacterium, resolution, metabolic injury

Advancing de novo metaproteomics with Kaiko 2.0.

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Abstract

Metaproteomics analysis using a matched metagenome database for search has remained the state-of-the-art for protein identification for several years. De novo peptide sequencing methods are considerably more challenging but enable peptide identification without a matched genome database. To facilitate downstream analysis in metaproteomics, we previously developed Kaiko, a tool for building a species-appropriate protein database for a given metaproteomic sample. Kaiko used a convolutional neural network, which predicted peptides de novo that were then aligned against the proteins from UniRef100. From these alignments, Kaiko successfully estimated species composition, a result we published in 2022 (https://doi.org/10.1021/acs.jproteome.2c00334).
Here we present Kaiko 2.0, an improvement on our previous pipeline, which uses a transformer-based architecture for its de novo peptide predictions. Transformers excel in learning short and long-distance relationships in structured data, and are thus ideal for mass spectra. To incorporate these advances into Kaiko, we chose Casanovo for our transformer architecture, and we trained a model from scratch using spectra from a diverse set of over 140 species, halving the peptide error rate observed with Kaiko. Kaiko 2.0 uses the _˜24000 reference proteomes in UniProt, eliminating confounding factors in estimating species composition when building the database.
In a matter of hours, Kaiko 2.0 produces a species appropriate FASTA database for a diverse metaproteomic sample. To use Kaiko 2.0, only docker and the Kaiko 2.0 database files are needed, which can be found on our GitHub page (https://github.com/microbiomedata/kaiko_metaproteome), along with detailed usage information.

Keywords: de novo sequencing, deep learning model, metaproteomics

*Speaker

Characterization of extracellular vesicles of intestinal microbiota using metaproteomics in patients with Crohn's disease.

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Abstract

Recent studies have indicated differences in the composition of the intestinal virome associated with chronic inflammatory bowel diseases (IBD), including Crohn's disease. The role of membrane vesicles secreted by both bacteria and human cells in the context of intestinal inflammation has also been recognized. The nanometric fraction of the microbiota, specifically extracellular vesicles (Evs) and viruses, is believed to play a crucial role in intestinal inflammation.
We have developed a technique for separating Evs and viruses from the intestinal microbiota based on their density and enhanced techniques for quantifying viruses and Evs in stools.
We have characterized EVs in the intestinal microbiota of both healthy subjects and Crohn's disease patients. Metaproteomics has been conducted on a timsTOF Pro (Bruker) coupled to nanoElute chromatography. Three-step interrogation of the databases IGC 10.4, Homo sapiens Swiss-Prot-TrEMBL, virus and contaminants was performed using using X!Tandem search engine. The grouping of proteins into protein subgroups was done using i2MassChroQ (http ://pappso.inrae.fr) based on the principle of parsimony. To compare protein number and abundance between clinical groups, we implemented a Wilcoxon test with Benjamini–Hochberg stepwise adjustment for comparison between clinical groups.

5% of proteins in Crohn's disease are human proteins and most of them are part of exosome, confirming the quality of the Evs preparation. In Crohn's conditions, new bacteria appear, such as Blautia compared to the Healthy's condition. Genera Megamonas and Fusobacterium show increased abundance in Crohn's conditions. Bacteroides have decreased in Crohn's condition. The Blastocystis genus is five times less abundant and Prevotella genus is twice times lower in the Crohn's conditions compared to Healthy conditions. In summary, the study employs advanced techniques to investigate the role of EVs and viruses in Crohn's disease, shedding light on potential mechanisms underlying microbiome disturbances in the hope of informing future therapeutic approaches.

Keywords: extracellular vesicles_ Crohn _metaproteomic

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Creating microbiome-model harmony between metaproteomics data and the ADM1da model for a two-step anaerobic digester

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Abstract

Effective operation, planning, and optimization of renewable energy production in anaerobic digestion (AD) plants rely on advanced process models, such as the Anaerobic Digestion Model No. 1 (ADM1). This study applies an ADM1-based model to a two-step digester in an industrial setting, revealing through simulations that 2.6% of methane is lost due to open hydrolysis, while the inclusion of a hydrolysis fermenter generally boosts methane production by 2.5%. Although ADM1-like models are widely recognized for accurately representing anaerobic digestion processes, their mechanistic insights into the microbiome have been limited by the absence of tools to analyze microbial composition and functionality at the time these models were developed.
To overcome this limitation, we utilized metaproteomics and metagenomics to assess the abundance and activity of microbial groups as defined by the model, aiming to bridge the gap between microbial ecology and bioprocess engineering in AD systems. We also developed and tested various methods to link specific microbial species to the model's functional groups.

Our analysis shows that while the model reflects stable microbiome composition in the main fermenter, it struggles to capture the dynamic behavior observed in the hydrolysis fermenter. Furthermore, the actual AD microbiome exhibits greater versatility than the model assumes, with microorganisms performing multiple functions rather than being restricted to single roles.
In conclusion, this study highlights the potential for improving AD models by integrating detailed biological knowledge, ultimately enhancing the performance and optimization of AD processes.

Keywords: Anaerobic digestion model 1, metaproteomics, modelling, microbiome, biogas plant

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Metaproteomics as a contributor to understanding rumen microbiome patterns

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Abstract

Improving sustainability is a major challenge for modern livestock production. One potential approach is mitigating methane emissions through rumen microbiome modulation. Performing such modifications in an efficient and reproducible manner requires a deeper understanding of the interactions between the host and the microorganisms inhabiting the rumen. While rapid progress has been made to characterize the bacterial and archaeal populations residing in the rumen, insight into how they interact with keystone protozoal species remains elusive. We performed a comprehensive multi-omic analysis of a controlled experiment of 24 beef cattle representing two different breeds using long-read metagenomics, (meta)transcriptomics, (meta)proteomics, and targeted and untargeted metabolomics. Our comparisons revealed two distinct rumen microbial community types that surprisingly were not strongly associated with breed or any animal performance trait, but linked to protozoal activity based on metatranscriptomic and metaproteomic data. The results from the two omics were complementary, supporting the same overall pattern, but also capturing different signals, with e.g. metatranscriptomics highlighting a difference in *Polyplastron multivesiculatum* abundance, and metaproteomics in *Isotricha* and *Entodinium* spp. Our results further suggested that the dominant *Epidinium* spp. in animals with community type B employ a plethora of fiber-degrading enzymes that present enriched *Prevotella* spp. a favorable carbon landscape to forage upon. Conversely, animals with community type A, dominated by genera *Isotricha* and *Entodinium*, harbor a more even distribution of fiber, protein, and amino acid fermenters, reflected by higher detection of metabolites from both protozoal and bacterial metabolism. In addition to important implications for future development of microbiome-based technologies, our results showcase the strength of multi-layered high-throughput molecular data, including metaproteomics, for interpreting patterns in complex host-microbiome systems.

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Keywords: rumen microbiome, multiomics, protozoa

Spirochetes in marine invertebrates: adversaries or allies?

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Abstract

Spirochete bacteria are often associated with animals, and the most well studied species are pathogens that cause a variety of mammalian diseases. Very little is known about spirochetes associated with marine hosts, and whether these associations are harmful or beneficial. A few examples of spirocheteal symbionts in marine animals - including gastropods, sponges, polychaetes, and oligochaetes - have been reported but the metabolic niche they inhabit in these hosts has not been studied in detail. We recently discovered a clade of spirochetes that is regularly present in the microbiome of gutless marine worms (oligochaetes). The dominant members of the microbiome of these small marine annelids are sulfur-oxidizing and sulfate-reducing bacteria that provide them with nutrition and recycle their waste products. To understand the role the spirochetes play in these symbioses, we analyzed metagenomes and metaproteomes and optimized a double extraction method to generate paired metaproteomes and bulk metabolomes from single host animals. Our metagenomic, metaproteomic, and metabolomic analyses revealed that the spirochetes ferment carbohydrates (including disaccharides) to acetate and hydrogen. These end products can be used by both the sulfur-oxidizing and sulfate-reducing symbionts of the host, thus contributing to the overall efficiency of the symbiosis. With this export of carbon and energy sources to neighboring bacteria as well as the lack of evidence for harmful effects to their hosts, we conclude that these spirochetes fill a mutualistic role within this symbiosis. Exploring the mechanisms of the mutualistic associations between spirochetes and other marine hosts will expand our understanding of these ubiquitous bacteria and how they interact with their partners, beyond pathogenicity.

