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*Comitato Nazionale per la Biosicurezza,
le Biotecnologie e le Scienze della Vita*



CONCEPT PAPER

**ITALIAN MICROBIOME INITIATIVE FOR
IMPROVED HUMAN HEALTH AND AGRI-
FOOD PRODUCTION**

2019

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1. Summary

Microbiomes (i.e. Bacteria, Archaea, Eukarya and viruses) are found in humans, plants, and animals as well as in terrestrial and marine environments, providing benefits to the planet as a whole and everything that lives on it. Their astonishing metabolic potential means microbiomes occupy a central position in the “One Health” framework, which approaches human, animal and plant health from a new integrated perspective that also encompasses biomedical systems.

Many recent research projects have offered new insights into the associations between microbiomes and a wide range of human diseases. Scientists are now using this knowledge to establish balanced conditions of health and to prevent and treat diseases through the development of personalised approaches and clinical tools. In parallel, ongoing studies are demonstrating that microbiome structures and dynamics across the food system (from soils and marine habitats to plants, animals and the foods produced from them) can have both direct and indirect effects on human microbiomes and health, in addition to their obvious impact on food quality, safety and sustainability.

Innovative technologies are providing us with more detail about microbiota. A multidisciplinary approach integrating these technologies gives us more information about the humans, plants and animals with which these microbes live in symbiosis. Microbiomes are emerging from the available studies as highly dynamic, changeable and adaptable systems. Further research is needed to fully clarify the interplay between microbiomes and environmental, nutritional and host variables.

To date, under FP7 and H2020 the European Commission has funded 216 projects to the tune of approximately €500 million to advance knowledge in this area (Hadrich, Front Genet, 2018). However, Italy’s role in this major EU-wide effort has so far been minor. Italian researchers have considerable expertise in the preclinical and clinical investigation of human microbiomes and viromes and in the study of microbiomes in the soil, plants, land and aquatic animals and foods and their relationship with the human gut microbiome and health. Nonetheless, their activity is largely fragmented and its social and economic impacts lower than should be expected. The main reason for this is that unlike other EU countries, Italy does not have a state-funded microbiome programme and this area is not a key priority in the national research plan or in the Italian technology clusters’ strategic R&I agendas. It is now imperative to promote a national plan for research on human, animal, plant and food microbiomes with the main objectives of:

- i) improving knowledge;
- ii) improving exploitation of natural resources;
- iii) sharing expertises and infrastructures; and
- iv) ensuring their efficient use in promoting health.

This will require a coordinated national initiative to assess the areas in which Italy’s existing knowledge, resources and biobanks might give it an advantage over other countries.

This should be done by:

- a) establishing a national network of experts and public and private organizations working in the sector in order to pool the existing national data, knowledge, infrastructure and skills;
- b) fostering cooperation between public and private sectors, in close cooperation with the Italian health and agri-food technology clusters, to maximize the leverage effects of public and private investments;
- c) planning and implementing joint regional and national R&I initiatives and policies by promoting investment in R&I and spin-offs/start-ups and facilitating their interconnection with international networks, to encourage the application of microbiome know-how as well as to promote best practices, standards and consistent protocols;

- d) promoting researcher careers, training and mobility and the development of skills in the different microbiome domains; and
- e) exchanging knowledge across the scientific and political community and ensuring the efficient use of the available resources, while advocating public understanding of the value of microbiomes for the health of the planet and all the humans, plants and animals that live on it.

2. Introduction

Complex microbial ecosystems, collectively referred to as microbiomes, inhabit and interact with living organisms and have co-evolved a range of symbiotic relationships with them, including mutualism, with ultimate beneficial outcomes for the host.

Due to their astonishing metabolic potential, microbiomes have a key role in human, plant, animal and, ultimately, planetary health, and occupy a central position in the “One Health” framework, contributing to a new integrated perspective of the health of living organisms (**Figure 1**).

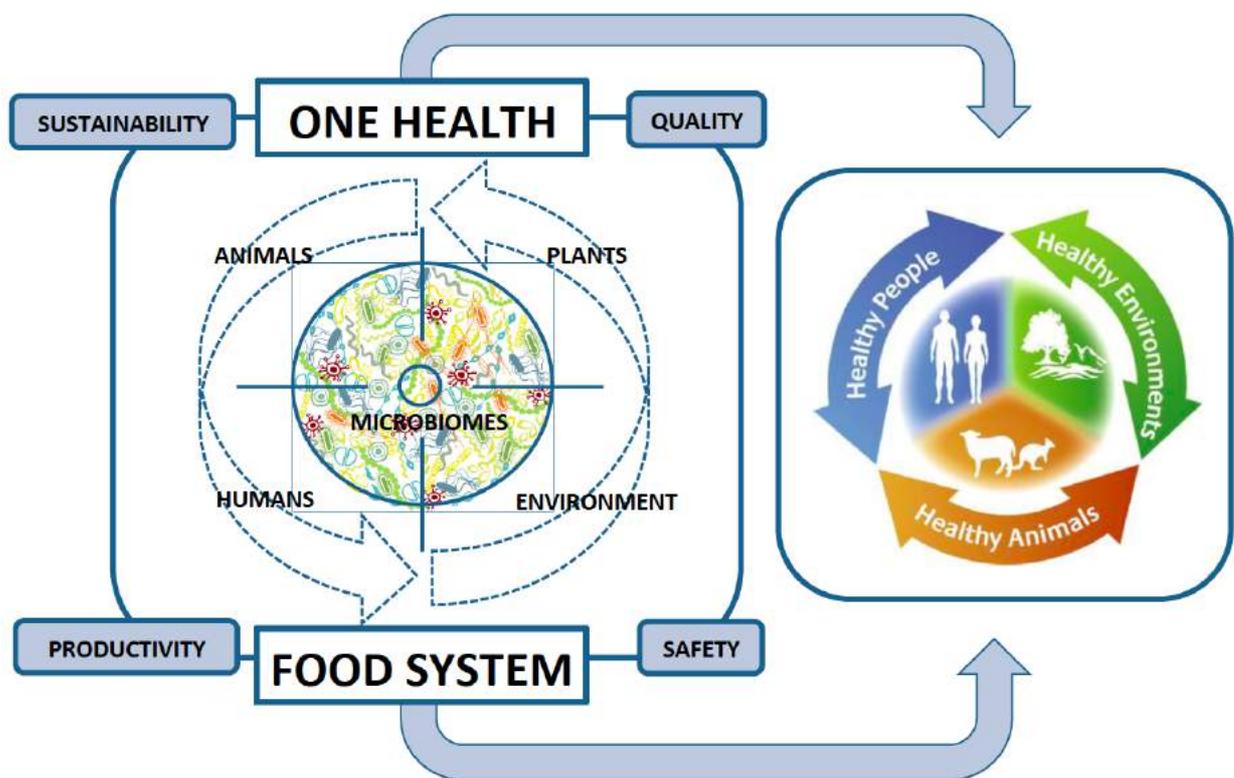


Figure 1. *The One Health framework offers a new integrated view of how microbiomes contribute to human, animal and plant health.*

The microbial ecosystem most explored to date is undoubtedly the microbiome of the human gut. The microbes in our gastrointestinal tract are ten-fold more numerous than all the cells that make up the human body (**Figure 2**) and include components from all three domains of life, i.e. Bacteria, Archaea and Eukarya, and their viruses.

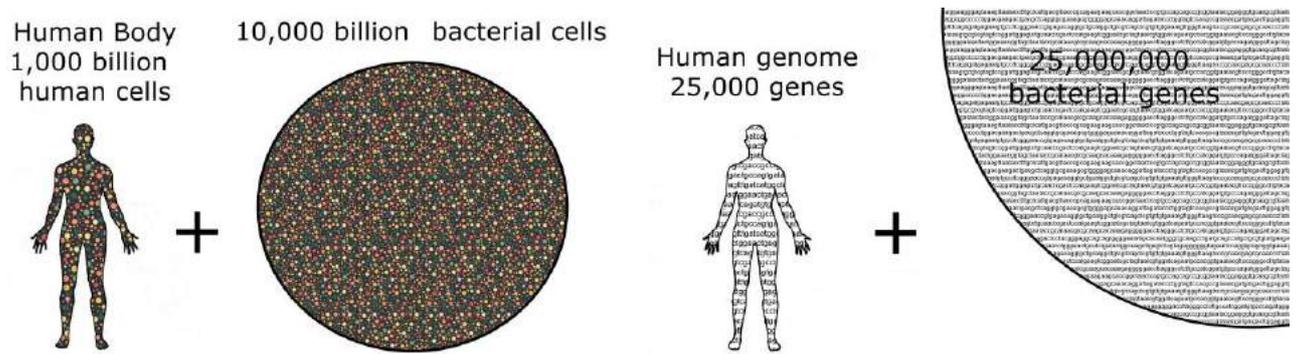


Figure 2. Estimated size of the human microbiota (the community of all microorganisms found in the human body) and of the human microbiome (the collective genomes of resident microorganisms in the human body).

This microbial community interacts constantly and extensively with the host at the intestinal mucosal surface and plays fundamental roles in several aspects of our physiology that are instrumental to the maintenance of metabolic and immunological homeostasis. Intestinal microorganisms are involved in the bioconversion of dietary components, leading to the production of a wide and diverse range of bioactive small molecules, including short-chain fatty acids (SCFAs), vitamins, amino acid metabolites, neurotransmitters and phytoestrogens, and interkingdom sensing molecules. They are thus fundamental to host nutrition, the function of the immune, endocrine and central nervous systems and protection against pathogenic colonization (Tilg and Moschen, *Gastroenterology* 2015; Sonnenburg and Bäckhed, *Nature* 2016). The crosstalk between the gut microbiome and the immune system is widely recognized as critical to human health (Honda and Littman, *Nature* 2016; Thaiss *et al.*, *Nature* 2016). The gut microbiome has in fact been considered a “forgotten” endocrine organ that acts as an endogenous circadian organizer and a key regulator of the central nervous system, with an ultimate capacity to modulate our behaviour and contribute to the pathophysiology of neurological disorders (Neuman *et al.*, *FEMS Microbiol Rev* 2015; Sharon *et al.*, *Cell* 2016; Thaiss *et al.*, *Cell* 2016; Tremlett *et al.*, *Ann Neurol* 2017). The individual gut microbiome may also affect the metabolism of xenobiotics, including drugs, with potentially important repercussions for their pharmacokinetic profile (Spanogiannopoulos *et al.*, *Nat Rev Microbiol* 2016).

The intestinal microbial communities are inherently dynamic and can fluctuate between different states of eubiosis over time. These fluctuations are closely linked to the person’s path through life and the communities maintain a mutualistic relationship with their human host that is essential for homeostasis and health (Halfvarson *et al.*, *Nat Microbiol* 2017). However, when challenged with various endogenous (e.g. Toll-like receptor polymorphisms) and/or environmental stressors, the composition and function of the gut microbiota may change considerably, resulting in a “dysbiotic” profile that compromises mutualism and may trigger or consolidate a disease state. There is in fact mounting evidence on the role of the gut microbiome in a number of enteric and systemic disorders, including gastrointestinal, metabolic, hepatic, autoimmune, respiratory, cardiovascular, neurological and even oncological diseases, often referred to as non-communicable diseases (NCDs) (Lynch and Pedersen, *N Engl J Med* 2016) (**Figure 3**).

