WHITE PAPER

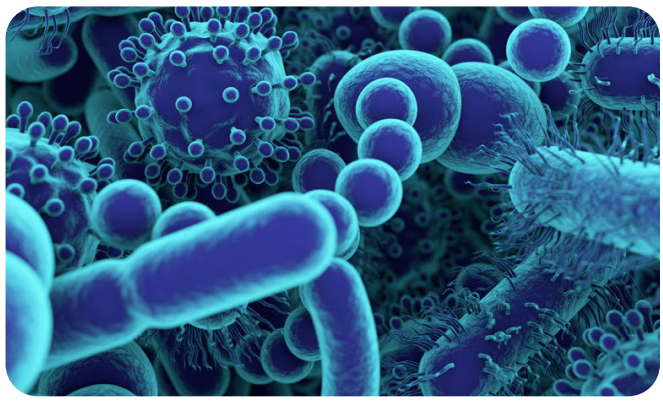
Postbiotics: A Paradigm Shift in Microbiome Research

Advancing our understanding of the microbiome and microbiota-derived metabolites

The human body is inhabited by a diverse and extremely active population of microorganisms known as the microbiota. These organisms exist on the skin and along mucosal surfaces such as the gastrointestinal tract, lungs, and genitourinary system and are critical for preserving homeostasis of the human host. The largest concentration and diversity of microbiota can be found within the human gut, especially in the colon. Here, microorganisms are provided with a stable, nutrient-rich habitat and in return they help stimulate the host's immune system, aid digestion, and provide otherwise unobtainable metabolites.

Several factors can affect the composition of the microbiota, including the host's environment, diet, and antibiotic intake.[1] Age is also a critical factor.[1] While a balanced microbiota plays an important role in maintaining human health, an imbalance in the composition and metabolic capacity of the microbiota can be detrimental. This state, known as dysbiosis, can disrupt gut homeostasis and has been linked to the onset or exacerbation of certain diseases, including Inflammatory Bowel Disease (IBD), Rheumatoid Arthritis (RA), diabetes, obesity, and certain cancers.[2]

Over the past few decades, researchers have attained a deeper understanding of the composition of the microbiota population and host-microbe interactions, especially in the context of human health and disease. There has also been increasing interest in therapeutic strategies and nutritional



preparations that modulate the composition of the gut microbiome. At the University of Milano-Bicocca, researchers in the laboratory of Professor Francesco Peri have been analyzing the mechanisms of microbiota-related functions in health and disease and their potential for clinical application. "We're interested in the dynamic molecular crosstalk between the host and the microbiota which is required to maintain homeostasis or prevent dysbiosis," he explained.

The team has been using high-content imaging and AlphaLISA™ immunoassays to explore the anti-inflammatory effect of molecules produced by microbiota metabolism, known as postbiotics, on human cells. "This is a fascinating new concept in the microbiota field," he enthused. In this article, Prof. Peri discusses the range of interventions currently being explored to reverse dysbiosis in the human gut and the work presently being conducted in the Peri Lab relating to postbiotics.

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Modulating the microbiota

The link between an imbalanced microbiome and the development of several diseases has led many researchers to explore ways to modulate dysbiosis to counteract these pathologies. "One of the most celebrated medical successes in the field is direct Fecal Microbiota Transplantation (FMT)," said Prof. Peri. This approach involves the administration of fecal matter from a donor into the intestinal tract of a recipient in order to change the recipient's gut microbial composition. "This has proved very successful, for example the FMT technique gave a 90% success rate as a treatment for *Clostridium difficile infection,*" he said. However, few long-term studies have been undertaken to assess the safety of FMT and so the theoretical risk of infection remains possible.[3].