Keywords: spirochetes, symbiosis, marine, metaproteomics, metabolomics

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Metaproteomics to investigate host-microbiota responses in a non-model sentinel species to environmental factors

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Abstract

The gut microbiota is critical for understanding contaminant fate, biotransformation, and toxicity in host organisms. Sentinel species like the amphipod *Gammarus fossarum* are widely used to assess chemical contamination impacts in rivers, yet little is known about the gut microbiota dynamics of *G. fossarum*.
To explore these phenomena, we combined metaproteomics and proteogenomics approaches to characterize host-microbiota responses to environmental factors in this non-model species. Our approach employs a multi-step search strategy and a tailored, sample-specific database, enabling us to overcome both the lack of host genomic data and the low-abundance microbial signal.

An initial study conducted under normal, contaminant-free conditions validated this approach by measuring taxonomic and functional changes in the gut microbiota, as well as host responses, across different diets. Our findings provide a comprehensive view of the gut microbial community, as well as the metabolic pathways and molecular processes involved in short-term dietary responses. A notable discovery is the role of foodborne microorganisms, which remain viable post-ingestion and contribute to food digestion. While functional stability was observed across diets, a protein-rich diet triggered adaptive shifts in both host and microbial functions.
Following this validation, we sampled eight wild populations of gammarids from rivers with varying cadmium pollution levels to investigate the role of the microbiota in host tolerance to this metal. Unexpectedly, inter-population comparisons revealed a homogeneous taxonomic and functional profile for the gut microbiota of gammarids, suggesting that cadmium tolerance may involve subtle molecular mechanisms.

Keywords: Proteogenomics, sentinel species, gut microbiota, diet, cadmium, ecotoxicology, environment

*Speaker

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Discovery of plastic degrading enzymes from the mealworm microbiome using metaproteomics and meta-chemoproteomics

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Abstract

Several species of insect larvae have emerged as potential solutions to plastic degradation, and mealworms (*Tenebrio molitor*) are of particular interest due to their ability to degrade multiple types of plastic. Plastic metabolism is facilitated by their gut microbiota, but the mechanisms behind this process remain unknown. If determined, the microbes or their enzymes can be used for efficient plastic metabolism. We synthesized a panel of chemical probes that mirror the plastics polyethylene, polystyrene, and polypropylene. The probes allow for the selective enrichment of proteins that bind to and likely metabolize these plastics. Probes also overcome significant challenges associated with very poor annotation of the mealworm microbiome. Proteins labeled by the probes can then be enriched and characterized by meta-chemoproteomics. Ultimately, this approach offers a way to reduce the dimensionality of proteomics data and elucidate target proteins that may not be detected using a global proteomic approach. Our focus has initially been on PE. Using global metaproteomics and meta-chemoproteomics, we identified novel proteins involved in polyethylene degradation. We also explored serine hydrolases that may be involved in secondary metabolism of plastics. Our data returned redox enzymes such as catalase and peroxidase that likely make initial modifications to the polymer chain, as well as hydrolases such as alpha-amylase, which is known to have activity against polyethylene. We also see proteins like apolipophorin which are involved in lipid transport. The identified proteins have low homology to currently annotated proteins and may consequently have poorly characterized functions, with some of our protein hits having no related proteins with annotation. These hits represent proteins that are likely understudied or undetected in global proteomic analysis. In summary, this approach helps to overcome the challenges of relative abundance and data dimensionality normally associated with bottom-up proteomics to identify proteins related to a function of interest.

Keywords: Chemoproteomics, Plastic, Holo, omics

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Temporal dynamics of bacterial species and CAZYmes involved in lignocellulose degradation by microbial consortia enriched from cow rumen and termite gut

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Abstract

Selectively enriched microbial consortia are potentially useful for the conversion of lignocellulose (LC) into biofuels and commodity chemicals. Microbial consortia are of interest to elucidate the roles of individual microorganisms and the dynamics of enzymes involved in LC deconstruction. Using metaproteomics, 16S rRNA gene amplicon sequencing and multivariate discriminant analysis, we revealed the temporal dynamics of microbial species and their CAZymes during anaerobic conversion of LC by microbial consortia derived from cow rumen (RWS) and termite gut (TWS) microbiomes. Bacteroidetes (Bacteroidota), Firmicutes (Bacillota) and Proteobacteria (Pseudomonadota) phyla were dominant, irrespective the inoculum origin, displaying functional complementarities. We identified a large variety of carbohydrate-active enzymes, distributed in 94 CAZy families, involved in biomass deconstruction. Additionally, proteins involved in short chain fatty acids biosynthesis were detected. Multivariate analysis clearly differentiates RWS and TWS metaproteomes, with differences originating in the initial inoculates. Further supervised discriminant analysis of the temporal succession of CAZymes revealed that both consortia consume easily accessible oligosaccharides during the early stage of incubation, degrading more complex hemicellulose and cellulose fractions at later stages, an action that pursues throughout the incubation period. Our results provide new insights regarding the functional roles and complementarities existing in lignocellulolytic consortia and highlight their potential for biorefinery applications.

Keywords: Lignocellulose bioconversion, anaerobic consortium, carboxylate production, CAZyme, metaproteomics, termite gut, cow rumen

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Metagenome-centric metaproteomic profiling of microbial dynamics in a full-scale biogas plant fed fish-ensilage

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Abstract

Accounting for more than 1.5 million tonnes annually, Norway is the world's largest producer of Atlantic Salmon. Marine fish residues represent a substantial side-stream from fish farming, in the form of fish ensilage. Fish ensilage is rich in lipids and proteins, making it a suitable substrate for biogas production. Biogas plants rely on the microbial process of anaerobic digestion for degradation of different organic wastes for production of methane and fertilizers. The microbial community driving anaerobic digestion represents a diverse mixture of anaerobic bacteria and methanogenic archaea, working symbiotically to ferment organic substrates into methane, which can serve as renewable energy source. Continued advances in meta-omic approaches, such as metagenomics and metaproteomics, increase our understanding of microbiomes. Moreover, integration of multiple meta-omics techniques provides detailed profiles of specific microbes present in a given environment and reveal their metabolic mechanisms. Here, we sampled a commercial full-scale biogas plant that treated fish residues once a month for a year (2020-2021). Process data like temperature, pH, volatile fatty acid concentrations and gas production were monitored. Through (meta)genome-centric metaproteomic, we connect and elucidate the relationship between physiochemical variables and changes in microbial composition and functions. In total, we recovered 188 high-quality metagenome-assembled genomes, which we applied as a sample-specific protein sequence database to recover > 6000 unique proteins. Metaproteomic analysis of the microbial community indicates stable methane production, while mechanisms for methane production vary across the year sampled, suggesting a dynamic adaptation to changes in substrate availability. This has potential implications not just for methane production, but for important microbial populations and key metabolic processes related to stability and efficiency of the reactor. By shedding light on complex microbial dynamics, our study contributes to valuable insights to the development of sustainable energy solutions, demonstrating how marine byproducts can be efficiently harnessed for renewable energy generation.

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Keywords: Anaerobic digestion, biogas, integrated meta, omics, fish, fish ensilage

Microbial interactions in a high-performance tubular foam-bed reactor for biomethanation

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Abstract

Biomethanation can be used to convert excess electrical energy into storable biomethane. Recently, a lab-scale high-performance tubular foam-bed reactor (TFBR) was developed to increase gas transfer which is a major limitation of this process. The addition of foam-stabilizing detergents to a microbial community (MC) consisting of methanogenic archaea and associated bacteria is thought to induce stress and trigger adaptation within the MC. To investigate this adaptation, the MC of the TFBR was analyzed, focusing on both structural and functional characteristics. Combined metagenomics and metaproteomics were applied to analyze the MC of TFBR.

The MC consisted of three archaeal MAGs assigned to Methanobactericeae (40%) and 60 % bacterial MAGs, predominantly Thiopseudomonas (12 %), Alkaligenes (5%), Natroninco-lacaea (5%), Massilibacterium (4%), Petrimonas (3%). The presence of bacteria, such as Petrimonas, feeding on archaeal biomass was observed in the stirred tank reactor for biomethanation and was further supported by the detection of extracellular hydrolases and proteases. However, the high abundance of Thiopseudomonas and Alkaligenes was surprising. Highly expressed proteins involved in oxidative phosphorylation, including complex IV of respiratory chain, implement metabolism using O2 as electron acceptor. The unexpected presence of O2 was confirmed by high expression of enzymes responsible for detoxifying reactive oxygen species (ROS) in most of the members of the MC. Although H2 produced by PEM electrolysis is considered to be very pure, the high abundance Thiopseudomonas and Alkaligenes clearly indicated a continuous contamination with O2. The oxidative metabolism Thiopseudomonas and Alkaligenes scavenged most O2 protecting the strict anaerobic archaea from damage by ROS.