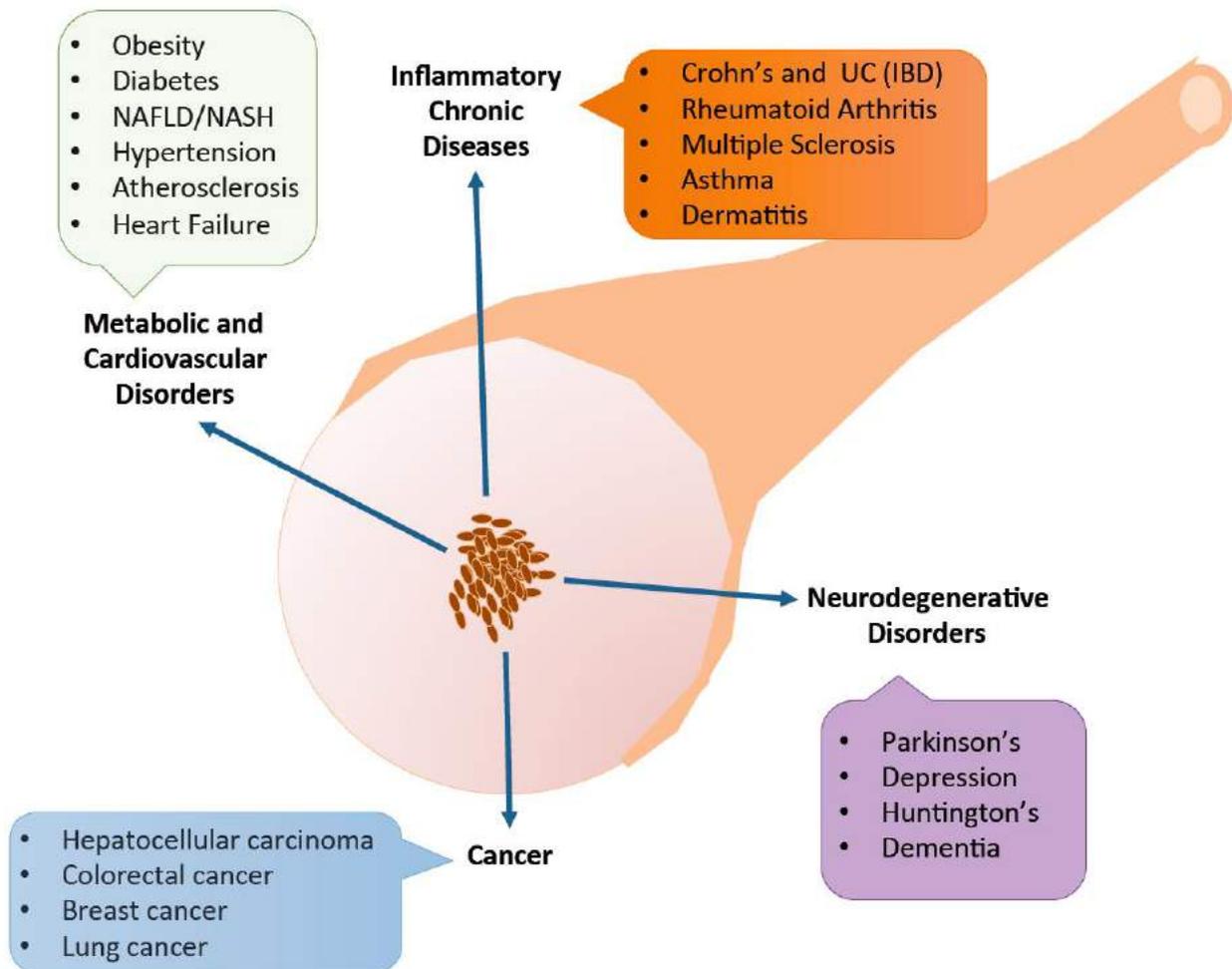


Figure 3. There is mounting evidence on the role of the gut microbiome in a number of enteric and systemic disorders.

Microbiome-disease associations typically involve the loss of beneficial, health-associated microbes (mostly producers of SCFAs), the bloom of opportunistic pathogens, or a broad restructuring of the gut microbial ecosystem, with clinically significant or even life-threatening consequences (Duvall *et al.*, *Nat Commun* 2017). Recent extensive surveys and meta-analyses exploring variations in the human gut microbiome in health and disease revealed that even microbiome dysbioses are highly personalized and follow distinctive temporal changes. A greater understanding of these could help guide more knowledge-based personalized intervention strategies (Zaneveld *et al.*, *Nat Microbiol* 2017; Koskella *et al.*, *Nat Ecol Evol* 2017). The loss of the microbiota-host equilibrium may also contribute to the onset of “pre-disease” states (pre-diabetes, pre-hypertension, etc.) and thus play a part in the rise of NCDs (van de Guchte M *et al.*, *Microbiome* 2018).

Studies characterizing the gut microbiome in thousands of people, in parallel with covariates assessing health status, diet, lifestyle, medication, biomedical parameters and genetics (Falony *et al.*, *Science* 2016, Wang *et al.*, *Nature Genetics* 2016; Rothschild *et al.*, *Nature* 2017), have demonstrated that these determinants explain only a small fraction of the total gut microbiome variation and that we are still missing important covariates when assessing drivers of this variation (**Figure 4**).

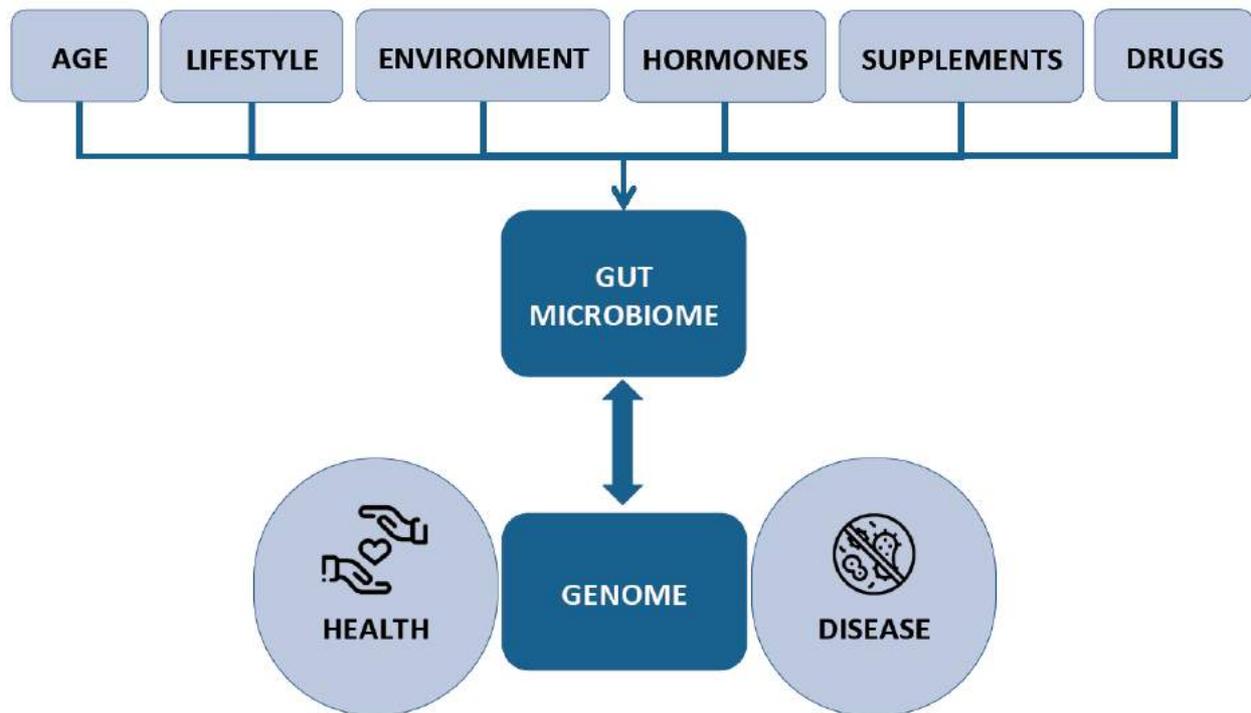


Figure 4. *The human gut microbiome has been the subject of extensive research, which has established its involvement in metabolism, nutrition, physiology and immune function. Determinants such as health status, age, environment, hormones, supplements and drugs explain only a small fraction of the total gut microbiome variation and its involvement in health and disease.*

The microbial communities of diverse anatomical sites, ranging from more obvious sites such as the skin and the genitourinary tract, to less obvious ones such as the airways, have now been characterized. Areas that were previously considered to be absolutely devoid of microorganisms, such as the placenta and the fetus, have also been characterized (Cao et al., 2014, Mor and Kwon, Am. J. Obstet. Gynecol., 2015). This broader perspective suggests that novel, previously neglected drivers of human gut microbiome variation could be sought using the One Health theoretical framework. These could include covariates in animal, plant and environmental domains that have never been explored. It is known, for instance, that microbiome structures and dynamics across the different components of the food system (soil, sediments, plants and animals, feed, food, farms, processing, workers and consumers) can directly and indirectly affect human microbiomes in many ways. For example, the soil/rhizosphere microbiome mediates the cycle of carbon and other nutrients, affects the maintenance of soil fertility and carbon sequestration, and sustains plant growth (Fiorilli *et al.*, Sci Rep 2018; Bulgarelli *et al.*, Cell Host Microbe 2015). At the same time, animals host diverse microbial communities that have evolved alongside them and play crucial roles in their biology and health (Walter and Ley, Annu. Rev. Microbiol., 2011, Hooper et al., Science, 2012) as well as in dairy and meat product fermentation processes. These activities have direct and indirect effects on both plant and animal growth and health in terrestrial and marine ecosystems and hence affect the quantity, quality and sustainability of primary production (Amundson *et al.*, Science 2015) and food processing, and, in turn, their final impact on human health.

Plants play a crucial role too, as they also possess their own microbiota, which has a strong influence on their health. Many physiological plant functions require the presence of

these mostly benign microbes and the establishment of specific plant–microbe relationships (Ash and Mueller, *Science*, 2016; Bonfante and Genre, *Nat. Commun.*, 2010). Hence, looking at microbiota from a One Health perspective, there are numerous important cofactors with closely interwoven relationships.

By far the greatest part of our current knowledge of microbiomes concerns bacteria. However, viruses are now receiving increasing attention, as they have been found to fill a variety of ecological niches within their host. Host-virome interactions can no longer be limited to a view of viruses as pathogens. Members of the eukaryotic virome that chronically infect both mammals (including humans) and plants have been found to exert significant effects on host physiology, far beyond the simple paradigm of invasion and tissue destruction (Foxman *et al.*, *Nat Rev Microbiol* 2011; Stelekati *et al.*, *Cell Host Microbe* 2012; Virgin *et al.*, *Cell* 2009).

Unlike some other EU countries, Italy does not have any state-funded national microbiome programme. Despite this, Italian researchers are active in various studies of microbiomes. These include preclinical and clinical studies of the human microbiome in various parts of the body, to characterize its role in physiological and pathological conditions in infants, children, adults and the elderly; investigation of the human virome; and investigation of the microbiome of soil, sediments, crops, aquatic plants and animals, and foods, and their relationship with human gut microbiome and health. However, their efforts are largely fragmented, limiting the potential contribution of their work to international knowledge of the microbiome and how it can be exploited. There is thus an urgent need for a coordinated national initiative.

3. Expertise, infrastructures and industrial sectors involved in microbiome-based knowledge exploitation

3.1 Microbiome Facilities and Expertise (Figure 5) rely on the genome-based analyses, resources and support needed to characterize and study microbiomes, including:

1. Advanced technological platforms for their phylogenetic and functional characterization, including **next-generation sequencing (NGS) platforms** such as Miseq, NextSeq and HiSeq (Illumina), Ion Torrent PGM (Life Technologies) and PacBio (Pacific BioSciences) that allow researchers to:

- rapidly sequence whole microbial genomes and metagenomes, enabling an overview of their functional capabilities;
- use RNA sequencing (RNA-Seq) for metatranscriptomic analysis.

2. **Quantitative Real-Time PCR platforms** for quantification of nucleic acids in different applications, for gene expression, genotyping, copy number variation, microRNA, pathogen detection, etc.

3. **Proteomics platforms**, including **LC-MS/MS Orbitrap** to quantify meta-proteomes and specific metabolic pathways.

4. **Metabolomics platforms: LC-MS/MS Quantum Access MAX triple quadrupole mass spectrometer** coupled to U-HPLC, **LC-MS/MS Orbitrap** and **NMR 600 Hz** for host/microbiome metabolomic profiling, quantification of microbiome metabolites and co-metabolites, measurement of trace-level environmental or food contaminants and detection of specific biomarkers.

5. **Phenotype Microarray** platform (e.g. **OmniLog®**) for genotype-phenotype characterization of microbes and determination of the optimal conditions for cell growth, sporulation and germination, production of secondary metabolites and enzymatic activities in cell lines.

6. **Simulator of the Human Intestinal Microbial Ecosystem (e.g. SHIME®)** to mimic the human gastrointestinal digestion in vitro. SHIME is one of the few gut models that simulates the entire gastrointestinal tract, incorporating the stomach, small intestine and different colon regions.

7. **Microbial biobanking**, under the umbrella of the pan-European research infrastructure MIRRI (Microbial Resource Research Infrastructure), for suitable long-term preservation of microbial isolates.

8. **Plant facilities** to mimic and predict the impact of climate change on crop production (e.g. FACE laboratory in open field).

