

COMMENTARY

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The Metaproteomics Initiative: a coordinated approach for propelling the functional characterization of microbiomes

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Abstract

Through connecting genomic and metabolic information, metaproteomics is an essential approach for understanding how microbiomes function in space and time. The international metaproteomics community is delighted to announce the launch of the Metaproteomics Initiative (www.metaproteomics.org), the goal of which is to promote dissemination of metaproteomics fundamentals, advancements, and applications through collaborative networking in microbiome research. The Initiative aims to be the central information hub and open meeting place where newcomers and experts interact to communicate, standardize, and accelerate experimental and bioinformatic methodologies in this field. We invite the entire microbiome community to join and discuss potential synergies at the interfaces with other disciplines, and to collectively promote innovative approaches to gain deeper insights into microbiome functions and dynamics.

Keywords: Microbiome, Metaproteomics, Multi-omics, Education, Interactions, Networking

Background

Microbial communities are major drivers of biogeochemical cycles, vital components of the biosphere including plants and animals, and key factors in human health. High-throughput genome sequencing technologies have revealed the immense diversity of microbes and the complexity of the ecological interactions within microbiomes [1]. In the past, deciphering the functioning of these complex microbial systems has been a daunting challenge, but is now becoming more approachable due to recent technological advances that allow diving

much deeper into the functional biomolecular machinery. Metaproteomics is one such emerging approach that is beginning to dramatically advance our understanding of microbiome functioning through analyzing the spatio-temporal expression of microbial genes and dynamics within a microbial consortium.

Proteins are the workhorses of metabolism, homeostasis, cell division, transport of nutrients, cell-cell communication, protein synthesis, and the construction of cellular and extracellular structures. While the proteome defines the set of proteins in a single organism, the metaproteome comprises the complete suite of proteins from the variety of microorganisms present in a sample or ecosystem. As a result, a myriad of proteins, some of which may share relatively similar sequences, should be identified and quantified to acquire phenotypically relevant knowledge of the

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functioning of microbiomes [2]. Importantly, metaproteomic information complements the genetic potential and gene expression information obtained through metagenomics and metatranscriptomics, respectively, by identifying the temporal and spatial abundance of metabolic enzymes and other proteins. Metaproteomics is also highly complementary to metabolomics in explaining the phenotype of biological systems. Importantly, due to sequence variation among metabolic enzymes, metaproteomics data provides insight into “which organism is doing what” in a microbiome ecosystem and enables identification of specific members of the microbiome that are responsible for a change in abundance of a given metabolite. Thus, metaproteomics is pivotally positioned amongst other multi-omics approaches to enable direct genotype-phenotype linkages. In particular, it facilitates the connection between nucleotide information and metabolic outputs. Furthermore, the identification of structural proteins, biofilm protein components, and other extracellular enzymes sets metaproteomics apart from the other omics layers, resulting in unique insights into microbiome functioning. Thereby, it provides robust data for unraveling microbial networks within microbiomes and contributes unique information to fully understand the interactions with their environment [3, 4]. Metaproteomics can capture data across all domains of life, which is crucial for example to understand host-associated ecosystems in which proteins and peptides represent essential molecular currencies of exchange. Hence, metaproteomics “snapshots” at the host-symbiont interfaces are unique in mapping out microbial and host functions that are reciprocally related. Improvements in sample preparation, high-resolution peptide/protein separation and analysis, and refined strategies for data interpretation have propelled metaproteomics dramatically forward in the past decade [4–7]. Indeed, metaproteomics has already helped answer a number of research questions that otherwise would have remained elusive, primarily by functional assessment of microbial communities based on the identification and quantification of taxon-specific peptides [8]. For example, whether polyphenols accumulated in soils are microbially unavailable under anoxia was challenged with metaproteomics on laboratory-controlled reactors [9]; unusual pathways for carbon and energy use were highlighted in *Olavius algarvensis*, a gutless marine worm relying on its bacterial symbionts to cope with nutrient-poor environments [10]; and fecal metaproteomics of patients with metabolic syndrome revealed the level of gut inflammation and the subtle changes in bacterial composition and their metabolism [11].

Introduction of the metaproteome initiative

While metagenomic and metatranscriptomic approaches share a common technical measurement platform that is fairly uniform and standardized, the experimental protocol for metaproteomics is much more broad and under active development. This presents challenges for assessing and comparing results and capabilities. In addition, metaproteomics currently benefits greatly by the sequencing, assembly, and annotation of metagenomes for accurate interpretation of the data. Thus, to propel metaproteomics to an unprecedented level of microbiome functional analyses requires an expanded and concerted stage of development where all possible synergies between analytical scientists, protein biochemists, bioinformaticians, statisticians, microbiologists, and other multi-omics specialists should be exploited. The current positive momentum of metaproteomics is particularly ripe for such a shift in gear. Importantly, the microbiome research community needs knowledge dissemination via improved educational resources so that community members are informed of the possibilities metaproteomics can offer and have an understanding of the realistic status of the field. Ideally, the microbiome and metaproteomics community should have easier access to the most recent updates of methodologies through specialized open platforms equipped with state-of-the-art instrumentation and strong expertise in data analysis. The full impact of active research to further improve metaproteomics in terms of methodology and data analysis will only be achieved if we improve its standards, educate the whole community, and foster new conceptual advances, thus facilitating the applicability of metaproteomics to solve a wide range of key biological questions. Clearly, metaproteomics has now matured sufficiently to be a viable component of experimental design in current and future microbiome research projects.