The oxidative metabolism of Thiopseudomonas and Alkaligenes protected the methanogenic archaea from damage by ROS by scavenging O2 from H2 supply. This process might be crucial for process stability when operating biomethanation as an industrial process.

*Speaker

Keywords: technical environment, biomethanation reactor, methanogenesis, oxidative stress, metagenomics

Profiling the Mushroom Microbiome: Impacts on Developmental and Disease Outcomes through Devome Manipulating

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Abstract

The impact of microorganisms as partners in host developmental crop phenotypes is a burgeoning field of study. The term 'devome' (developmental microbiome) describes microbiomes that are necessary for or contribute to the development of a host organism. Cultivation of *Agaricus bisporus* involves changes in the compost and casing microbiome that are crucial for productive mushroom development. The casing layer hosts beneficial bacteria that are essential for this development. We compositionally and functionally characterized the casing devome using integrated metagenomics and metaproteomics. Casing material was manipulated by collecting the substrate at the point of pinning (mushroom primordia initiation) and adding it to fresh standard casing material (ratio 1:10). The passaged casing triggered early pinning of mushrooms and demonstrated disease suppression to a common commercial disease, bacterial blotch. High quality, nearly complete genome-resolved metaproteomics was derived from a database constructed from 229 metagenome-assembled genomes (MAGs). Greater biomass contributions were identified from key genera in passaged casing, including *Pseudomonas*, *Flavobacterium*, and *Brevundimonas*. Biological processes related to monoatomic ion transport, transmembrane transport and cell adhesion were highly represented among taxa. A differential protein abundance analysis revealed that nearly 10% of the identified protein biomass was related to TonB receptors, whose role in casing is previously unknown. Taken together, manipulating the casing devome offers advantages in shorter cropping cycles and suppression of blotch. This work aims to identify the compositional and functional role of a microbial cohort from passaged casing with potential commercial benefits. While our understanding of devomes remains in its early stages, their intersection with microbiome sciences and developmental biology provides new prospects for the field of crop production.

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Keywords: Mycology, Soil Microbiome, Developmental Microbiome, Disease Dynamics, Mushroom Science, Agricultural Microbiomes

Metaproteomic profiling of the secretome of a granular biofilm forming enrichment

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Abstract

Microbes in nature are often embedded in self-produced extracellular matrices known as biofilms, which provide protection and enhance survival in competitive environments (1). Extracellular proteins play critical roles in the formation and structure of these biofilms and aggregates. However, identifying and characterizing these proteins remains challenging due to their specific properties and the complexity of biofilms, leaving many questions about their specific contributions to the formation of biofilm unanswered (2).
We present a metaproteomic study designed to identify the extracellular metaproteome of a *Ca.* Accumulibacter enrichment, which serves as a proxy for granular biofilms commonly found in biological wastewater treatment (2,3). We performed metaproteomics on the cell culture medium combined with limited proteolysis of intact granular biofilms, followed by functional and physicochemical classification based on properties such as charge, hydrophobicity, and beta-sheet content. This approach identified over 1200 secreted and extracellular proteins, of which a large fraction originated from multiple organisms and exhibited physicochemical characteristics suggesting involvement in aggregate formation, including filamentous, beta-barrel, and cell surface proteins (3).

This study enhances our understanding of proteins involved in the formation and structure of granular biofilms, thereby providing valuable insights for applications in engineering, medicine, and environmental management.

References

(1) Philipp, L. A., Bühler, K., Ulber, R., & Gescher, J. (2024). Beneficial applications of biofilms. Nature Reviews Microbiology, 22(5), 276-290.

(2) Aqeel, H., Weissbrodt, D. G., Cerruti, M., Wolfaardt, G. M., Wilén, B. M., & Liss, S. N. (2019). Drivers of bioaggregation from flocs to biofilms and granular sludge. Environmental Science-Water Research & Technology, 5(12), 2072-2089.
(3) van Olst, B., Eerden, S.A., Estok, N.A., Roy S., Abbas, B., Lin Y., Loosdrecht, M.C.M, Pabst, M. (2024). Metaproteomic profiling of the secretome of a granule-forming Ca. Accumulibacter enrichment. bioRxiv.

Keywords: Secretome, biofilms, aggregate forming proteins, enrichment culture

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Sample preparation and 4D-Omics approaches for metaproteomics

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Abstract

In this presentation, we will highlight the use and benefits of some available industrial solutions for metaproteomic analysis. More specifically, we will cover sample preparation (homogenization, protein extraction, purification and digestion), data collection (highlighting new acquisition modes, allowing for DeNovo sequencing while maintaining optimal data completeness) and data processing (use of a real-time De Novo approach)

*Speaker

The Peptonizer2000: Confident Taxonomic Insights, Now Just a Click Away!

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Abstract

The accurate taxonomic identification of microbial communities remains challenging in metaproteomics due to the complex nature of peptide data and the limitations of traditional analysis methods. We introduce an enhanced version of the Peptonizer2000, a novel tool that integrates peptide scoring from any proteomic search engine with taxonomic data from Unipept. The Peptonizer2000 employs Bayesian modeling to generate high-confidence taxonomic identifications by calculating probabilities for microbial taxa presence, thus providing more reliable results than conventional methods based solely on the counting of peptide-spectrum matches.

In this latest version, the Peptonizer2000 has been adapted to run directly in a web browser, eliminating the need for local installation and making it accessible across platforms. The browser-based interface is designed for user-friendly interaction, offering streamlined data input, parameter configuration, and visualization. These features allow users to conduct metaproteomic analysis without requiring extensive bioinformatics expertise.

We demonstrate the performance of the Peptonizer2000 web tool using publicly available datasets, revealing its capacity to deliver high-resolution taxonomic identifications with robust confidence scores in a user-friendly way. This user-centric design, combined with a robust statistical framework, makes the Peptonizer2000 an accessible and powerful tool for advancing taxonomic analysis in metaproteomics. By enabling efficient analysis through a web interface, the Peptonizer2000 broadens access to reliable microbial identification, thus supporting microbiome research across diverse scientific domains.

Keywords: bioinformatics, Unipept, bayesian statistics, taxonomic identification

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Unipept in 2024: Expanding Metaproteomics Analysis with Support for Missed Cleavages, Semi-Tryptic and Non-Tryptic Peptides

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Abstract

Unipept (https://doi.org/10.1021/acs.jproteome.8b00716), a pioneering tool in metaproteomics, has significantly advanced the analysis of complex ecosystems by facilitating both taxonomic and functional insights from environmental samples. Initially, Unipept's capabilities focused on tryptic peptides, utilizing the predictability and consistency of trypsin digestion to efficiently construct a protein reference database. However, the evolving landscape of proteomics and emerging fields like immunopeptidomics necessitate a more versatile approach that extends beyond the analysis of tryptic peptides.
In metaproteomics, samples are typically prepared using trypsin which cleaves proteins at specific cleavage sites (https://doi.org/10.1038/s41467-021-27542-8). Occasionally, trypsin skips a cleavage site, resulting in fragments composed of multiple tryptic peptides, referred to as peptides with missed cleavages. Semi-tryptic peptides, produced when one terminus is generated by trypsin cleavage and the other is not, were previously undetectable by Unipept. Accurately identifying semi-tryptic peptides is crucial for understanding the complex proteolytic activities of the gut microbiome (https://doi.org/10.1186/s40168-020-00967-x).

Unipept's limitations to match only tryptic peptides constrains its utility in the use-cases described earlier. Over the past year, we have integrated a sophisticated index structure from the field of metagenomics, the Sparse Suffix Array (SSA), to accommodate for these shortcomings and improve Unipept's capability in identifying substrings in large text bodies efficiently.

Unipept Next is the next-generation of Unipept, capable of analyzing peptides regardless of how their originating proteins were cleaved. Utilizing an Enhanced Suffix Array, Unipept Next can identify the proteins from which a peptide originates in milliseconds, enabling rapid taxonomic and functional analysis of protein samples.