9. **Animal Facilities** to perform studies in animal models (e.g. mice and rats). Authorized facilities are available for the conduct of specific challenge models in controlled conditions, as are infrastructures to produce germ-free and specific pathogen-free animals, facilities for the conduct of small and mid-scale trials in farm animals (e.g. pigs, poultry, dairy cows and goats), and experimental tanks for aquaculture studies.

10. **High-performance computers (HPCs) and infrastructure** as essential tools to organize and exploit big databases for microbiome knowledge.

11. **Databases and bioinformatics pipelines** for metataxonomic and metagenomic data analysis, and **multidimensional statistical approaches** for the integration of microbiome datasets, host phenotyping and environmental metadata, from a systems biology perspective.

12. **Human, animal and plant model phenotyping**: body composition analysis (dual-energy X-ray absorptiometry, bioimpedance analysis, air displacement plethysmography), energy expenditure evaluation (indirect calorimetry, movement actigraphy), energy and nutrient intake (eating pattern and behaviour evaluation), and functional assessment (handgrip and leg-extension strength tests, submaximal exercise test, physical performance battery).

13. **Platforms for controlled microscale food fermentation**, to determine the dynamics of controlled fermentation of food products under different operating conditions and microbiome configurations.

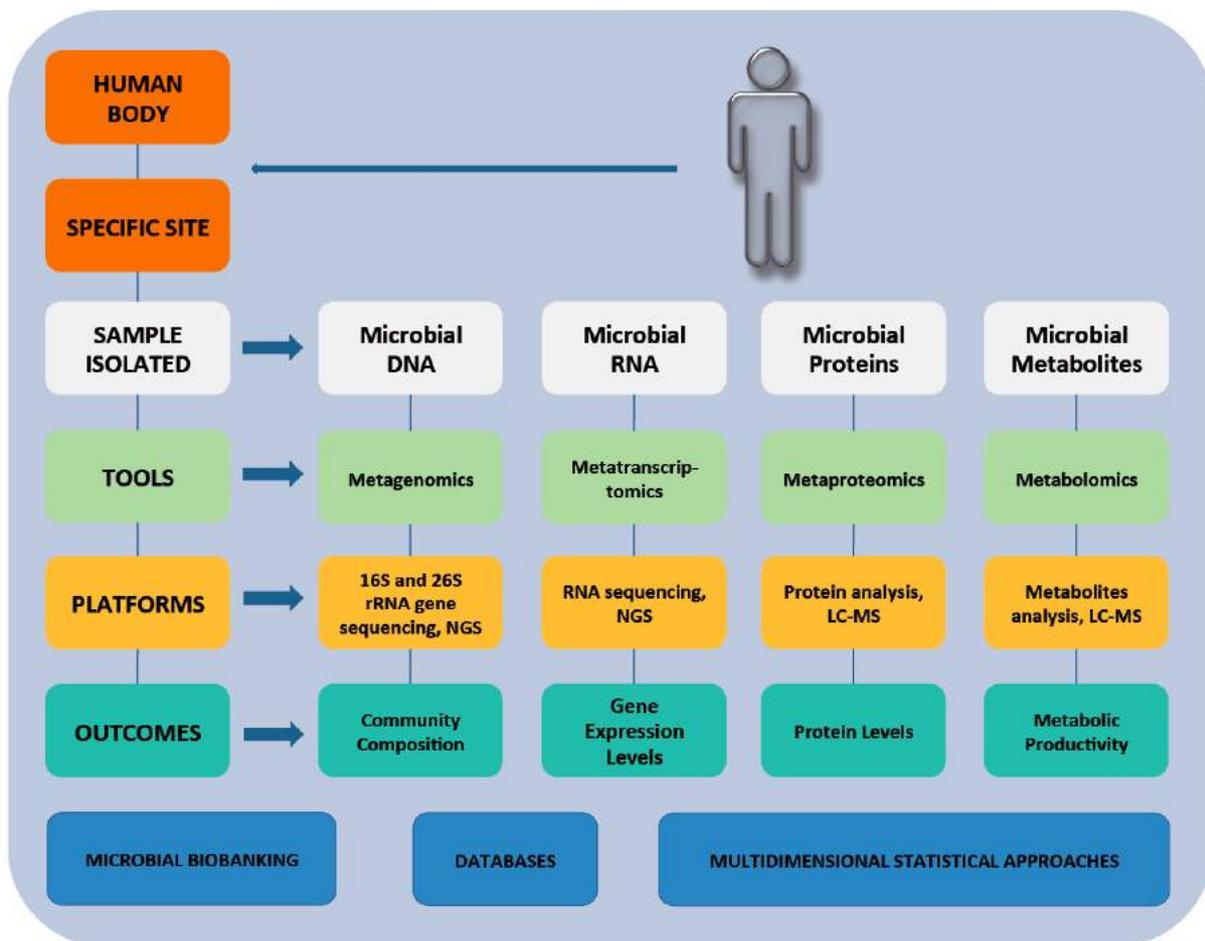


Figure 5. Some of the Microbiome Facilities and Expertise used to characterize and study microbiomes.

Based on: Al Khodor S, Reichert B and Shatat IF (2017) The Microbiome and Blood Pressure: Can Microbes Regulate Our Blood Pressure? *Front. Pediatr.* 5:138. doi: 10.3389/fped.2017.00138

3.2. Italian involvement in EU projects and networks

Italian researchers have participated in or are currently contributing to a number of past and ongoing collaborative EU projects dealing with nutrition, microbiome and human, animal and plant host health, as summarized below.

Nutrition and host health

- “New dietary strategies addressing the specific needs of elderly population for a healthy ageing in Europe” (**NU-AGE**) consortium: a collaborative project for the development of functional food prototypes and the improvement of traditional foods tailored for the elderly; https://cordis.europa.eu/result/rcn/155983_it.html (COORDINATION)
- “European network on the factors affecting the gastro-intestinal microbial balance and the impact on the health status of pigs” (**PiGutNet**) consortium: a collaborative project for genome/metabolome-wide association studies and the provision of a road map to increase pig resistance against GIT infections. COST Action FA1401; <http://www.pigutnet.eu/> (COORDINATION)

- "Ecology from farm to fork of microbial drug resistance and transmission" (**EFFORT**) consortium: collaborative project to inform decision makers, the scientific community and other stakeholders about the consequences of anti-microbial resistance in the food chain; https://cordis.europa.eu/project/rcn/111140_it.html
- "Reducing egg susceptibility to contaminations in avian production in Europe" (**RESCAPE**) consortium: collaborative project for the reduction of food safety risks by optimizing hen cages and egg collection management; https://cordis.europa.eu/project/rcn/80032_it.html
- "European animal disease genomics network of excellence for animal health and food safety" (**EADGENE**) consortium: collaborative project for strengthening the implementation of durable integration in FP6 Networks of Excellence; https://cordis.europa.eu/project/rcn/74158_en.html
- "Interplay of microbiota and gut function in the developing pig – Innovative avenues towards sustainable animal production" (**INTERPLAY**) consortium: collaborative project for the improvement of animal health and food safety through investigating the gut function of farm animals, the gastro-intestinal microflora and their interactions; https://cordis.europa.eu/project/rcn/92294_en.html
- "Connecting the animal genome, gastrointestinal microbiomes and nutrition to improve digestion efficiency and the environmental impacts of ruminant livestock production" (**RUMINOMICS**) consortium: collaborative project for the development of models and tools to enable the livestock industry to reduce environmental damage from methane and nitrogen emissions and to improve feed efficiency; https://cordis.europa.eu/project/rcn/101163_it.html
- "The role of intestinal microflora in non-alcoholic fatty liver disease (NAFLD)" (**FLORINASH**) consortium: collaborative project addressing the role of intestinal microflora activity in the pathogenesis of NAFLD; https://cordis.europa.eu/project/rcn/93672_en.html
- "New E-Services for a dietary approach to the elderly" (**RISTOMED**) consortium: collaborative project addressing the common problem of delivering innovative nutraceuticals for the control and prevention of age-related diseases; https://cordis.europa.eu/project/rcn/92439_en.html (COORDINATION)
- "Metagenomics of the Human Intestinal tract" (**METAHIT**) consortium: collaborative project to establish associations between the genes of the human intestinal microbiota and our health and disease; https://cordis.europa.eu/project/rcn/87834_en.html
- "Microbiome Influence on Energy balance and Brain Development-Function Put into Action to Tackle Diet-related Diseases and Behaviour" (**MyNewGut**) consortium: collaborative project for the development of new approaches to prevent diet-related diseases and behavioural disorders through lifestyle changes and dietary interventions with new food products; https://cordis.europa.eu/project/rcn/111044_it.html
- "Genomic and nutritional innovations for genetically superior farmed fish to improve efficiency in European aquaculture" (**AqualIMPACT**) consortium: collaborative project responding to the need for innovative feed formulations for fish reared in aquaculture systems; Horizon 2020. Call: H2020-BG-2018-2020 (Blue Growth) Topic: DT-BG-04-2018-2019; Type of action: IA
- **MIC CERES** international project: Microbial eco-compatible strategies for improving wheat quality traits and rhizospheric soil sustainability.

Food processing

- "Controlling Microbiomes Circulations for Better Food Systems" (**CIRCLES**) consortium: collaborative project to discover, translate and communicate innovative, microbiome-based applications to enhance food system performances and their sustainability; H2020-SFS-2018 (COORDINATION)

- "Sustainable innovation of microbiome applications in food system" (**SIMBA**) consortium: Innovative action to harness complex soil and marine microbial communities (microbiomes) for the sustainable production of food. This project has just been accepted for a 4-year period in response to call LC-SFS-03-2018 "Microbiome applications for sustainable food systems".

- "Microbiome applications for sustainable food systems through technologies and enterprise" (**MASTER**) consortium. This project has just been accepted in response to call LC-SFS-03-2018 "Microbiome applications for sustainable food systems".

- "SOURDOugh biotechnology network towards novel, healthier and sustainable food and bloproCesseS" (**SOURDOMICS**) consortium. This project has just been accepted in response to call OC-2018-1 "RP3 Towards a sustainable future, the aim of the present: a focus on Agriculture, Biological sciences, Environment and Chemistry. COST action.

- "Biotransformation of brewers' spent grain: increased functionality for novel food applications" (**FUNBREW**) consortium: collaborative project to establish bioprocessing technologies for the synthesis in situ of functional compounds in BSG, such as exopolysaccharides (EPS) and antioxidants. COST ACTION 2017. [https://tuhat.helsinki.fi/portal/en/projects/biotransformation-o\(ea2896e5-a08a-4cd4-a131-ddc6faf52098\).html](https://tuhat.helsinki.fi/portal/en/projects/biotransformation-o(ea2896e5-a08a-4cd4-a131-ddc6faf52098).html)

- "Inflammatory Bowel Disease: an EU-NZ integrated approach for characterizing its molecular multifactorial mechanisms" (**REINFORCE**) consortium: collaborative project aiming to improve the understanding of IBD and train the scientists involved and expand on their technical knowledge; FP7- PEOPLE. https://cordis.europa.eu/result/rcn/165580_en.html

- "Novel multifunctional plant protein ingredients with bioprocessing" (**BIOPROT**) consortium: collaborative project to boost scientific cooperation between EU member and associated states in order to maximize the contribution of research to the development of more sustainable food systems; FP7-ERANET-2011-RTD. <https://www.era-learn.eu/network-information/networks/susfood/1st-joint-call-for-proposals/novel-multifunctional-plant-protein-ingredients-with-bioprocessing>

- "Innovative biotechnological solutions for the production of new bakery functional foods" (**Bake4Fun**) consortium: collaborative project to respond to the needs of food SMEs for innovative food formulations and technologies to overcome the negative effect of iron fortification of bakery products; FP7-SME-2013. https://cordis.europa.eu/project/rcn/110204_it.html

Soils, sediments and the rhizosphere

- "Unravelling and exploiting Mediterranean Sea microbial diversity and ecology for xenobiotics' and pollutants' clean up" (**ULIXES**) consortium: collaborative project aimed at the bioremediation of the Mediterranean Sea by exploring its microbial diversity – SICA; FP7 https://cordis.europa.eu/project/rcn/97824_it.html (COORDINATION)

- "Marine microorganisms: cultivation methods for improving their biotechnological applications" (**MACUMBA**) consortium: collaborative project to improve the isolation rate and growth efficiency of marine microorganisms from conventional and extreme habitats and the use of automated high throughput procedures; FP7 https://cordis.europa.eu/project/rcn/104389_it.html

- "Linking belowground biodiversity and ecosystem function in European forests" (**Biolink**) consortium: collaborative project to fully explain the connection between diversity, stability and ecosystem function - COST ACTION FP1305 (2013-2018); http://www.cost.eu/COST_Actions/fps/FP1305