An alternative approach to modulate the gut microbiota is through the ingestion of nutrients utilized by host microorganisms, known as prebiotics. Prebiotics are nondigestible compounds that, through their metabolization by microorganisms in the gut, modulate the composition or activity of the gut microbiota and exert a beneficial effect on the health of the host. The most well-known prebiotics are inulin, fructooligosaccharides (FOS), lactulose, and galactooligosaccharides (GOS). Fermentation of these prebiotics by gut microbiota produces short-chain fatty acids (SCFAs), which have a number of beneficial effects, including inflammatory regulation and maintenance of intestinal barrier integrity.[4]

Another way that non-healthy, dysregulated microbiota can be replaced with healthy microbiota is through the administration of probiotics. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host. "The most commonly used probiotic microorganisms are *Lactobacillus* and *Bifidobacterium*, which are important to maintain gut homeostasis," explained Prof. Peri. But despite the health benefits of probiotics, some reports have questioned their effectiveness and safety, especially in high-risk patients. Concerns include the maintenance of viability and stability in the production process, the presence of antibiotic resistance genes that could be transmitted via horizontal gene transfer, and unpredictable strain-specific behaviors.[1,5]

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Postbiotics

Postbiotics include any substance released by or produced through the metabolic activity of the microorganism, which exerts a beneficial effect on the host, directly or indirectly.

The postbiotic revolution

Recently, attention has shifted towards the gut microbiota's production of bioactive metabolites, known as postbiotics, as an alternative clinical approach of microbiota modulation. Examples of postbiotics include SCFAs, microbial cell fractions, extracellular polysaccharides, and cell lysates. "Through the action of several enzymes, the microorganisms composing the gut microbiota have the capacity to perform a series of chemical modifications on natural products present in the diet," explained Prof. Peri. "Sometimes these chemical modifications generate compounds that have better absorption and distribution properties than the parent molecules. These compounds fall in the class of postbiotics, together with the metabolites directly produced by microbiota."

Many potential benefits of postbiotics have been described, including modulation of the resident microbiota, enhancement of epithelial barrier functions, modulation of local and systemic immune responses, and systemic signaling via the nervous system.[6] It has also been suggested that certain metabolites can alter the effect of anti-tumor therapies. "This means the effect of microbiota is not only positive – sometimes it can be negative in the sense that some microorganisms could induce resistance to certain anti-cancer therapies," said Prof. Peri. "It is therefore very important to know about any kind of chemical

transformation made by microbiota on drugs, foods, or natural compounds that enter our body – this is of high interest to us, and a new frontier for medicinal chemistry." Indeed, an appealing perspective is to develop functional foods enriched with these potentiated bioactive metabolites produced by microbiota. Prof. Peri notes that one of the advantages of postbiotics is that they are expected to have a better safety profile than probiotics. "In principle, as postbiotics do not contain live organisms, the risks associated with their intake are minimized," he said. They are also likely to be more stable and have a longer shelf life than live, active probiotics, which makes them rational candidates for use in functional foods.

Understanding the anti-inflammatory effects of urolithin A

The current work of the Peri Lab is focused on validating the anti-inflammatory effect of urolithin A (UA), a natural polyphenol metabolite compound resulting from the transformation of ellagitannins by the gut bacteria. Polyphenols are phytochemicals in foods which are present in abundant levels in fruits and vegetables. Ellagitannin-rich foods have beneficial effects on inflammatory diseases, including IBDs and cancer, however, without the contribution of the microbiota their bioactive components have poor absorption and distribution. "The microbiota digestion to simpler molecules, mainly UA, make these compounds drug-like molecules which could be used to prevent the mentioned diseases," said Prof. Peri.