The latest iteration of Unipept is now integrated with the Peptonizer2000, a tool aiming

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to bring confidence to taxonomic analyses in metaproteomics. By integrating both tools we enable seamless peptide analysis within Unipept, optimizing workflow efficiency for researchers.

Keywords: taxonomic analysis, non, tryptic, missed cleavage, peptonizer, taxonomic analysis, confidence score

MetaPepView: a web interface for visualization and evaluation of metaproteomic data

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Abstract

Numerous tools are available for processing proteomics data. However, there is a lack of user-friendly platforms that offer interactive visual analysis of community microbiomes and allow easy evaluation of metaproteomic performance parameters. For example, metaproteomics reports often do not include key performance factors such as sample complexity, MS spectral quality, completeness of reference protein databases, and validation of database search results. Here, we showcase MetaPepView, a user-friendly dashboard interface designed for visualization and quality evaluation of metaproteomic data. The tool can be flexibly integrated into various metaproteomic workflows. For example, MetaPepView can process database search results and *de novo* sequencing outputs directly from the freely available tools SearchGUI and DeNovoGUI, as well as from other common software suites like PEAKS, MaxQuant and Proteome Discoverer. The microbiome visualization pipeline extends metaproteomics data with taxonomic (from NCBI or GTDB) and functional classifications (from KEGG or EggNOG) to provide interactive community insights across multiple experiments. The quality evaluation pipeline provides useful metaproteomic quality indicators, including benchmarking against an experimental reference dataset. Moreover, the interface supports reporting using the standard mzQC file format established by the Quality Control working group of the Human Proteome Organization. MetaPepView is packaged as a Docker image for easy setup on any local desktop platform.

Keywords: MetaPepView, Metaproteomics quality, Data visualization, Bioinformatics, Dashboard

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Evaluation of statistical approaches for differential metaproteomics

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Abstract

There is a long-standing debate about which statistical methods are suitable for discerning differentially abundant protein groups in metaproteomics samples. While statistical methods used for proteomics have been employed in metaproteomics, it is unclear whether they work as expected in these complex samples. Challenges of statistics in metaproteomics datasets include data sparsity, compositionality and variability (biological as well as caused by sample preparation and analysis). Various processing steps, including a multitude of possibilities for imputation, normalization, and transformation, are attempting to address these challenges. This creates a complex matrix of analysis options, which will then impact the results of statistical tests. An in-depth, ground-truth based method evaluation and comparison is as yet lacking. To address this, we generated a set of metaproteomics samples with known composition and abundance changes of organisms. These samples consist of 13 mixes: Into three different complex metaproteomic matrices (mouse faeces, plant, and gnotobiotic mouse faeces), we added a total of five different pro- and eukaryotic pure cultures in different ratios. Four of the five strains were grown under two different conditions, inducing differential protein abundance and adding an additional layer of complexity. These artificial microbiomes address, beyond the general challenges outlined above, various scenarios in metaproteomics data analysis and statistics, including: (i) small (but biologically relevant) protein group abundance changes, (ii) very large abundance changes, (iii) mis-identifications or mis-assignments of protein groups for different species. We compared regression-based tools, general statistics inference methods (e.g., t-test), and machine learning techniques. We show that some regression-based approaches provide a good balance between false positives and false negatives, while machine learning techniques are well suited for data exploration under relaxed assumptions.

Keywords: regression, machine learning, random forest, differential abundance, statistical inference

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MetaLab Platform Enables Comprehensive DDA and DIA Metaproteomics Analysis

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Abstract

Microbial communities are intricate ecosystems that play vital roles in nutrient cycling, disease resistance, and ecological stability. Metaproteomics, which examines protein expression within these communities, provides deep insights into their metabolic functions, interactions, and adaptive mechanisms. Despite advancements in mass spectrometry-based techniques like Data-Dependent Acquisition (DDA) and Data-Independent Acquisition (DIA), challenges remain. DDA offers high accuracy but may miss low-abundance proteins, while DIA captures a broader protein spectrum but demands robust computational methods for precise data interpretation.

This paper presents enhanced versions of MetaLab-MAG and MetaLab-DIA, tools optimized for comprehensive metaproteomic analysis. MetaLab-MAG now supports timsTOF data, utilizing improved ion mobility and sensitivity, and features a novel scoring system to boost genome-level identification accuracy. MetaLab-DIA, tailored for DIA data, uses a neural network model to predict peptide candidates from prior quantitative data, facilitating efficient library construction for DIA searches. This approach significantly increases peptide identifications, nearly doubling those achievable in DDA mode across various datasets.

The MetaLab suite thus offers a versatile, efficient solution for metaproteomic studies, enabling comprehensive, high-resolution analysis across diverse mass spectrometry platforms. By integrating advanced data analysis techniques, MetaLab addresses key challenges in microbial community proteomics, empowering researchers to explore microbial dynamics with unprecedented detail and reliability.

Keywords: gut microbiome, mass spectrometry, DIA, software

*Speaker

Increased insight into metaproteomics data obtained by advanced bioinformatics tools and modeling

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Abstract

Metaproteomics provides a robust approach for in-depth microbiome analysis, critical for advancing our understanding of human health, environmental ecosystems, and biotechnological applications. Recent advances in experimental workflows and mass spectrometry have greatly enhanced the resolution of metaproteomics, allowing for more extensive protein identification and delivering a richer perspective on microbial phenotypes. Researchers leverage these protein datasets to reconstruct the taxonomic and functional composition of various microbiomes, perform statistical analyses to detect significant variations, and map these insights onto metabolic pathways to uncover underlying biological mechanisms.
To progress beyond traditional microbiome characterization, innovative computational and modeling tools are essential. Building on the MetaProteomeAnalyzer-Cloud for mass spectrometry-based metaproteomics data analysis (accessible at https://mdoa-tools.bi.denbi.de/home), we have developed additional prototypes, such as Metaforge for metaproteomics metadata management, specialized tools for research data handling, and frameworks for integrating proteomics data into modeling workflows. Moreover, we are harnessing the potential of knowledge graphs to enhance metaproteomics data analysis. Knowledge graphs bring unique advantages, including the ability to identify links between microbiomes, proteins, diseases, and drugs, and to apply graph machine learning for detecting novel patterns within complex datasets.
In summary, the development of tailored metaproteomics software is driving forward microbiome research across health, environmental, and biotechnological domains, providing deeper insights into complex microbial communities and their functional dynamics.

Keywords: Bioinformatics, Modeling, Knowledge graphs, Software development

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Metadata in metaproteomics: integrating MIxS into SDRF-Proteomics

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Abstract

Sharing data and resources has brought new opportunities to the life sciences, enabling deeper insights and discoveries. However, the lack of detailed metadata often limits the utility of publicly available datasets. In metaproteomics, advancements in sample preparation, instrumentation, and data analysis have pushed microbiome research forward, but challenges with metadata management and standardization persist, as shown in the CAMPI benchmark study. The Sample and Data Relationship Format for Proteomics (SDRF-Proteomics) provides a foundation for metadata organization but does not yet address the specific needs of metaproteomics. By integrating MIxS environmental extensions commonly used in metagenomics, SDRF-Proteomics can be adapted to better describe microbial environments. This pilot project proposes SDRF-Metaproteomics frameworks for ecosystems such as the human gut, soil, and oceans. These tailored standards aim to improve metadata annotation in metaproteomics, making it easier to align with multi-omics approaches and advance microbiome research across different environments.

Keywords: metadata, SDRF, metaproteomics, standardization

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Meta-analysis of fecal metaproteomic datasets reveals robust human and microbial signatures for inflammatory bowel disease

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Abstract

Recent advances have deepened our understanding of the relationship between the gut microbiome, gut immune system, and inflammatory bowel disease (IBD). However, the role of protein biomarkers in IBD diagnosis and prognosis remains insufficiently studied. In this work, we developed a metaproteome-based panel for diagnosing and monitoring IBD, drawing on metaproteomic data from approximately 600 fecal samples, including IBD and non-IBD cases across six independent studies.
Initially, we analyzed 120 samples to identify 59 metaproteins whose variation was disease-related rather than study-dependent. Among these, 10 metaproteins, including clostridial ribosomal proteins and human immunoglobulins, showed significantly different abundances between IBD patients and controls across two independent datasets not used in the discovery phase. Additionally, 23 metaproteins, primarily from human neutrophil vesicles, demonstrated significant changes associated with remission during treatment, suggesting their potential utility for disease monitoring.