- "Industrial applications of marine enzymes: innovative screening and expression platforms to discover and use the functional protein diversity from the sea" (**INMARE**) consortium: collaborative project to streamline the pathways of discovery and industrial applications of new marine enzymes and bioactives for the targeted production of drugs and fine chemicals; H2020 https://cordis.europa.eu/project/rcn/193292_it.html
- "Marine Microbial Biodiversity, Bioinformatics and Biotechnology" (**MICRO B3**) consortium: collaborative project for the development of innovative bioinformatic approaches to make large-scale data on marine viral, bacterial, archaeal and protists genomes and metagenomes accessible for marine ecosystems biology and to define new targets for biotechnological applications; FP7 https://cordis.europa.eu/project/rcn/101555_en.html
- "Increasing Value and Flow in the Marine Biodiscovery Pipeline" (**PHARMASEA**) consortium: collaborative project for the production of new products with desirable characteristics for development by SME partners in three accessible market sectors, health (infection, inflammation, CNS diseases), personal care and nutrition; FP7 https://cordis.europa.eu/project/rcn/104338_it.html
- "DEvelopment Of innovative Tools for understanding marine biodiversity and assessing good Environmental Status" (**DEVOTES**) consortium: collaborative project for the development, testing and validation of innovative integrative modelling tools to further strengthen our understanding of ecosystem and biodiversity changes; FP7 https://cordis.europa.eu/project/rcn/105613_en.html
- "Biotechnology from desert microbial extremophiles for supporting agriculture research potential in Tunisia and Southern Europe" (**BIODESERT**) FP7 report https://cordis.europa.eu/project/rcn/93561_en.html (COORDINATION)

3.3 Industrial sectors involved in microbiome-based knowledge exploitation

The knowledge generated around microbiomes is now attracting the interest of several industries, which need to be engaged to effectively and responsibly apply this new knowledge. The National Technology Clusters CLAN (Agri-Food) and ALISEI (Life Sciences) are supporting initiatives to open up ways to modify microbiomes and develop microbiome-based applications.

Biotechnology industry

- Several recently established companies aim to develop new therapies that alter the microbiome for the benefit of human health. For instance, probiotics and prebiotics are now in common use and are routinely included in foods, drinks and supplements.

Pharmaceutical and diagnostic industry

- The development of alternative interventional measures (prophylaxis/prevention or treatment) to limit antimicrobial use has a high translational potential. The development and validation of modulators of host immunity and/or microbial ecosystems, feed additives and novel molecules are key research targets. Inter-sectorial collaboration could open new avenues for the exploitation of residual bioactive compounds and agro-industry by-products.

- A major expectation for microbiome research is the identification of biomarkers to enable the reclassification of comorbidities by their mechanism and the development of new therapeutic strategies. New validated biomarkers will greatly facilitate the management of metabolic diseases and their comorbidities by providing new possibilities

for diagnostic and predictive medicine and qualified strategic therapies, thus paving the way for a new direction in clinical research on new targets.

- New diagnostic tools and methods for point-of-care diagnostics, cheap, user-friendly innovative devices, high throughput microbial detection and characterization and antibiotic resistance monitoring.

Food industry

The food we eat, and the way we eat it, are changing. The consumption of processed foods with longer shelf lives and ready-to-eat foods is on the rise, and snacking is becoming ever more dominant. At the same time, the human population is rapidly expanding and the great challenge is now sustainable agriculture. The combination of these forces is creating a need for sustained innovation in the identification of affordable new food sources and highly nutritious foods. Studies of microbiomes could help in the integration of agricultural policies and practices, food production and distribution and nutritional recommendations for consumers of different ages, lifestyles, geographies and states of health.

Agro-industry: primary production

Plant and animal microbiomes can be manipulated for a more sustainable production of high-quality foods by modulating plant and animal growth, controlling their physiological development, boosting their defences against pathogens and insect pests, improving their nutrient quality and controlling resistance to stress.

4. State-of-the-art, research requirements and actions needed to boost the sector in Italy

4.1. Knowledge and impact

4.1.1. Microbiome and human health

Microbiomes colonize a number of different body regions including the nose, mouth, throat, skin, vagina, urethra and intestine. To date, the gut microbiome has received the most attention. Its immense gene catalogue is almost 3 orders of magnitude larger than that of the human body, and it has a profound influence on human physiology and metabolism. Gut microorganisms complement several gaps in human metabolic pathways, producing essential vitamins (mainly B vitamins) and enabling energy to be extracted from otherwise indigestible carbohydrates (dietary fibre), thus playing a major role in host energy balance and nutrition. Intestinal microbes are also involved in the bioconversion of dietary components, with major effects on immune functionality and metabolic homeostasis (Tilg and Moschen, *Gastroenterology* 2015; Sonnenburg and Bäckhed, *Nature* 2016).

It is therefore not surprising that several metabolic disorders, including obesity, type 2 diabetes and NAFLD, are associated with microbiome dysbiosis. The altered pattern of bacterial taxa and microbiome-derived biomolecules this entails may promote or further aggravate disease processes (Hartstra *et al.*, *Diabetes Care* 2015; Tripathi *et al.*, *Nat Rev Gastroenterol Hepatol* 2018). For example, the dysregulation of the microbiota-host co-metabolism of bile acids may impair glucose homeostasis and liver and pancreatic function and lead to adipose tissue inflammation (Wahlström *et al.*, *Cell Metab* 2016). High-fat, low-fibre Western diets have been repeatedly shown to affect the gut microbiome structure, with detrimental effects on intestinal permeability, lipid accumulation and inflammatory

state (Jena *et al.*, FASEB J 2018). In contrast, a Mediterranean-style diet with a higher intake of high-fibre food is known to beneficially modulate host-microbiome interactions, helping to prevent a number of diseases (De Filippis *et al.*, Gut 2016) (**Figure 6**).

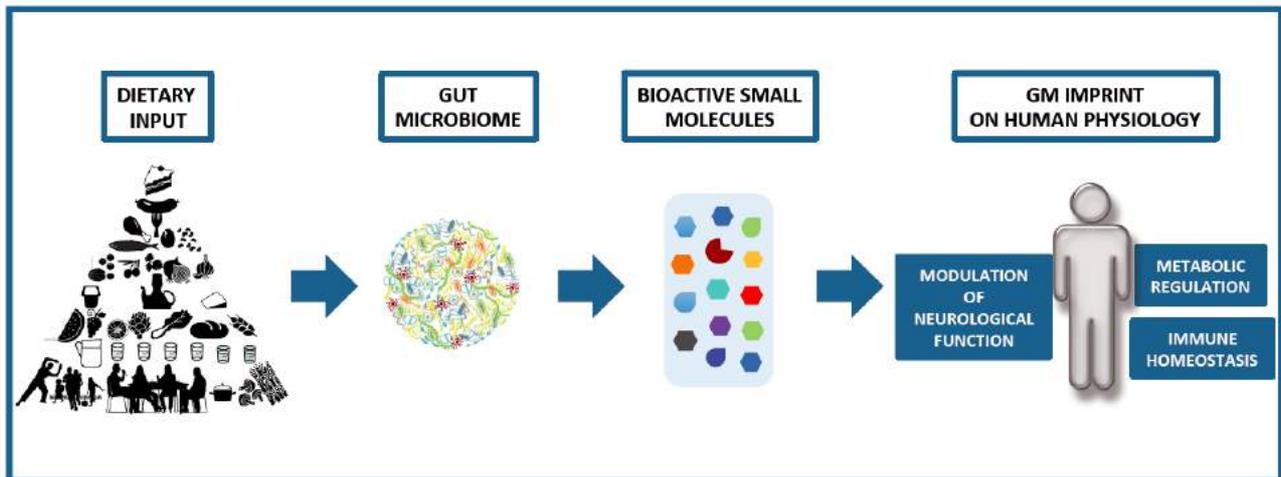


Figure 6. Microbiota-host transgenomic metabolism and its impact on human health (GM: gut microbiome)

However, the health-promoting features of the gut microbiome as shaped by the Mediterranean diet require further investigation.

Recent evidence suggests that physical activity has a role in the modulation and shaping of gut microbiomes (Cronin *et al.*, mSystems 2018; Greenhill *et al.*, Nat Rev Endocrinol 2018).

Metagenomic analysis in obese Italians has revealed specific signatures associated with different metabolic traits. An interplay has been demonstrated between increased proteobacteria and phenylacetic acid, a microbial metabolite that interferes with branched chain amino acid catabolism (Hoyles *et al.*, Nature Medicine 2018). In subjects with glucose intolerance, derangements in microbial tryptophan metabolism have been observed, with a specific induction of metabolites of the kynurenine pathways and a reduction in indoles (Laurans *et al.*, Nature Medicine 2018).

The gut microbiota is widely recognized as an integral component of the human immune system, finely calibrating both innate and adaptive immune responses throughout life. The intimate interplay between gut microbes and mucosal immune cells is crucial for the maintenance of immune homeostasis and, above all, for the education of the immune system during infancy (Honda and Littman, Nature 2016; Thaiss *et al.*, Nature 2016). Specific gut microbiome alterations in the first months of life, a critical time window for long-term health, have been associated with the development of allergy and other immunological/metabolic disorders later in life (Arrieta *et al.*, Sci Transl Med 2015; Cox *et al.*, Cell 2014). Regardless of age, disruption of the delicate balance of commensal bacteria (dysbiosis) may lead to impaired local and systemic immune responses, with breakdown of mucosal barriers, translocation of gut bacteria beneath the epithelium and a change in the cytokine milieu and T cell ratio towards an inflammatory phenotype, thus eliciting a profound inflammatory state both locally and systemically (Gopalakrishnan *et al.*, Cancer Cell 2018) (**Figure 7**).

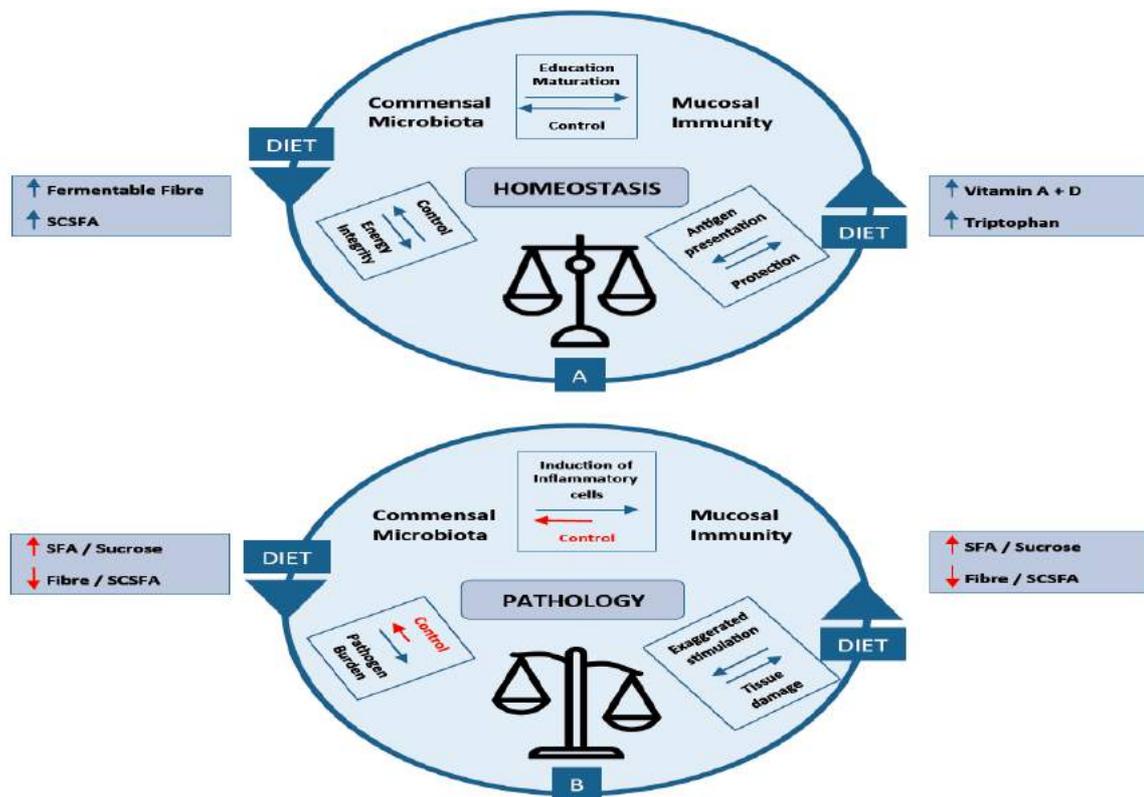


Figure 7. Interaction between diet, microbiota and immune response at mucosal sites. A. To maintain a healthy state, the local microbiota and mucosal immune system are in homeostasis at mucosal sites. The microbiota educates and promotes the maturation of the immune system by inducing pro-inflammatory and anti-inflammatory immune cells; B. During pathological conditions, such as inflammatory bowel disease and asthma, the homeostasis at the mucosal barrier is disrupted.