A growing community of researchers using metaproteomics had the opportunity to meet at several international symposia (Magdeburg, Germany in February 2016, Alghero, Italy, in June 2017, Leipzig, Germany in December 2018, an online edition in June 2021, and Luxembourg City, Luxembourg in September 2021) and launched specific actions such as training sessions and interlaboratory comparisons. Over the past year, members of this community have regularly discussed a variety of subjects, such as the need for a place where newcomers and experts can discuss and initiate new projects, the desire to forge metaproteomics as a critical methodology of choice for functional analyses, and the need to establish and disseminate the best options and standards for this methodology. This has enabled the development of a roadmap in order to assess the possibilities of tackling more complex projects, such as meta-omics projects and

spatio-temporal studies of hundreds of differing conditions involving a large number of samples and replicates to be analyzed at increasing depth and throughput.

This international community, which at the time of writing brings together over 120 members from 48 research groups from 15 countries, has proposed a new initiative under the aegis of the European Proteomics Association (EuPA, <https://eupa.org>), the federation of European national proteomics societies, with a clear mission and vision statement: “the Metaproteomics Initiative promotes dissemination of metaproteomics fundamentals, advancements, and applications through collaborative networking in microbiome research. We aim to be the central information hub and open meeting place where newcomers and experts interact to communicate, standardize, and accelerate experimental and bioinformatic methodologies in this field”. This will be achieved initially through a dedicated website (<https://metaproteomics.org>) and Twitter account (@MetaP_Init), presentations and other educational resources, an online communication channel, inter-laboratory comparisons, and regular symposia. We are delighted to announce that as of February 2021 the Metaproteomics Initiative was officially launched and supported by EuPA. We are confident that this Initiative will benefit from an open structure, facilitating exchanges and interactions with other disciplines, and favouring additional synergies at the interfaces with other omics, data science, and microbiology. Through this commentary, we invite the entire microbiome community to join this Initiative and promote collective innovative and holistic approaches to understand how microbiomes function.

The formulation and execution of a community-driven, multi-lab comparison of established metaproteomic workflows, the “Critical Assessment of MetaProteome Investigation” (CAMPI) study, is the first tangible action of the Metaproteomics Initiative [12]. CAMPI has compared sample preparation, mass spectrometry analysis, and bioinformatics workflows for data analysis. This benchmarking effort has led to findings which have highlighted the importance of each step of the analytical pipeline. Such a benchmarking effort calls for discussion not only on the needs of standardized experimental procedures and data treatment workflows, but also specific guidelines in data reporting and data sharing. The CAMPI effort has also paved the way for subsequent inter-laboratory assays where advanced methodological questions will be tackled by the strength and multiple expertises of the whole community. In this regard, it is clear that the definition of appropriate standardized biological samples or benchmark datasets presents important tasks for the community.

Among the other major activities already initiated by the Metaproteomics Initiative are (i) an inaugural online symposium in June 2021, (ii) its promotion among the microbiome community by means of active communication channels, (iii) gathering tutorials and lectures on metaproteomics for offering simple and free access to high-standard educational resources, and (iv) establishment of a consensual framework to stimulate, initiate and coordinate open working groups conducting projects with defined objectives and outputs. To achieve its objectives, the Metaproteomics Initiative will favor the organization of training courses for newcomers and advanced users, further promote its vision in regions currently under-represented, regularly organize new CAMPI editions open to all volunteers, propose brainstorming sessions and hackathons for solving specific bottlenecks, arrange specific round-tables for improving guidelines, and recommending metrics for high-quality results, and establish a platform where scientists with key scientific questions can meet metaproteomics specialists for smooth and efficient collaboration.

As pointed out above, the benefits of a united and stronger metaproteomics community and its successful insertion at the crossroads of many disciplines are huge. Although under the EuPA umbrella, the Metaproteomics Initiative is not limited to a given geographical area and welcomes all positive ideas and feedback. As the puzzling complexity of microbiome samples has been an important driver of methodological improvements in meta-omic sciences, open challenges in metaproteomics are expected to further stimulate significant advancements in mass spectrometry-based proteome analysis and proteome bioinformatics [12, 13]. Due to the myriad of possible applications, metaproteomic approaches are increasingly used and the field is becoming attractive to young scientists. Indeed, a new generation of scientists trained in one or more of the interrelated areas of meta-omics, data science, modeling, and microbiology is emerging. Engaging, inspiring, and educating early-career researchers are undoubtedly a rewarding prospect for the microbiome community.