Further validation across samples from other disease contexts-such as non-alcoholic steatohepatitis, diabetes, and colorectal cancer-highlighted the need for complex biomarker panels, as no single marker consistently differentiated IBD from all other conditions.
In summary, we identified universal human and microbial metaproteins for IBD diagnosis and monitoring. Our findings underscore the value of meta-analyses in metaproteomics for discovering and validating biomarker panels and for evaluating their disease specificity.

Keywords: Inflammatory Bowel Disease, Clinical Metaproteomics, Meta, Analysis

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The use of probiotics to reduce antibiotic resistance gene carriage in the infant gut

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Abstract

The human microbiome plays a crucial role in infection prevention, as the resident microorganisms can shield their host from harmful pathogens. However, the microbiome also harbors microbes with pathogenic potential. The rise in antibiotic-resistant opportunistic pathogens is a major global challenge in the fight against infections. This issue is particularly pressing for infants, who are among the most vulnerable groups due to their developing immune systems and common exposure to hospitals during birth. Moreover, infants exhibit a relatively higher gut carriage of antibiotic resistance genes (ARGs) than that of adults, even for infants never exposed to antibiotics.

Our research explores the potential of probiotic bacteria in reducing gut colonization with antibiotic-resistant bacteria. As a first step, we characterized the factors that influence the ARG carriage by analyzing metagenomic data from 14 cohorts, covering 3,981 stool samples of 1,270 infants. We identified factors that significantly influenced the gut resistome, including birth mode, gestational age, antibiotic exposure, and geographical location. A gradual decrease of *Escherichia coli* was a key factor contributing to the reduction in ARGs as the infants matured.

Next, we examined metagenomic data from 800 Tanzanian newborns who participated in a clinical trial investigating the potential of probiotic use for infection prevention (ProRIDE). Our initial results indicate that probiotic use reduces colonization by antibiotic-resistant bacteria without raising any safety concerns. In parallel, our group has developed a method for quantitative detection of short-chain fatty acids (SCFA). SCFAs are products of microbial fermentation that are suggested to confer colonization resistance against pathogens. We are currently measuring SCFA levels in the ProRIDE samples. This work aims to clarify whether the observed decrease of drug-resistant bacteria is linked to altered fecal metabolome. The results may support the use of probiotics as part of strategies to combat antimicrobial resistance and related infections in infants.

Keywords: Antimicrobial resistance, Metagenomics, Metabolomics, Infants, Infections, Probiotics

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A new platform to study gut microbiota-derived extracellular vesicles and their impact on the host physiology through a metaproteomics approach

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Abstract

Extracellular vesicles are membrane-embedded particles which are carriers of different molecules released by all kind of cells including gut bacteria. However, an extensive knowledge on how gut microbiota EVs (GM-EVs) affect host physiology is still lacking.
For this purpose, we developed a method to produce, isolate, characterize and assess the biological and physiological functions of GM-EVs from fresh fecal samples. GM-EVs were fully characterized through the analysis of their size and morphology (NTA and SEM), the detection of typical EVs markers (Western Blotting), the analysis of membranes (Lipidomics) and of EVs content and origin (Metaproteomics). Two bacterial stimulation (pH 6 and pH 4.5) were then used to investigate how GM-EVs change and to study their impact on gut-immune, gut-brain and gut-bone axes in vitro and in vivo.

GM-EVs isolated from cultured gut-microbiota maintained size, shape and microbial taxonomy of original fecal EVs. Membrane markers (LTA, hopanoids and LPS) were present, while proteomic analysis showed that GM-EVs proteins are mainly involved in intercellular communication. Metaproteomics showed that the production of GM-EVs is not directly correlated to the abundance of individual bacteria. The stimulation of gut bacteria using different pH levels strongly impacted EVs production, bacterial origin, and their biological and molecular functions.

Moreover, GM-EVs affected the release of TNF-α by THP-1 cells in a bacterial origin-dependent manner, suggesting their involvement in the inflammatory processes and responses. The stimulation of neural cells with GM-EVs of different origin altered cells global protein synthesis rate, suggesting their potential role in the communication between gut and brain. These results were also confirmed in *in vivo* mice model. GM-EVs were also able to

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induce osteogenic differentiation and bone mineralization in vitro.
We demonstrated that the proposed platform is particularly suitable to study gut microbiota-derived EVs, the EVs-microbiome relation and to investigate GM-EVs impact on the host.

Keywords: Gut microbiota, extracellularr vesicles, metaproteomics, gut, brain axis, gut, bone axis, inflammation

Airway microbiota dynamics in response to ETI therapy: a metaproteomic perspective

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Abstract

Cystic fibrosis (CF) is a progressive genetic disease characterized by thick and sticky mucus secretions that cause multisystem complications. Progressive airway damage and chronic lung infections are among the disorder's most common signs and symptoms. The airway microbiota is among the factors that may contribute to symptom exacerbation, treatment outcomes, and infection risk. Current therapies target the cystic fibrosis transmembrane conductance regulator (CFTR), the primary cellular defect in CF. Elexacaftor-Tezacaftor-Ivacaftor (ETI), a novel CFTR-modulating therapy, has demonstrated significant improvements in lung function and symptoms, yet its impact on airway microbiota dynamics remains largely unexplored. This study investigates the effects of ETI on airway microbiota and its relationship with CFTR function restoration and infection dynamics.

We profiled 180 sputum samples from 36 CF patients, collected before and up to 12 months following ETI initiation. Extracted proteins were subjected to deep metaproteomics measurements in DIA mode to thoroughly characterize microbial communities. Raw data were interpreted against a customized database of respiratory tract microorganism genomes, complemented by data obtained from the without a priori proteotyping of CF sputum samples. Taxonomic description was refined with LineageFilter, our new python-based AI software for complex sample proteotyping.

Longitudinal microbiota profiles were integrated with clinical and microbiological data. Microbiota diversity revealed four distinct patient clusters with unique microbiota configurations that could not be explained by clinical parameters alone. Each cluster exhibited a unique microbiota remodeling timeline post-ETI, marked by unique community reorganization and a common significant decline in strictly anaerobic bacteria, suggesting a pivotal role of anaerobes in microbiota restructuring. We will discuss how taxonomical and functional metaproteomic profiles reveal the influence of initial microbiota configurations on community reorganization following ETI. Insights from this study may support the discovery of molecular signatures predictive of long-term therapy efficacy and infection risk, addressing critical challenges in CF management.

Keywords: Metaproteomics, Cystic Fibrosis, microbiota, DIA, proteotyping, machine learning

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Functional biogeography of the colorectal cancer-associated microbiota

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Abstract

Recent studies have provided evidence of a relationship between gut microbiota dynamics and colorectal carcinogenesis. Although feces are typically used as a proxy for the gut microbiota, it remains unclear whether this type of sample can fully represent the original complexity of the intestinal microenvironment, particularly of the mucosa. Additionally, while a few studies have compared the metagenomes of the gut mucosa, lumen, and feces in human subjects, a systematic study based on a metaproteomic approach is still lacking.
In this study, paired tumor-associated mucosa (MT), adjacent normal mucosa (MN), luminal contents (L), and feces (F) were collected from ten patients diagnosed with colorectal cancer and subjected to surgical resection. Protein extraction was carried out using a combination of bead beating, heating, and an SDS-based buffer, while a FASP-based protocol was employed for clean-up and digestion. Peptide mixtures were separated using a 78-min liquid chromatography gradient and analyzed with an Exploris 480 mass spectrometer in data-dependent acquisition mode. Peptide identification and label-free quantification were performed using Proteome Discoverer, with Sequest-HT as the search engine and four sequence databases (including public and custom human gut metagenomes). Unipept and eggNOG-mapper were employed for taxonomic and functional annotation, respectively.

MT samples exhibited lower richness and alpha-diversity values, while PCA revealed two distinct clusters for MT/MN and L/F samples. Differential analysis identified numerous taxa (including the well-known *Fusobacterium*) and functions (including several enzymes involved in amino acid and sugar metabolism) with significantly different abundance between the sample types. Additionally, a considerable number of features were detected in all samples with comparable abundance levels.
In conclusion, we present a biogeographic characterization of the colorectal cancer-associated microbiota, which may assist in elucidating the functional relationships between gut microbes and the tumor microenvironment, as well as the degree of similarity between microbiota-enriched clinical sample types.