Based on: Statovci D, Aguilera M, MacSharry J and Melgar S (2017) The Impact of Western Diet and Nutrients on the Microbiota and Immune Response at Mucosal Interfaces. *Front. Immunol.* 8:838. doi: 10.3389/fimmu.2017.00838

This is the case with several NCDs whose common basis is inflammation, including inflammatory bowel disease and irritable bowel syndrome. It is also seen in other disorders with a strong immunological component. In autoimmune diseases such as type I diabetes and rheumatoid arthritis, the host can no longer control commensal bacteria, while in immunosuppressive diseases such as HIV infection, or in patients with or undergoing haematopoietic stem cell transplantation for blood cancers, it may enable pathogen invasion and infection (Lynch and Pedersen, *N Engl J Med* 2016; Zaneveld *et al.*, *Nat Microbiol* 2017).

Mounting evidence on the bidirectional gut-brain axis also supports the role of the intestinal microbiome in the regulation of anxiety, cognition, pain and behaviour and its possible contribution to the pathophysiology of central nervous system disorders, such as multiple sclerosis, autism spectrum disorders and major depression (Sharon *et al.*, *Cell* 2016). The gut is the largest nervous system outside the CNS that is autonomously active and in close interplay with its microbiota. This enteric nervous system is also called “the second brain”.

The microbiota has been shown to influence multiple aspects of neuropathophysiology, including neurotransmission and the behaviours often associated with neuropsychiatric conditions, such as anxiety and depressive-like behaviours (Kim *et al.*, *Curr Neuropharmacol* 2018). In addition, convincing evidence points to a malfunction

of the intestinal barrier as the mechanism underlying the immune dysfunction that ultimately leads to neurological disorders such as the demyelinating lesions seen in multiple sclerosis (Camara-Lemarroy *et al.*, Brain 2018). There is also mounting evidence on the role of the microbiota-gut-brain axis in neurodegenerative conditions (including Parkinson's disease, multiple sclerosis, Alzheimer's disease and amyotrophic lateral sclerosis) and psychiatric disorders (including anxiety, depression, schizophrenia and autism spectrum disorders) (Sampson *et al.*, Cell 2016; Pellegrini *et al.*, Acta Neuropathologica 2018). Recent experimental and clinical investigations have also shown that the gut microbiota influences the outcome of cerebral ischemia (Singh *et al.*, J Cereb Blood Flow Metab 2018). In addition, the functional output of the gut microbiome, including SCFAs and amino acids, and the microbiome's ability to alter the bioavailability of aromatic and branched chain amino acids, confirm the existence of a gut-muscle communication pathway and seem to be involved in the mechanisms leading to the development of sarcopenia, sarcopenic obesity and, possibly, cancer cachexia (Picca *et al.*, Mediators Inflamm 2018; Ticinesi *et al.*, Nutrients 2017; Inglis *et al.*, Curr Osteoporosi Rep 2015; Klein *et al.*, Curr Opin Support Palliat Care 2013).

4.1.2 The human gut microbiome and disease prevention and therapy

There is an urgent need to translate the data and knowledge on the gut microbiome generated in recent years into concrete actions. The growing body of literature on the transition of the human gut microbiota from health to disease will help in the design of knowledge-based, personalized intervention strategies to manipulate the intestinal microbial community towards a more "favourable" profile, enabling an enhanced therapeutic response. These strategies will include:

- i) dietary interventions, as a simple, safe and accessible adjunct to current therapies;
- ii) administration of bacterial consortia or "designer probiotics", selected on the basis of the growing understanding of the complex syntrophic connections between microbiota components and the dysbiosis in question; and
- iii) faecal microbiota transplantation, as the most direct means of manipulating an impaired gut microbiome.

Future research will have to take into account the inherent variability of the gut microbiome by monitoring the individual trajectory over time in states of eubiosis and dysbiosis. Similarly, novel, microbiome-specific endpoints must be implemented to assess the efficacy of a given probiotic therapy. The success of a probiotic course depends on the supported recovery of a state of microbiome eubiosis and hence on the probiotics' ability to redirect the individual dysbiotic trajectory to within the range of normal variation ("the healthy plane" – Halfvarson *et al.*, Nat Microbiol 2017; Zaneveld *et al.*, Nat Microbiol 2017). Re-analysis of the gut microbiome at the end of any probiotic course will thus be essential to evaluate their efficacy in terms of recovery of a healthy microbiome configuration.

Culturomics, an approach that aims to cultivate all the components of the human gut microbiome through the use of optimized selective and/or enrichment culture conditions coupled with metagenomic identification, is expanding the narrow range of probiotics currently available to consumers. Other microorganisms are being revealed as providers of new, more effective probiotic functions - the "next-generation probiotics" (O'Toole *et al.*, Nat Microbiol 2017; Kim *et al.*, Crit Rev Food Sci Nutr 2018). For example, the bacteria *Christensenella* and *Faecalibacterium prausnitzii* show a greater anti-inflammatory potential than the traditional bifidobacteria and lactobacilli, while *Akkermansia muciniphila* has promising potential for the treatment of obesity. Human microbiomes are also receiving significant attention thanks to their influence on cancer therapy. Several recent studies have demonstrated the existence of a "more favourable" gut microbiome configuration predictive of a positive long-term response, strongly suggesting that

manipulating the gut microbiota could be an important adjunct to current multimodal therapeutic anti-cancer strategies to improve response and circumvent primary resistance, disease recurrence and treatment toxicity (Gopalakrishnan *et al.*, Cancer Cell 2018; Thomsen *et al.*, Benef Microbes 2018; Pouncey *et al.*, Ecancermedicallscience 2018; Viaud *et al.*, Oncoimm 2018).

In any case, despite the many advances in metagenomics achieved in recent years, the human gut microbiome still harbours a large amount of uncharacterized functional diversity, with an immense potential for the discovery of new health-promoting biological molecules. Such molecules could offer innovative possibilities in medicinal chemistry, leading towards the identification of novel drug candidates and targets and more generally, new ways to treat unmet medical needs. Microbiota-derived metabolites are now emerging as promising starting hit compounds to modulate human targets, hence triggering certain pharmacological responses. Robust campaigns of metagenome-based microbiome bioprospecting that synergize complementary approaches (such as functional omics, systems medicine, *in silico* drug discovery, target fishing and quantum biomolecular modelling) must thus be prioritized to enable the implementation of innovative, precise strategies to steer the contribution of the gut microbiome towards human health. At the same time, increasing knowledge of the impact of the microbiome on pharmacokinetics and hence on individual drugs is instrumental to the implementation of specific approaches of rational drug design. These are aimed at removing or modifying chemical and functional groups known to undergo microbial metabolism in the gut, in order to increase drug bioavailability and reduce drug toxicity. Research in this direction will enable the best intervention strategies to be established in line with the individual gut microbiome functional configuration, for more precise, knowledge-based and cost-effective drug use.

4.1.3. Soils, sediments and sustainable agriculture and food processing

Relevance of primary production and food processing for the Italian economy.

In 2017, the Italian food industry in 2017 had a turnover of 137 billion euros (approximately 32 billion euros from exports) and involved 385,000 employees and 58,000 companies, making it the second largest manufacturing sector in Italy. It is also the third largest food industry in Europe, behind Germany and France (Italian bioeconomy strategy http://www.agenziacoesione.gov.it/opencms/export/sites/dps/it/documentazione/S3/Bioeconomy/BIT_v4_ENG_LUGLIO_2017.pdf). The 18th census of the Italian Ministry of Agricultural, Food and Forestry Policies (Official Gazette of the Italian Republic, General Series no. 57 of 09/03/2018 – Ordinary Supplement no. 11) lists more than 4,800 traditional foods and beverages, the main pillars of the regional economies. Italy is the European leader for foods and beverages with legal recognition status: 294 foods have the status of DOP, IGP or STG, and 523 wines have DOCG, DOC or IGT. As declared by UNESCO, the Mediterranean diet, mainly based on traditional foods and beverages, is an Intangible Cultural Heritage of Humanity.

In 2016, the value of arable, fodder and tree crop production in Italy was approximately 27 billion euros, half of the value of all agriculture, silviculture and fishery sectors combined (CREA, 2017 <https://www.crea.gov.it/wp-content/uploads/2017/03/Itaconta-2016-x-WEB.pdf?x99213>; the Italian Bioeconomy strategy http://www.agenziacoesione.gov.it/opencms/export/sites/dps/it/documentazione/S3/Bioeconomy/BIT_v4_ENG_LUGLIO_2017.pdf). Italy is a European leader in high-quality certified agri-food products, with exports increasing from 33 to 41 billion euros since 2013. The demand for high quality Italian agri-food products is increasing both in Europe and further afield. Farmers, especially those producing high quality products, are paying increasing

attention to sustainable solutions that adopt microbial-based fertilizing, N-fixing Rhizobia (Mathu et al., 2012), plant growth-promoting, plant bio-conditioning and biocontrol products (Bashan *et al.*, Plant Soil 2014; Bashan *et al.*, Biol Fertil Soils 2016). This interest is markedly supported by the ongoing growth of organic farming in Europe and worldwide. Despite recent concerns on the economic sustainability of organic farming and its impact on forestry and biodiversity (Seufert *et al.*, Nature, 2012), this market grew to 29.8 billion euros in Europe in 2015, an increase of 13% over the previous year. Italy is one of the ten largest world producers of organic products and has the second largest surface area dedicated to organic farming in Europe, after Spain. In 2016, the Italian organic farming surface area increased by 20% over 2015, amounting to 3.5% of the total world organic farming surface area (1.8 M hectares), while 14.5% of the Italian surface was devoted to agriculture (CREA, 2017 <https://www.crea.gov.it/wp-content/uploads/2017/03/Itaconta-2016-x-WEB.pdf?x99213>).

In this context, microbiome studies may provide new insights that improve the productivity of organic farming and overcome its limitations through the use of eco-compatible solutions (e.g. microbial-based fertilizers, plant growth-promoting bacteria, plant bio-conditioning and biocontrol products).

Microbiome relevance in terrestrial primary production. The soil/rhizosphere microbiome mediates the cycle of carbon and other nutrients, affects the maintenance of soil fertility and soil carbon sequestration and sustains plant growth (**Figure 8**).