Valentina Artusa and Ana Rita Franco, both co-workers in the Peri Lab, explained how they are exploring the impact of UA on immune cells, specifically macrophages. "We pretreat macrophage-like cells with UA and then challenge cells with lipopolysaccharides (LPS), a component of the cell wall of gram-negative bacteria which is known to have a proinflammatory effect, " said Artusa. "The aim was to assess what the impact of this pretreatment was." They explored which pathways were activated or inhibited, and the quantity or quality of the proinflammatory mediators that were released upon LPS stimulation in the presence or absence of this metabolite deriving from the microbiota. The team used high-content imaging to study the intracellular pathways involved, as well as AlphaLISA assays to quantify cytokine release. Commenting on the potential of this work, Prof. Peri said: "What has been observed and described for the microbiota-transformed ellagitannins could in the future be extended to a series of other natural compounds, whose biological activity is well documented while it is known that they are poorly absorbed and distributed."

Addressing the challenges of microbiota research

According to Franco, one of the greatest challenges in this area of research is the availability of microbiome-related models. "We need to develop a model that mimics the part of the body we want to study, for example the intestine, and represents how the microbiota digests a certain type of food," she said. "But not everyone digests food in the same way because they don't have the same microorganisms. So, the real challenge is to develop a model that is complex, reproducible, and that can give us reliable answers. Of course, we will never have a model that can be translated to everyone, but ideally we want something that is as uniform as possible."

Another challenge of microbiota studies is the complexity and inter-individual variability in the composition of the microbiome and its response to dietary interventions. "We also face the challenge that the *in vitro* situation is always different from what is happening in the organism," said Prof. Peri. These observations have led to the concept of metabotyping, which is the investigation of the relationship between the metabolic phenotype and the microbiome-derived metabolites that characterize an individual. Metabotyping can be used to group individuals with similar metabolic profiles and is emerging as a key approach for the delivery of personalized nutrition.

Other tools that Prof. Peri predicts will aid microbiota research are computational and artificial intelligence algorithms. He believes such approaches could be used to predict all metabolic transformations by microbiota that could generate potent bioactive compounds from food components, thus providing a powerful tool to predict the beneficial or therapeutic effect of the parent molecules. "We will need very powerful computational tools to take into account all the factors that influence the composition of microbiota and its metabolism in every individual."

Prof. Francesco Peri

Francesco Peri is Full Professor of Organic and Medicinal Chemistry at the Department of Biotechnology and Biosciences, University of Milano-Bicocca. Prof. Peri also has a permanent professorship at the École Normale Supérieure (ENS) de Lyon (France) where he teaches a course of Medicinal Chemistry (Master's level). Prof. Peri's research interests in the field of drug development include small molecular TLR4 modulators, new leads to treat heart failure and new methods for synthesis of antibody-drug conjugates (ADCs). He has published 105 peer-reviewed papers in international journals, totaling over 3150 citations, H-index of 33 (21 since 2014) and an i10-index of 71. Prof. Peri has filed 10 international patents so far and has led the foundation of University for Innovation (U4i, http://www.u4i.it/), an initiative that facilitates the licensing of inventions and scientific innovations. Between 2015 and 2018, he coordinated the H2020-funded MSCA-ETN project TOLLerant ("Toll-Like Receptor 4 activation and function in diseases: an integrated chemical-biology approach", GA number 642157).

Valentina Artusa

Valentina Artusa is a Post-Doctoral Researcher. After a Master's Degree in Plant, Food and Environmental Biotechnology, she joined Prof. Peri Lab for her doctoral training. She received her Ph.D. in Converging Technologies for Biomolecular Systems from University of Milan-Bicocca in 2022. Her investigation mainly relates to Inflammation and Pharmacology, with a particular focus on the characterization of the bioactivity of plant-derived natural compounds. Her publications include the evaluation of the anti-inflammatory and immunomodulatory potential of coffee beans extracts, chlorogenic acid and palmitoylethanolamide.

Ana Rita Franco

Ana Rita Franco is a PhD student in Prof. Peri's lab. Besides her interest and work in microbiome, she is also part of BactiVax ITN network where she focuses on the development of new adjuvants for antibacterial vaccines. She got her integrated masters degree in Pharmaceutical Sciences from the University of Porto in 2019.

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