Keywords: colorectal cancer, gut microbiota, intestinal mucosa, feces, metaproteomics

*Speaker

Metaproteomic Approach to Detect Key Host and Microbial Peptides from Oral Leukoplakia samples.

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Abstract

Oral leukoplakia is a potentially pre-cancerous condition where thick, white patches develop in the oral cavity. There is a need for risk stratification biomarkers for precancerous oral leukoplakia patients. While host biomarkers are available for detection, there is a need to expand the biomarker panel to improve predictive performance. We have used the latest methods for enrichment of low-abundance proteins from non-invasively collected samples, sensitive mass spectrometry (MS) methods, and bioinformatic analysis to delve deeper into microbial proteome along with host and variant proteins from precancerous patients.
Enrichment of low abundance proteins coupled with DIA-PASEF MS allowed for deeper quantitative analysis of oral host proteome and taxonomic and functional analysis of oral microbiome. Several human and microbial proteins were detected to be differentially abundant in pretreatment and treated samples. Out of the 5894 human proteins quantified across the six replicates, 81 proteins were differentially abundant (31 in treated samples and 23 in pretreatment samples). Proteins associated with coagulation and complement cascade were upregulated and apoptosis and inflammasome pathways were downregulated after treatment. Metaproteomics analysis detected 6830 microbial protein groups, out of which 38 microbial proteins were detected to be differentially abundant amongst treated and untreated samples. Notably, dextransucrase (glycotransferase involved in exopolysaccharide synthesis and promotes biofilm formation) and D-Ala D-Ala carboxypeptidase (involved in peptidoglycan synthesis) from *Streptococcus salivarius* were abundant in treated samples. Alkyl hydroperoxide reductase subunit C (catalytic subunit responsible for the detoxification of reactive oxygen species) from the genus *Veillonella* was found to be abundant in pretreated samples. Taken together we have more than 74 peptides (62 host and 12 microbial peptides) that will be prioritized to generate a biomarker panel which can be later assessed for a) risk stratification in oral leukoplakia and b) deciphering mechanisms for host-microorganism interaction in oral carcinogenesis.

Keywords: Oral Leukoplakia, Host proteins, Microbial proteins, Clinical metaproteomics, Peptide panel

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Metaproteomics Uncovers Distinctively Abundant Metabolic Enzymes that Exhibit Correlation With Response to Cancer Immunotherapy

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Abstract

The gut microbiome plays a crucial role in the host's response to cancer immunotherapy. However, the metagenomic studies that identified the link have been limited in providing insights into the microbiome's activity. For the first time, to the best of our knowledge, we employ a metaproteomic approach to identify differentially abundant proteins and metabolic pathways in stool samples from advanced melanoma patients undergoing immune checkpoint blockade (ICB) therapy. Two patient cohorts, one at the Sheba Medical Center in Israel and the second at the University of Pittsburgh Hospital, were included in the analysis. Patients were classified as ICB responders or non-responders based on the RECIST 1.1 criteria. DNA and protein extractions were performed, and cohort-specific protein databases were generated using shotgun DNA sequencing. Tryptic peptides were labeled with tandem mass tags (TMT) for multiplexed LC-MS3 analysis. Fragpipe was used for quantitative analysis, and statistical analyses were conducted using MSstatsTMT in R. Our study reveals several significant findings. Firstly, we observed that the most abundant bacteria based on DNA data did not necessarily exhibit the highest protein abundance. Secondly, we identified hundreds of differentially abundant microbial and host proteins in responder vs. non-responder stool samples. Distinct expression patterns in bacterial metabolic pathways, including glycolysis, were observed between the microbiomes of responders and non-responders. This observation is biologically intriguing and highlights the strengths of our approach, as metagenomic analyses alone would not be able to identify glycolysis enrichment due to the widespread presence of glycolysis genes in bacteria. Furthermore, we found a robust and significant enrichment of methanogenesis proteins in the microbiomes of non-responders, supported by studies demonstrating the anti-inflammatory properties of methane, which can be detrimental to immunotherapy. We are currently setting up melanoma immunotherapy gnotobiotic mouse models to study the impact of methane and methanogenic archaea on response to immunotherapy.

Keywords: Immunotherapy, Melanoma, Microbiome, Metaproteomics, Methane

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Insights into protein annotation, pathway reconstruction, and species resolved function from the paper "Dietary protein source strongly alters gut microbiota composition and function"

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Abstract

The source of protein in a person's diet affects their total life expectancy. However, the mechanisms by which dietary protein sources differentially impact human health and life expectancy are poorly understood. Dietary choices have major impacts on the composition and function of the intestinal microbiota that ultimately modulate host health. This raises the possibility that health outcomes based on dietary protein sources might be driven by interactions between dietary protein and the gut microbiota. In this study, we determined the effects of seven different sources of dietary protein on the gut microbiota of mice. We applied an integrated metagenomics-metaproteomics approach to simultaneously investigate the effects of these dietary protein sources on the gut microbiota's composition and function.

Different dietary protein sources significantly altered the species composition of the gut microbiota. Yeast and egg-white protein had the greatest effect on the composition of the gut microbiota driven by an increase in the abundance of *Bacteroides thetaiotaomicron*. The abundance of enzymes associated with different broad functional categories also significantly changed due to dietary protein sources. In particular, the abundance of amino acid degrading enzymes increased in the presence of brown rice and egg white protein, while glycoside hydrolases increased in the presence of yeast and egg white protein. The glycoside hydrolases increased in the yeast and egg white protein diets were mostly *B. thetaiotaomicron* enzymes previously associated with the degradation of yeast cell-wall glycoproteins in the case of the yeast diet, and the degradation of mucins in the case of the egg white diet. We validated that *B. thetaiotaomicron* expresses these glycoside hydrolases when grown on mucin, yeast, and egg white protein in vitro.

These results show that the source of dietary protein can alter the composition and function of the gut microbiota through the specific glycosylations present on dietary glycoproteins. Both amino acid degradation and mucin metabolism by the microbiota have been previously linked to playing a role in modulating gut health. Our study is important because it shows that dietary protein sources should be considered, in addition to fiber and fat, when designing diets for a healthy gut microbiome.

*Speaker

Poster Abstracts

Metaproteomic insights into diurnal variability of bacterioplankton communities during a spring phytoplankton bloom in the North Sea

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Abstract

Phytoplankton blooms create a substrate-rich environment that supports the growth of bacterial planktonic heterotrophs. Previously, we studied the dynamics of such bacterioplankton at a long-term ecological research site near the coast of Helgoland Island (North Sea) once a day (e.g. (1, 2)). Therefore, diurnal changes have been out of the scope of these studies.
Here, we present a novel dataset indicating significant differences at the protein level in a semidiurnal analysis. Using metaproteomics, we studied changes in the free-living (0.2-3 μm) bacterial community that occurred between early (7 am) and late (9 pm) sampling over three days. The results highlight the sensitivity, robustness and reproducibility of mass spectrometry-based metaproteomic analyses to assess changes in the activities of the bacterioplankton communities. Our comprehensive metaproteome dataset uncovered significant changes between the early and late samples in the abundances of distinct bacterial protein groups at both, the taxonomic and functional levels. Proteins from genera such as *Ulvibacter_B* and *Aurantivirga* were significantly more abundant in the late samples. Moreover, we identified 422 significantly changed protein groups, including 47 TonB-dependent receptors which can mediate the import of oligosaccharides into the periplasm of Gram-negative polysaccharide-degrading bacteria.

These results highlight semidiurnal changes in bacterial community composition and metabolic activity during a phytoplankton bloom that would have remained undetected with a once-per-day sampling approach. We anticipate that this study will pave the way towards diurnal metaproteome studies (in temperate marine systems) and hope that the presented results will be a useful foundation for researchers.

References:

1. Teeling, H., *et al.* (2012). Substrate-controlled succession of marine bacterioplankton

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populations induced by a phytoplankton bloom. *Science, 336*(6081), 608-611.
2. Wang, F.-Q., *et al.* (2024). Particle-attached bacteria act as gatekeepers in the decomposition of complex phytoplankton polysaccharides. *Microbiome, 12*(1), 32.