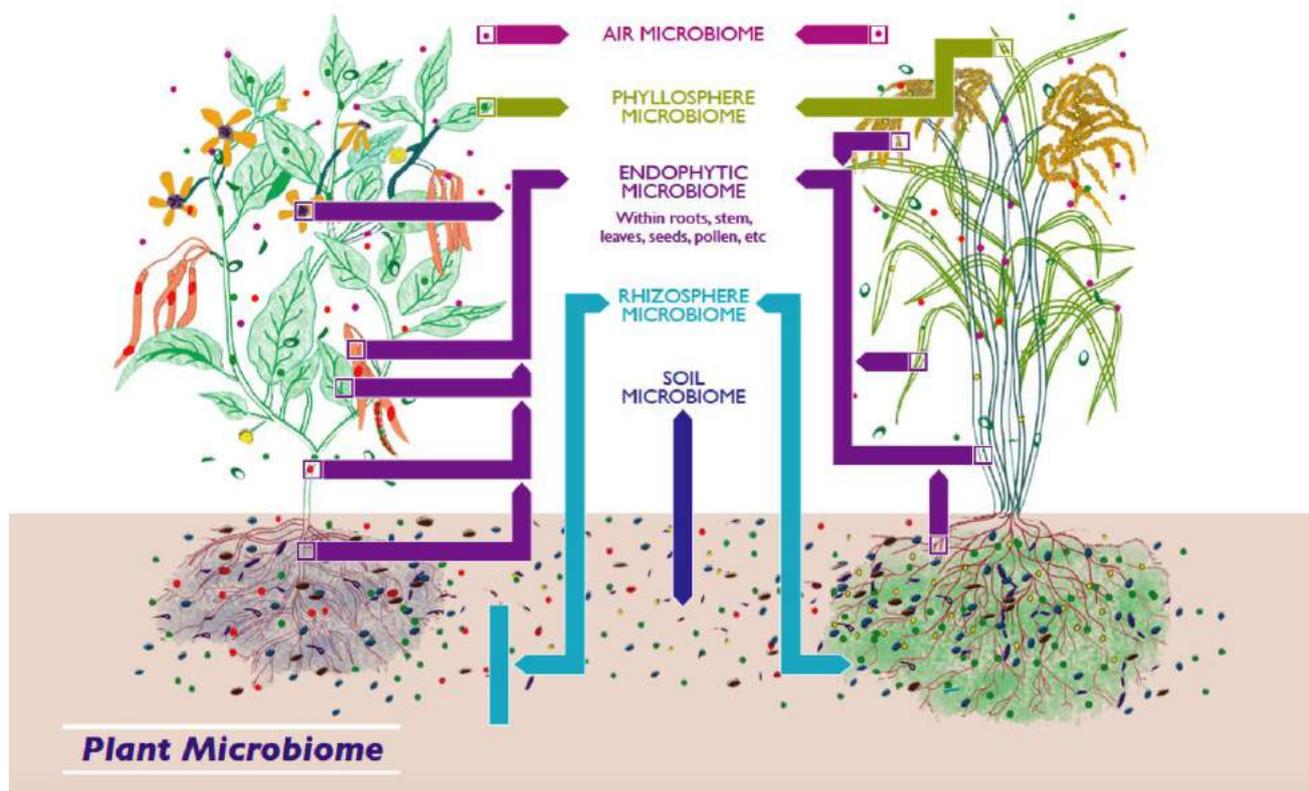


Figure 8. The plant microbiome can be described as the sum total of the genomic contribution made by the diverse microbial communities that inhabit the surface and internal tissues of the plant parts. The microbial communities are mainly found in the rhizosphere, endosphere and phyllosphere. The soil microbiome is the main source from which the plant selects and builds its microbiome profile.

Based on: Gopal M and Gupta A (2016) Microbiome Selection Could Spur Next-Generation Plant Breeding Strategies. *Front. Microbiol.* 7:1971. doi: 10.3389/fmicb.2016.01971

All these activities have direct and indirect effects on plant and animal health in terrestrial ecosystems and hence affect the quantity, quality and sustainability of primary production (Amundson *et al.*, Science 2015). Given the great heterogeneity of soil and the fluctuating environmental conditions across microbial habitats (e.g. the rhizosphere, preferential water flow paths, animal burrows and intra- and inter-aggregate environments), there is no “typical” soil microbiome and the relative abundances of the major bacterial and archaeal taxa can vary considerably within just a few centimetres (Fierer *et al.*, Nat Rev Microbiol 2017; Bulgarelli *et al.*, Nature 2012).

The functions of soil microbes are still largely unknown. The available data suggest that the soil microbiota is a complex and dynamic ecological community that interacts with animals and plants and influences their physiology, health, production efficiency and behaviour. Host fitness is determined by the microbiomes associated with the plant rhizosphere and tissues in the root system and other plant organs. They support the plant’s nutrition, health and resistance to biotic and abiotic stress. These microbiomes are highly diversified and provide a plant-independent secondary genome that delivers key ecological functions favouring the fitness of the plant host (Rosenberg, Zilber-Rosenberg, MBio 2016). These interactions mean that the soil and plant microbiotas are key to the quality and safety of plant primary production, including fruits, and related processed foods (Gilbert *et al.*, Proc Natl Acad Sci USA 2014; Marasco *et al.*, Microbiome 2018). Italian researchers have investigated microbial resources from desert and arid environments as well as worldwide mycorrhizal fungi for the alleviation of abiotic plant stress.

The profile and functions of the microbiome in **food-producing animals (Figure 9)** have been investigated in a number of species. The use of advanced molecular methods in recent years has enabled a more detailed understanding of this area.

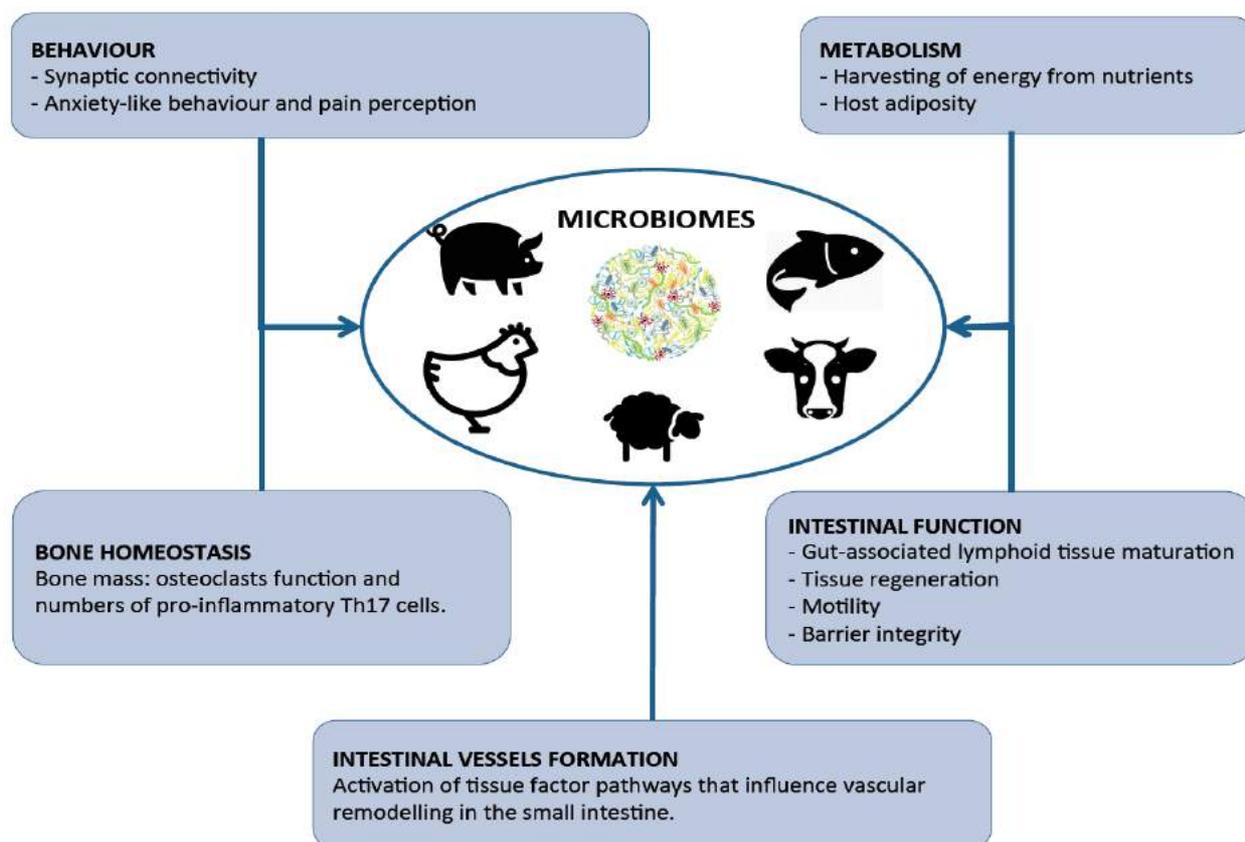


Figure 9. Factors of animal physiology and development affected by the microbiome.

It is now clear that animals host a wide diversity of microbial communities that have evolved alongside them as a result of complex and mutualistic interactions and play crucial roles in their biology and health (Walter and Ley, *Annu. Rev. Microbiol.*, 2011, Hooper et al., *Science*, 2012). The gastrointestinal microbiota offers a typical example of such an interconnected community (Hamady and Knight, *Genome Res.*, 2009; Savage, *Annu. Rev. Microbiol.*, 2003; Gill et al., *Science*, 2006; Sender et al., *Cold Spring Harbor Labs Journals*, 2016). However, the microbial communities of other anatomical sites have also been characterized in recent years. These range from more obvious sites such as the skin and the genitourinary tract, to less obvious ones such as the airways, and including areas that were previously considered to be devoid of microorganisms, such as the placenta and the fetus (Cao et al., 2014, Mor and Kwon, *Am. J. Obstet. Gynecol.*, 2015). The aim is to exploit this knowledge to facilitate the transition of livestock production from a system strongly oriented towards maximizing productivity to a more efficient system that is less dependent on the use of antibiotics, and hence more sustainable for the environment and for society.

Although different species seem to partly share their microbiota (Milani *et al.*, *ISME J* 2017), monogastric (e.g. pigs, chicken), ruminant (e.g. dairy cows, sheep) and lagomorph (e.g. rabbits) animals have well distinguished ecosystems that have co-evolved with their own microbiota and are characterized by species-specific enterotypes. This is due to their different food sources (e.g. herbivorous vs. omnivorous) and feeding behaviours. Several studies of rumen microbiota (Wallace *et al.* *BMC Genomics* 2015; Ben Shabat *et al.* *ISME J* 2016) have investigated their evident symbiosis with ruminants and their role in greenhouse gas production. However, there is a lack of information on pigs and poultry, and no significant relationship between the gut microbiome and animal phenotypes has yet been established (Stanley *et al.*, *Appl Microbiol Biotechnol* 2014; Yulixaxis Ramayo-Caldas *et al.*, *ISME J* 2016). Nonetheless, the available data suggest that the livestock

microbiota plays a significant part in the ultimate health, production efficiency and behaviour of animals.

Relevance of microbiomes in marine primary production.

Fish is an invaluable source of food and nutrition that offers an income and livelihood to communities worldwide. The worldwide fish consumption per capita reached 20 kg in 2014 thanks to the growth of aquaculture, which now provides half of all fish for human consumption (FAO 2016).

The microbial communities found in marine sediment and seawater ecosystems are numerically, taxonomically and functionally diverse (**Figure 10**).

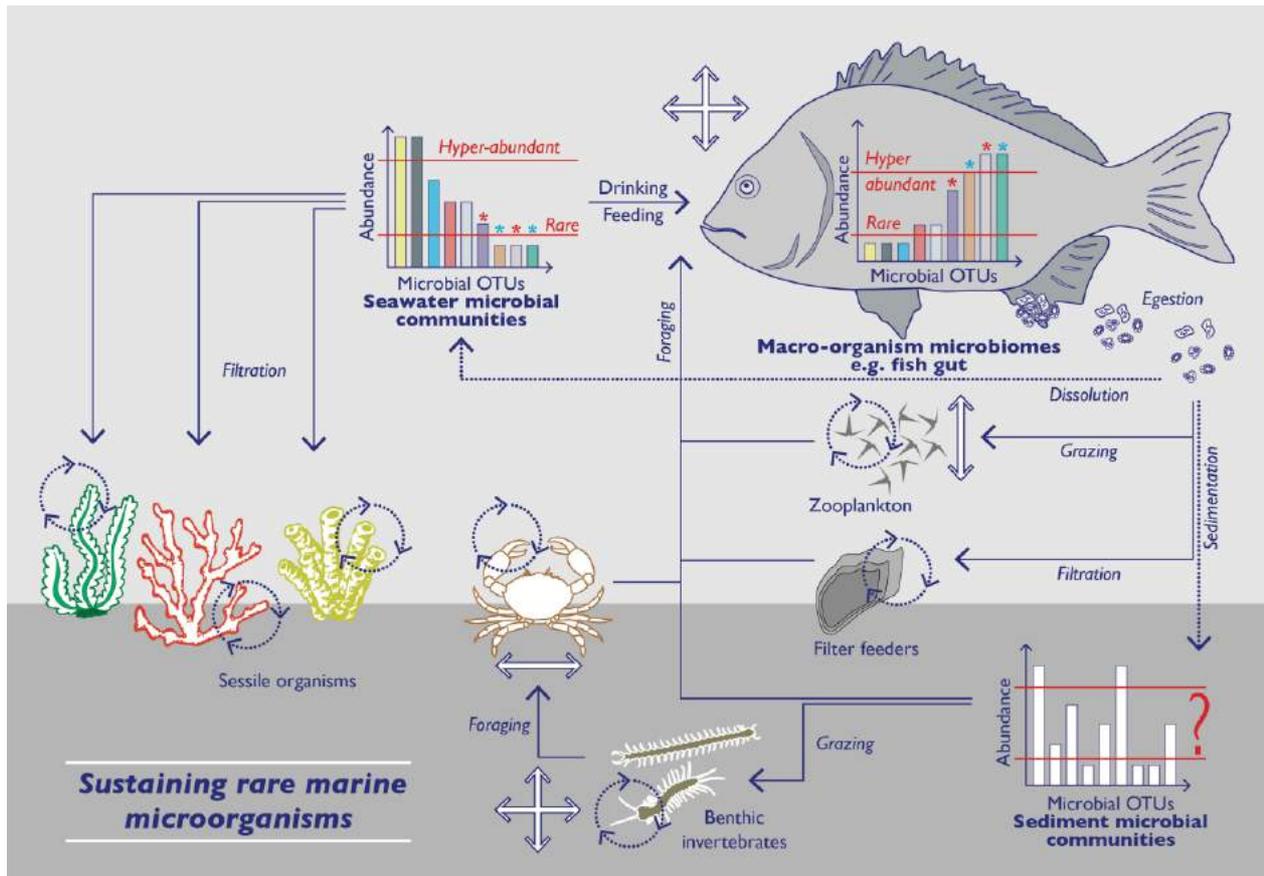


Figure 10. Sustaining marine microorganisms: schematic view of macroorganisms, and especially fish, as key contributors to the maintenance of microbial diversity.