Conclusions

Ambitious long-term plans such as the Human Genome Project, the European Green Deal, and the Human Brain Project have pushed the boundaries of science. The multidisciplinary giant Tara Oceans project aimed at deciphering the complexity of ocean life by means of planetary exploration sampling and huge metagenomics efforts is an emblematic model for environmental microbiology [14]. The International Human Microbiome Standards Project is also a pivotal project for improving data quality and optimizing omics comparability in the

human microbiome field. Such long-range frameworks offer challenging objectives for many researchers, cutting-edge research infrastructure to advance methodologies and knowledge, and a visibility that is attractive for stakeholders, media, and the public. The alliance of the Metaproteomics Initiative and other microbiome disciplines could become the cornerstone of such a long-term, cutting-edge project on the comprehensive molecular analysis of microbiome ecosystem functioning and adaptation. Through its unique positioning, we are confident that the Metaproteomics Initiative will contribute to the best possible returns on investment for microbiome research.

Supplementary Information

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Authors' contributions

T.V.D.B, R.L.H, and J.A coordinated development and construction of the manuscript, with input from all co-authors. All co-authors contributed edits and input throughout the manuscript construction, and all have read and approved the final version.

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Competing interests

All authors declare that they have no competing interests.

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References

- Berg G, Rybakova D, Fischer D, Cernava T, Vergès M-CC, Charles T, et al. Microbiome definition re-visited: old concepts and new challenges. *Microbiome*. 2020;8:103.
- Heintz-Buschart A, Wilmes P. Human gut microbiome: function matters. *Trends Microbiol*. 2018;26:563–74.
- Zhang X, Li L, Butcher J, Stintzi A, Figeys D. Advancing functional and translational microbiome research using meta-omics approaches. *Microbiome*. 2019;7:154.
- Schiebenhoefer H, Van Den Bossche T, Fuchs S, Renard BY, Muth T, Martens L. Challenges and promise at the interface of metaproteomics and genomics: an overview of recent progress in metaproteogenomic data analysis. *Expert Rev Proteomics*. 2019;16:375–90.
- Karaduta O, Dvanajscak Z, Zybailov B. Metaproteomics—an advantageous option in studies of host-microbiota interaction. *Microorganisms*. 2021;9 Available from: <https://doi.org/10.3390/microorganisms9050980>.
- Saito MA, Bertrand EM, Duffy ME, Gaylord DA, Held NA, Hervey WJ 4th, et al. Progress and challenges in ocean metaproteomics and proposed best practices for data sharing. *J Proteome Res*. 2019;18:1461–76.
- Issa Isaac N, Philippe D, Nicholas A, Raoult D, Eric C. Metaproteomics of the human gut microbiota: Challenges and contributions to other OMICS. *Clin Mass Spectrom*. Elsevier BV. 2019;14:18–30.
- Kleiner M. Metaproteomics: much more than measuring gene expression in microbial communities. *mSystems*. 2019;4 Available from: <https://doi.org/10.1128/mSystems.00115-19>.
- McGivern BB, Tfaily MM, Borton MA, Kosina SM, Daly RA, Nicora CD, et al. Decrypting bacterial polyphenol metabolism in an anoxic wetland soil. *Nat Commun*. 2021;12:2466.
- Kleiner M, Wentrup C, Lott C, Teeling H, Wetzel S, Young J, et al. Metaproteomics of a gutless marine worm and its symbiotic microbial community reveal unusual pathways for carbon and energy use. *Proc Natl Acad Sci U S A*. 2012;109:E1173–82.
- Biemann R, Buß E, Benndorf D, Lehmann T, Schallert K, Püttker S, et al. Fecal metaproteomics reveals reduced gut inflammation and changed microbial metabolism following lifestyle-induced weight loss. *Biomolecules*. 2021;11 Available from: <https://doi.org/10.3390/biom11050726>.
- Van Den Bossche T, Kunath BJ, Schallert K, Schäpe SS, Abraham PE, Armengaud J, et al. Critical assessment of metaproteome investigation (CAMPI): A multi-lab comparison of established workflows. *bioRxiv*. 2021; Available from: <http://biorxiv.org/lookup/doi/10.1101/2021.03.05.433915>.
- Wilmes P, Heintz-Buschart A, Bond PL. A decade of metaproteomics: where we stand and what the future holds. *Proteomics*. 2015;15:3409–17.
- Sunagawa S, Acinas SG, Bork P, Bowler C, Tara Oceans Coordinators, Evelard D, et al. Tara Oceans: towards global ocean ecosystems biology. *Nat Rev Microbiol*. 2020;18:428–45.

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