Keywords: Aurantivirga, day/night variability, metaproteomics, phytoplankton bloom, Ulvibacter

AI-powered design of increasingly simplified microbial communities

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Abstract

The gut microbiota, a diverse community of bacteria, viruses, archaea, and fungi within the human body, plays a crucial role in maintaining health and influencing disease processes. Advances in omics technologies, particularly metaproteomics, have opened new avenues to explore these microbial populations, providing valuable insights into the functions and interactions. This work leverages metaproteomic data and AI-driven approaches to design simplified microbial consortia that could mimic key traits of the gut microbiota. By using functional insights, we aim to reduce the number of taxa while preserving the community's overall functional diversity. For this purpose, human gut metaproteomic profiles from large cohorts were analyzed to retrieve the functions expressed by the different microbial components. Identified proteins were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) databases, achieving functional annotation for over 70% of the detected proteins. Functional diversity across samples was measured using Rényi entropy metrics and compared with taxonomic composition to assess the influence of specific subset of taxa on the model functional diversity. Machine learning models, including the Gaussian Process Regression, were trained. Results revealed that a subset of 15 microorganisms could recapitulate the functional diversity observed across the analyzed fecal samples. Complementary results were obtained by adapting the Genetic Algorithm to metaproteomics data. This approach showed that about 95% of the observed functional diversity could be reproduced using combinations of 13 microorganisms. Similarly, combinations of as few as 7 microorganisms could capture around 90% of the functional diversity observed across fecal samples. Examples of these simplified consortia will be presented, alongside strategies to predict their functional output under specific cultivation conditions. These tailored, simplified communities of human gut microbiota hold significant importance for advancing our understanding of microbial ecosystems, enabling targeted studies, and catalyzing therapeutic innovations.

Keywords: Microbial communities, Artificial intelligence, Microbiota, Metaproteomics

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Isolation strategies for nitric oxide dismutase (nod) producing bacteria

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Abstract

Nitrous oxide (N2O) is a significant greenhouse gas with a century-long atmospheric lifetime. It has a 300-times greater global warming potential than carbon dioxide and causes the depletion of the ozone layer. Naturally, N2O is mainly produced through microbial denitrification; however, human activities, such as fertilizer use, animal manure, and land cultivation, significantly enhance denitrification and increase N2O emissions. Recent studies have revealed a novel nitrogen metabolic pathway, oxygen-producing denitrification, in which a postulated protein termed nitric oxide dismutase (NOD) directly transforms nitric oxide (NO) into dinitrogen (N2) and oxygen (O2) gas, thereby circumventing the production of N2O. Although several NOD genes have been isolated from diverse environments, limited knowledge exists regarding the bacteria undergoing oxygenic denitrification. Consequently, this study aimed to examine bacteria capable of denitrification under anaerobic conditions and to isolate potential NOD-containing bacteria.

Keywords: Nitric oxide dismutase (NOD), denitrification

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Enhancing proteome analysis of environmental microorganisms: FISH-FACS and label-free quantitative proteomics from ultra-low cell numbers.

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Abstract

Metaproteomics is an essential approach to investigate microbial metabolic activity in diverse environments. However, functional analysis of specific microorganisms is often underexplored by the protein inference problem due to sequence homologies among closely related species. This challenge limits our understanding of the role of particular microbes in complex environmental samples. In this study, we have developed a workflow that combines fluorescence in situ hybridisation (FISH) and fluorescence-activated cell sorting (FACS) with mass spectrometry-based proteomics to analyse proteins from non-culturable bacteria directly from environmental samples. The workflow was first optimised using the culturable model bacterium *Polaribacter* sp. KT25b, isolated from the North Sea. This allowed for assessment of cell number requirements for robust protein identification and quantification following FISH and FACS. We determined that samples containing 100K cells are sufficient for reliable qualitative protein identification, while samples with 500K to 1000K cells enable reproducible protein quantification. Furthermore, the use of a taxon-specific database improves data analysis by significantly reducing the size of protein groups compared to metaproteomics data. Additionally, we tested this workflow to gain deeper insights into the activity of key bacterioplankton species involved in North Sea phytoplankton blooms. Overall, this workflow addresses a major methodological gap, providing a broad application for directly enriching non-culturable microbes from their habitat and enabling downstream proteome analysis.

Keywords: Enrichment, Polaribacter, FISH, FACS, DIA, Spectronaut

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Metaproteomics of the equine stomach: effects of hay, hay-oat diets and pasture feeding

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Abstract

Introduction: The equine stomach is characterized by a demanding interplay of microbial and auto-enzymatic digestion within a complex biochemical environment (1). Metaproteomic analysis can provide insight into digestive functions, dietary effects and microbial interactions. The aim was to characterise the proteome of the equine stomach under different feeding conditions relevant to practice.

Material and Methods: 24 horses were allocated to four diets: hay *ad libitum* (HAY), hay *ad libitum* with oats at 1 or 2 g starch/kg body weight/meal=day (OS1; OS2) or 24h/day pasture (PST) (TVV 63/21). They had free access to water, salt lick and straw bedding within the stable. After $>$ 34 days, the horses were euthanized, dissected and digesta were sampled from *pars nonglandularis* (PNG) and *pars glandularis* (PG) of the stomach. Samples were prepared as previously described (2) and analysed using a timsTOF Pro mass spectrometer.

Results: Proteins of equine (EPs) and microbial (MPs) origin were higher in PNG than PG. Highest levels of dietary plant proteins (PPs) were found in PG. EPs were substantially higher in oat *vs.* forage only diets, while PPs and MPs were particularly high in HAY and PST. MPs mainly belonged to the *Streptococcaceae* and *Lactobacillaceae* families, with lower *Streptococcaceae* proteins in OS2. Horses fed oats showed a higher abundance of keratins in their digesta.

Conclusion: Results suggest that both anatomical region and diet influence protein composition in the equine stomach. Whether elevated EPs and in particular keratins are part of either a problematic or even protective response to starchy feed is subject of ongoing investigations at the functional level.

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(1) Chaucheyras-Durand et al. (2022). Microorganisms 10:2517.
(2) Heyer et al. (2019). Front Microbiol.10:1883.

Keywords: horse, stomach, high starch diet, protein composition, microbiota

The microbiome of a biofilter

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Abstract

Paper production consumes large amounts of water and energy. This can be reduced by using a closed water cycle and producing primary energy by anaerobic water treatment. Besides the paper production the closed water treatment cycle consists of UASB reactors for anaerobic water treatment, a subsequent stripping of carbon dioxide and waste gases, a lime trap and the final treatment of waste air using a trickle bed reactor and a biofilter. The biofilter successfully removes hydrogen sulfide, ammonia and odor from waste gas stream but the removal of methane is incomplete.

The study aims a better understanding of microbial methane removal in the biofilter and to identify key targets for process optimization.

Combined metagenomics and metaproteomics were applied to analyze the microbial community of the biofilter. Extracted microbial were tryptically digested, transferred to nanoHPLC-MS/MS (TimsTOF Pro) and identified by search against a protein sequence database based on corresponding metagenome data.

The combined metagenomics and metaproteomics approach showed a simple microbial community that was dominated by the orders *Rhizobiales* and *Methylococales*. The genus *Methyllocella* accounted for 40 % of the microbial community.

Functional analysis showed enzymes involved in methane oxidations and assimilation. Key enzymes for methane oxidation such as methane monooxygenase and PQQ dependent alcohol dehydrogenase were highly abundant. Formaldehyde was further metabolized via formaldehyde activating enzyme, glycine hydroxymethyltransferase and glycine cleavage system. The presence of malyl-CoA lyase indicated the assimilation of carbon via the serine pathway. The high abundance of a polysaccharide biosynthesis/export protein could be correlated with biofilm formation on the biofilter by *Methyllocella*.

The metaproteome analysis confirmed the microbial degradation of methane on the biofilter. The results could be used as a starting point for process optimization.