Full arrows depict transfer of microbes through trophic processes and dashed arrows depict microbial release from macroorganisms and passive processes. Grey arrows correspond to the horizontal and/or vertical transfer potential of macroorganisms. Asterisks on the abundance distributions represent two types of host-associated microbes: symbionts/mutualists (blue) and opportunist copiotrophs (red). Question marks represent ecosystem compartments for which there is currently insufficient data to generalize the proposed mechanisms of the macroorganisms' impact on microbial diversity. OTU = operational taxonomic unit.

Based on: Troussellier M, Escalas A, Bouvier T and Mouillot D (2017) Sustaining Rare Marine Microorganisms: Macroorganisms As Repositories and Dispersal Agents of Microbial Diversity. *Front. Microbiol.* 8:947. doi: 10.3389/fmicb.2017.00947

These communities are the primary contributors to marine/ocean biogeochemical cycles and thus the drivers of other trophic levels, including aquatic animal production and health (Moran, Science 2015). The marine microbiome responds to many natural and anthropogenic environmental pressures (Bourlat *et al.*, Mar Pollut Bull 2013; Goodwin *et al.*, Front Mar Sci 2017; Buttigieg *et al.*, Curr Opin Microbiol 2018) and has been proposed as an ecosystem indicator to assess the quality and productivity of seawater and sediments (Nogales *et al.*, FEMS Microbiol Rev 2011).

Few studies have investigated the role of the microbiome in fish health, physiology, ecology and behaviour. Pioneering molecular studies have described the functional diversity of the sea bream microbiome (Xing *et al.*, FEMS Microbiol Ecol 2013; Ni *et al.*, FEMS Microbiol Ecol 2014), the role of the microbiome in the developmental stages of fish (Califano *et al.*, Front Microbiol 2017) and how nutrition affects the gut microbiome and, in turn, fish growth and efficiency (Parma *et al.*, Anim Feed Sci Tech 2016; Piazzon *et al.*, Microbiome 2017). Other studies have focused on developing effective strategies to manipulate gut microbial communities through diet, to promote fish health and improve productivity (Rimoldi *et al.*, PeerJ 2018; Rimoldi *et al.*, PLoS ONE 2018). Such strategies could contribute to the goal of antibiotic-free aquaculture production.

While there is a growing market demand for high-quality fish products, competition for land and water is increasing. Open-sea fish cages are thus key to the future growth of aquaculture. In fact, the main issue with coastal aquaculture is benthic organic enrichment caused by the settlement of solid waste (faeces and feed) on the seabed, which can lead to eutrophication problems in enclosed and semi-enclosed coastal environments (Price *et al.*, Aquacult Environ Interact, 2015; Ballester-Moltó *et al.*, Mar Environ Res 2017). Ultimately, the loss of marine environmental quality also affects aquaculture fish quality and productivity. Bioremediation technologies can be employed to prevent or mitigate such consequences.

Relevance of microbiomes in food processing and nutrition. Microbes in food and beverages are key factors in their ultimate quality, authenticity, nutritional properties, safety and preservation. Food microbiomes derive from natural contamination from primary food matrices and the environment (house microbiome) and from deliberately added natural or commercial starters, which undergo pressures from stochastic, deterministic and temporal drivers. Microbial fermentations are complex systems where bacteria, yeasts and filamentous fungi communicate and interact. These microbial contributions and activities enable raw materials to be processed into products with added nutritional value. Microbial fermentation is the most natural, sustainable, low-cost, functional and user-friendly biotechnology for the production of foods and beverages with improved shelf life, safety, nutritional, functional and organoleptic properties and health-promoting potential (Macori and Cottar, Curr Opin 2018; Filannino *et al.*, Curr Opin Biotechnol 2018) (**Figure 11**).

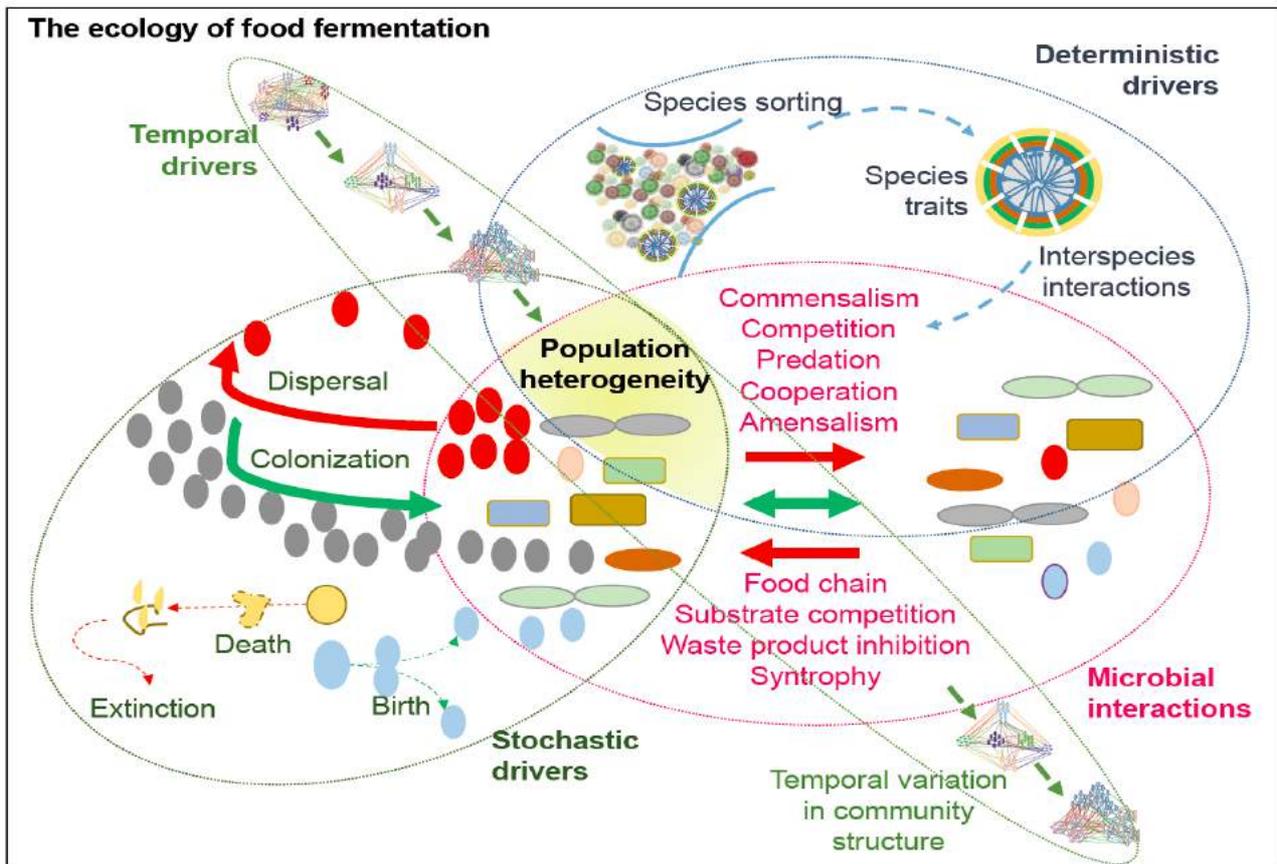


Figure 11. *The ecology of food fermentation.*

The most intriguing and promising prospect in food processing is the establishment, assembly and shaping of the microbiome. This plays fundamental roles in any food matrix, ranging from fermentation to contamination and spoilage (Filannino *et al.*, *Curr Opin Biotechnol* 2018).

The way food is produced and consumed has changed rapidly even over the last 20 years. Since the 1990s, food production and consumption has shifted predominantly to ready-to-eat foods for consumption both at home and elsewhere. It is predicted that many consumers will return to their kitchens in years to come, further modifying the food production–consumption paradigm, and become food producers in their homes, with increasing food manipulation through fermentation (Cocolin *et al.*, *Nat Microbiol* 2016). Most scientific evidence suggests that fermented foods should be included in national dietary recommendations (Chilton *et al.* *Nutrients* 2015), because they are sources of beneficial microbes able to synthesize bioactive or bioavailable metabolites/compounds.

From this perspective, understanding food microbiomes will be necessary for the better control of foodborne pathogens and the protection of public health. A coherent new vision of microbiome management in foods and beverages must consider the contribution not of individual aspects, but of the community as a whole. Microbial diversity should thus be investigated from an interactive community-based perspective rather than focusing on individual species.

4.2 Constraints, needs and opportunities in relation to R&I and policies

4.2.1 Microbiomes and host health

Expanding microbiome research to other body sites

Although the gut microbiome is the largest and most complex host microbial community, other body sites, including the mouth, skin, urogenital and respiratory tracts, have their own specific microbiome that is intimately associated with various physiological functions and is often involved in conditioning local or systemic host health. During development, the host microbiome follows a body-site-specific trajectory, with each body site developing a specific “biogeography”.

The oral microbiome is considered one of the most complex and variable host-associated microbial communities. Its complexity is due to the great number of distinct niches present in the oral cavity (Xu *et al.*, *Environ Microbiol* 2015), while its composition varies enormously between individuals and over time (Hall *et al.*, *NPJ Biofilms and Microbiomes* 2017). The finely tuned ecosystem in the oral cavity can shift to a dysbiotic profile, with a loss of community balance or diversity and just one or a handful of species predominating. Different studies have shown a relationship between changes in the oral microbiota composition and host health in relation not only to disorders strictly associated with the oral cavity (Kilian *et al.*, *Br Dent J* 2016), but also to systemic diseases (Jia *et al.*, *Br Dent J* 2018).

The skin also harbours a myriad of microbial communities. These live on the skin surface as well as in hair follicles and sebaceous glands (Belkaid and Segre, *Science* 2014). Microbes in the skin control the expression of various innate immune factors (Gallo *et al.*, *Nat Rev Immunol* 2012), limit pathogenic microbial invasion and reinforce the skin’s own stability and containment (Belkaid and Segre, *Science* 2014). However, their specific contribution to host health still needs further investigation.

The vaginal microbiome has a certain degree of stability and can be classified into discrete states during disease processes. Individual species of *Lactobacillus* as well as diverse additional anaerobic taxa tend to dominate the vaginal microbiota in asymptomatic subjects (Ma *et al.*, *Annu Rev Microbiol* 2012). The lactobacilli are believed to benefit the host by lowering vaginal pH and reducing allochthonous microbial colonization or pathogen invasion. Microbial composition varies over days to weeks within an individual subject (Ravel *et al.*, *Microbiome* 2013).

The respiratory tract is a complex organ system that reaches from the nostrils to the lung alveoli, and has a succession of ecological niches with distinct but related bacterial communities. Knowledge about the composition and function of these communities is still limited in comparison with other host microbiomes. The anterior nares harbour a relatively simple microbiota (Zhou *et al.*, *Genome Biol.* 2014), whereas the nasopharyngeal microbiota is more complex and dynamic. The latter undergoes important changes over time (Perez-Losada *et al.*, *PLoS One* 2017) and is a well-known reservoir for potential pathogens that cause respiratory tract infections.