[*]Speaker

Modulation of gut microbiota of children with Down syndrome

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Abstract

Down syndrome (DS, OMIM # 190685) is a chromosomal abnormality that results from the trisomy of the 21st chromosome. It is one of the most common chromosomal abnormalities, with an incidence rate of approximately 1–2 per 1,000 live births globally across the world. The condition is characterized by a particular combination of phenotypic features, including cognitive delay and characteristic facies. Previous studies have demonstrated differences in the gut microbiota (GM) of individuals with DS in comparison to healthy controls, both at the metagenomic level and in terms of functional metabolites. Indeed, the GM has been shown to play a vital role in human health, and can be affected by genetic and environmental factors. In this context, we investigated the GM structure in children with DS by a metaproteomic approach.
Stool samples were collected from 48 paediatric patients; bacteria fraction was enriched and proteins were extracted. After enzymatic digestion, performed according to filter-aided sample preparation protocol, nanoLiquid Chromatography-ElectroSpray Ionization-tandem mass spectrometry (nLC-ESI-MS/MS) of obtained peptides was carried out. MS data were analyzed by MetaLab-MAG software to perform both the label-free quantification and the functional annotation analysis. The taxonomic (by LCA) assignments were retrieved by Unipept. The subsequent bioinformatic pipeline was performed by R and Python *ad hoc* scripts developed by our study group.

To the best of our knowledge, this study represents the first investigation of the GM metaproteome of individual with DS. Metaproteomics provided evidence of a T21 GM's distinctive disease related-pattern linked to the different clinical manifestations of DS, such as intellectual disability, behavioral issues, and autoimmune comorbidities, from both a functional and an LCA-derived taxonomic point of view.

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Keywords: Down syndrome, Gut microbiota, pediatrics, MetaLab MAG, Unipept

PathwayPilot: A User-Friendly Tool for Visualizing Metabolic Pathways and Navigating the KEGG Database

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Abstract

Deciphering metabolic pathways is essential for understanding biological processes, yet navigating the extensive Kyoto Encyclopedia of Genes and Genomes (KEGG) database can be complex. Transitioning from identified peptides to actionable insights within these pathways often proves challenging. To overcome this, we introduce **PathwayPilot**, a streamlined and user-friendly web application designed to bridge this gap. PathwayPilot efficiently maps identified peptides or proteins to Enzyme Commission numbers and taxon identifiers, supporting both peptide-centric and protein-centric analyses. Its intuitive interface provides clear visualizations, highlighting relevant proteins within pathways to uncover meaningful insights. Additionally, the tool enables targeted analysis of specific organisms and supports intra- and inter-sample comparisons, empowering researchers to explore metaproteomic data with precision and ease.

Keywords: Meta, omics, metaproteomics, pathways, visualization, bioinformatics, software

*Speaker

Proteome Plasticity in Synthetic Microbial Communities: Insights into Ecosystem Functionality

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Abstract

Microbial communities play pivotal roles in biological and ecological processes, yet our understanding of how microbes perceive their environment within communities remains limited. This gap hinders our ability to fully harness these processes. While it is well established that individual microbial protein expression is influenced by environmental factors, exploring gene expression at the community level could enable the development of predictive models. In this study, we constructed synthetic microbial communities that mimic the phylogeny and functionality of gut microbiomes. Using these models, we analyzed proteomic responses under both limited and rich carbon regimes. Our findings revealed remarkable proteome plasticity at both the individual and community levels, particularly in dense and competitive environments. These analyses uncovered repetitive patterns of protein production that drive ecosystem functionality.

Keywords: Synthetic communities, metaproteomics, varying carbon regimes

*Speaker

Stepping into the Meta-Verse: A Proteomics Platform Perspective on Approaching Metaproteomics

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Abstract

The study of microbiomes is gaining interest for their critical role in health, disease, and the environment. In the context of climate change, reducing greenhouse gas emissions is a major objective. Livestock, particularly ruminants, are significant sources of these emissions due to ruminal fermentation by the microbiota, essential for feed digestion. Over the past decade, metaproteomics has developed significantly, becoming a valuable tool for studying microbiomes. While this technique has matured significantly over the past years, challenges remain, especially in the study of highly complex communities like the rumen microbiome. Therefore, the objective of my PhD project is to develop a metaproteomic approach to support research aimed at enhancing the efficiency of ruminant animals and therefore reducing their carbon footprint.

To explore this emerging technique, resources from www.metaproteomics.org, including tutorials, lectures, and newsletters, were utilized, as well as comprehensive reviews and method chapters. Several valuable resources, such as general metagenomic databases from MGnify, were highly beneficial for studying prokaryotes. Information on metaproteomic-specific techniques, like iterative search strategies and specific sample preparation protocols was accessible and detailed. Additionally, bioinformatic tools such as Unipept, iMetaLab, and others simplified the approach to this complex analytical technique.

However, challenges were encountered when digging deeper into our research topics, particularly related to the complexity of the rumen microbiome. The integration of eukaryotes like protozoa and fungi into databases was laborious, with the first publicly available FASTA file including these species published only recently. Peptide-centric tools for taxonomic and functional analysis often rely on resources with limited information about under-researched microorganisms, hampering their application in studies of highly diverse microbiomes. Furthermore, guidance in LC-MS/MS methods to optimally analyze these complex samples and was limited. With this perspective, we give an overview of our experiences with implementing and employing metaproteomic pipelines in our proteomics laboratory

Keywords: Metaproteomics, Rumen Microbiome, Database, LC, MS/MS methods, Bioinformatics

*Speaker

The National Microbiome Data Collaborative and standardized bioinformatic workflows for FAIR processing of mass spectrometry omics data

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Abstract

The National Microbiome Data Collaborative (NMDC) is a *sustainable data discovery platform* that promotes open science and shared ownership across a broad and diverse community of researchers, funders, publishers, societies, and other collaborators. The NMDC aims to enable multi-omic research to accelerate scientific discovery in environmental microbiome science by connecting data, people, and ideas to create an inclusive and interdisciplinary community. To achieve this goal, we are creating a FAIR (Findable, Accessible, Interoperable, Reuseable) microbiome data sharing network, through infrastructure, data standards, and community building, that addresses pressing challenges in environmental sciences. The NMDC is a U.S. Department of Energy funded program that is a collaboration between the Lawrence Berkeley, Los Alamos, and Pacific Northwest National Laboratories. As part of this effort, we aim to develop metadata-informed, standardized and automated bioinformatics workflows for processing mass spectrometry-based omics data. The goal of these workflows is to provide baseline information regarding the data contents of microbiome datasets and host that information on the NMDC data portal thereby making it findable to the community. To the extent possible, we also seek to make MS data interoperable through standardized processing protocols. Our first platform supports the processing of data dependent acquisition (DDA) proteomics data and is based on the established workflow used by the Environmental and Molecular Sciences Laboratory (EMSL) user facility. We have also begun work on the development of a workflow to enable the processing of data independent acquisition (DIA) metaproteomics data and gas chromatrography-mass spectrometry (GC-MS) metabolomics data.

This presentation will outline the current and in development workflows available in the NMDC with the goal of generating discussion about the challenges, best practices, and value to the community in the development of metadata-informed, standardized bioinformatic tools in the metaproteomics and broader mass spectrometry-based omics research field.

*Speaker

Keywords: FAIR data, Bioinformatics, metadata

Metaproteomics approach to study long COVID microbiome

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Abstract

Nearly 10% of people who recover from COVID-19 develop what is known as long COVID. Long COVID is the development of new symptoms 3 months after the SARS COV-2 infection and lasting at least 4 weeks. Previous research has confirmed a relationship between altered gut microbiota and COVID-19, suggesting a similar association may exist with long COVID. In this study, our main objective was to investigate changes in microbiota composition and function in patients with long COVID using metaproteomics.
A cohort of 100 patients with long COVID and 34 controls was recruited. Fecal and saliva samples were collected from each patient and control. Peptides were analyzed on a timsTOF Pro2 using MSFragger for protein and peptide identification. The IGC and the Human Oral Microbiome Database were used to identify microbial protein in gut and oral samples. Metalab program was used for the taxonomic and functional annotation of gut microbiota samples and the taxonomic analysis of oral samples. The functional analysis of salivary microbiota was performed using the Unipept Desktop application.

We identified 7.437 protein groups, corresponding to 295 species in the gut, and 1.154 protein groups corresponding to 156 species in the oral microbiota. We observed differences in the relative abundance of different taxa between groups in both samples. For example, *Akkermansia* was significantly more abundant in control samples, while members of Proteobacteria were enriched in long COVID saliva samples. Functional analysis of human proteins in saliva revealed interesting results too. The String analysis showed a significant cluster associated with stress response in Long COVID samples.
In conclusion, our findings highlight the importance of studying the microbiota to understand and potentially manage the complexities of long COVID. This research provides valuable insights into the disease that may improve patient outcomes.

Keywords: long COVID, oral microbiota

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