The healthy lung sterility model has repeatedly been challenged since the introduction of culture-independent techniques (Dickson *et al.*, *Lancet Respir Med* 2014; Taylor and Wesselingh, *Cell Microbiol* 2016). From a functional perspective, the respiratory tract microbiome could help maintain the homeostasis of respiratory function by acting as a gatekeeper that resists colonization by respiratory pathogens and probably shapes local immunity (Man *et al.*, *Nature Reviews Microbiology* 2017). Understanding the respiratory microbial composition is particularly relevant in chronic lung diseases such as cystic fibrosis and chronic obstructive pulmonary disease (Huang YJ, LiPuma JJ. *The Microbiome in Cystic Fibrosis. Clin Chest Med.* 2016 Mar;37(1):59-67), in which personalized microbiome-modifying approaches could have an impact on disease progression.

Viromes

The term “virome” has been coined to describe the total collection of viruses in a given organ, usually at a single time point. It encompasses a sort of viral "flora" that includes bacteriophages, endogenous retroviruses and eukaryotic viruses, which might be unassociated with any disease or capable of causing acute, self-limiting, persistent or latent infections (Virgin *et al.*, Cell 2009; Focosi *et al.*, Clin Microbiol Infect 2016). Virome characterization is key to the investigation of disease transmission - a growing risk for public and veterinary health - across humans, wildlife and domesticated animals (Miller *et al.*, Sci Rep 2017; Olival *et al.*, Nature 2017). Bats and rodents have been established as the main reservoirs for emerging cross-species viral transmission (Han *et al.*, Science 2015; Luis *et al.*, Proceedings of the Royal Society B, 2013). For this reason, characterization of their virome is one of the objectives of the global virome project, which aims to describe the majority of currently unknown viruses (Carroll *et al.*, Science 2018). However, how viruses are transmitted between wildlife reservoirs and livestock animals is largely unknown, except in a few specific cases (Zhou *et al.*, Nature 2018).

The characterization and role of human and animal viromes in health and disease is largely unknown. It has now been demonstrated that viruses are present in the host not only in association with disease, but also in healthy conditions and in various sites. The recent application of metagenomic approaches to the characterization of human and livestock viromes has identified a considerable number of unknown viruses in healthy host conditions (Shan *et al.*, J Virol 2011; Lima *et al.*, J Gen Virol 2017; Day *et al.*, Virol J 2010). However, further studies are needed to expand and confirm the characterization of livestock viromes, including their involvement in NCDs such as IBD, diabetes and cardiovascular disorders.

There is also increasing evidence that virome fluctuations, interactions with the host, changes in the bacterial microbiome and environmental factors have profound effects on virome composition and host physiology (Freer *et al.*, Curr Med Chem 2017; Freer *et al.*, Front Microbiol 2018, Hurwitz *et al.*, ISME J 2015; Anderson *et al.*, Microbiome 2017) (Figure 12).

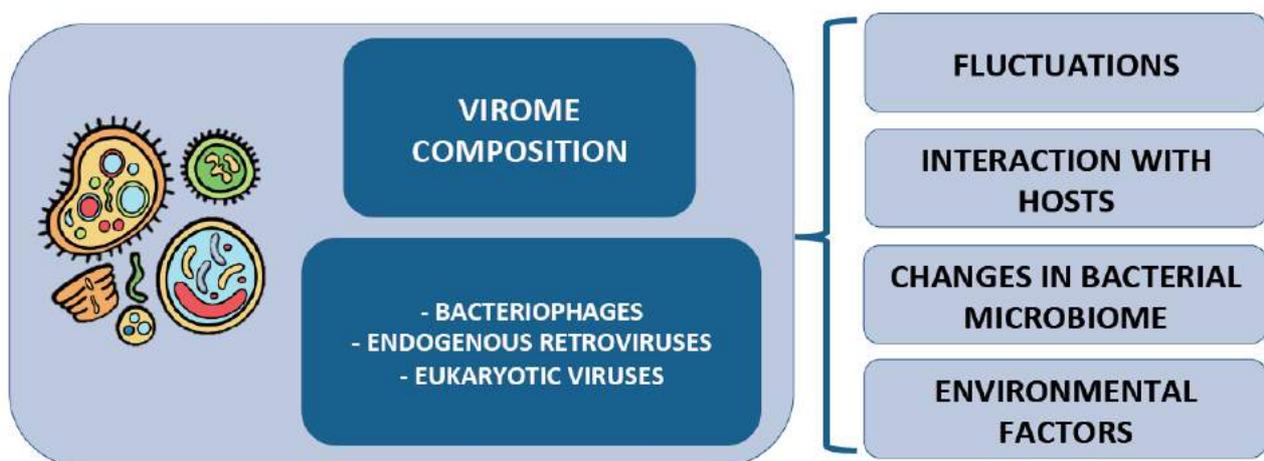


Figure 12. Increasing evidence demonstrates that virome fluctuations, interactions with the host, changes in the bacterial microbiome and environmental factors have profound effects on virome composition and host physiology.

Environmental factors include the modulation of livestock gut and ruminal viromes through the use of different diets. This could offer new prospects for livestock farming by

improving host health and productivity, but to date, only a handful of studies have investigated this opportunity, and only in ruminants (Anderson *et al.*, Microbiome 2017).

It was recently suggested that monitoring the intra-host dynamics of the human virome may be a promising area of research for the identification of new biomarkers predictive of an ailment or that help physicians to manage patients with a disease (De Vlaminck *et al.*, Cell 2013; Carding *et al.*, Aliment Pharmacol Ther 2017; Legoff *et al.*, Nat Med 2017; Albert *et al.*, J Clin Virol 2017; Focosi *et al.*, J Gen Virol 2015; Focosi *et al.*, J Infect Dis 2014). This could also prove a fruitful field of research in livestock animals, although no investigation into potential biomarkers of disease has yet been proposed. The in-depth and large-scale investigation of the virome and its evolution is therefore crucial to increase our knowledge of its composition, fluctuation and symbiotic relation with the host microbiome. Characterization of intra- and inter-host viral diversity could also vastly increase our knowledge of how variations in virome composition affect host health, as well as aid the discovery of new markers to monitor wellbeing and predict the onset and progression of diseases. Finally, more knowledge is also needed of the viromes of plants, fungi and marine organisms, whose impact is ever more relevant. All these areas of research are made possible by the advanced techniques available today.

Mycobiomes

The abundance of the different microbial species in the gut varies, which means that less common species can be overlooked. These rarer components include fungi, i.e. yeasts and moulds, or the “mycobiome”. The few available studies have consistently shown that healthy individuals have a low-biodiversity community that is dominated by yeasts (mainly *Saccharomyces* but also *Malassezia* and *Candida*) and that shows high temporal and inter-individual variability. Its composition is closely related to eating habits and lifestyle as well as to the bacterial component of the microbiome, which can modulate the fungal fraction both directly and indirectly, through modulation of the host immune system (Nash *et al.*, Microbiome 2017).

Research into the human gut mycobiome is increasing because it can act as a reservoir for opportunistic pathogens and is thus a risk factor for both gastrointestinal and systemic diseases, especially when the mucosal barrier is compromised (Iliev and Leonardi, Nat Rev Immunol 2017). Changes in the fungal community have been found in association with inflammatory bowel disease, colorectal adenomas, post-operative complications, *Clostridium difficile* infection, obesity, type-1 diabetes and various types of disorder in immunocompromised subjects (such as haematological and infectious diseases). Analysis of the gut mycobiome could be of particular relevance for women, especially when pregnant or lactating, when control of fungal infections is important for both their own and their child’s wellbeing. It may also be important for premature and underweight neonates. Antibiotic treatments can considerably increase the risk of imbalance in the mycobiome, thus contributing to the onset of specific mycoses (Huseyin *et al.*, FEMS Microbiol Rev 2017).

The mycobiome of livestock animals is considered a new field of research. The fungal community has been investigated in a handful of animals, including bats (Li *et al.*, Current Microbiology 2018), mice, pigs (Donovan *et al.*, PLOS ONE 2018) and ruminants (Dias *et al.*, Front Microbiol 2017; Ishaq *et al.*, Front Microbiol 2017), although to our knowledge, no consistent information is available. The role of the fungal community in livestock disease is still largely unknown, with the few studies mainly focusing on clinical mastitis (Wen *et al.*, Adv Microbiol 2018) and sub-acute ruminal acidosis (Ishaq *et al.*, Front Microbiol 2017) in ruminants.

Even though a large number of yeasts have been used as probiotics in livestock animals, with beneficial effects (Hatoum *et al.*, Front Microbiol 2012), there is still a lack of

information on their effect on the intestinal and ruminal mycobiome and their interaction with the microbial community. Specific studies of the fungal community in livestock have been conducted in relation to animal waste (Heaney *et al.*, *Sci Total Environ* 2015) and farm air quality for humans (Kumari *et al.*, *Sci Rep* 2016).

The role of fungi in the production of fermented foods should not be underestimated. *Saccharomyces cerevisiae* and other yeasts are responsible for alcoholic fermentation in wine and beer making, but also play a part in cheeses and fermented vegetables and meats. Yeasts and moulds are also powerful spoilers of food. A better understanding of the food mycobiome will therefore also enable better control over the spoilage processes, with a clear benefit for food producers, while also reducing waste.

Culturome research

A bottleneck in microbiome research is the difficulty of its translation to laboratory models for experimental verification. This is mainly due to problems with the in-vitro culturing of the majority of bacterial species and the difficulties of reproducing inter-microbial and microbe-host interactions in the laboratory (**Figure 13**).

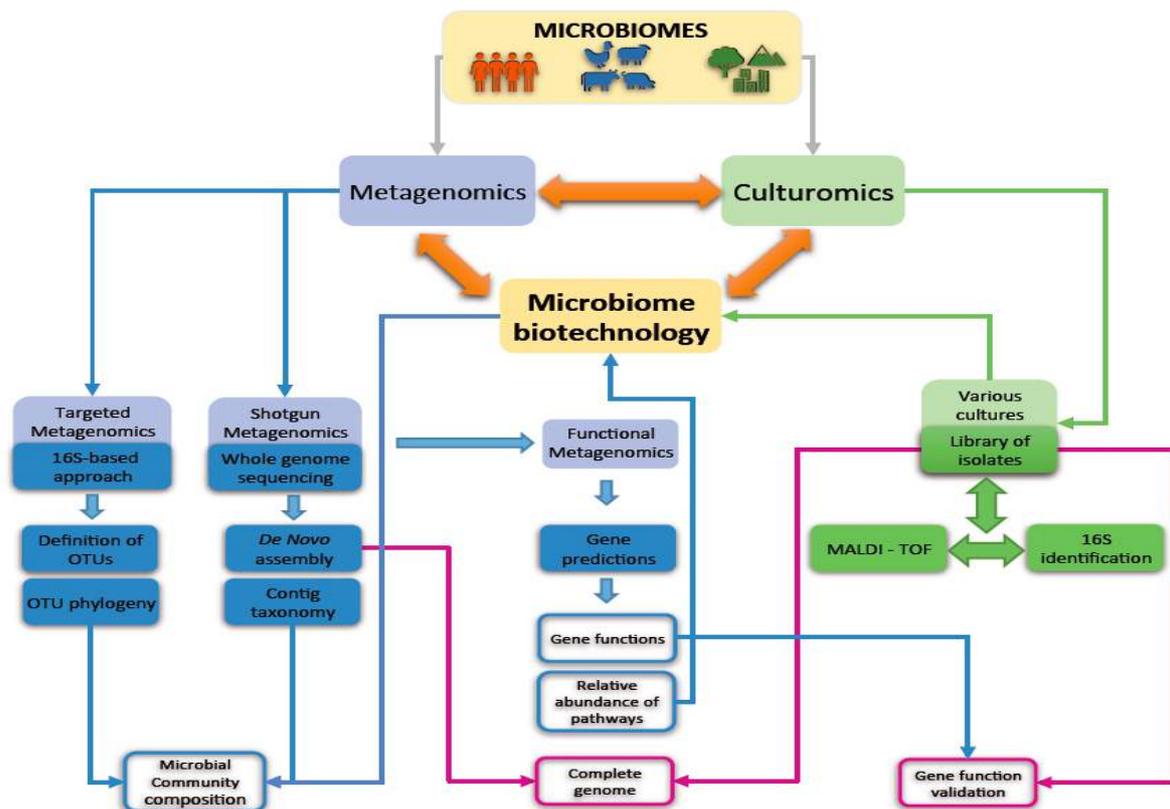


Figure 13. Workflow integrating metagenomic and culturomic analysis of microbiomes.

Just over 7,000 of the approximately 1 million bacterial species on Earth have been correctly classified to date (<http://www.bacterio.net/>). From the 11 phyla (the highest-level division within the bacterial kingdom) described in the 1980s, the number of bacterial phyla identified through metagenomic analyses has now grown to more than 80, the majority of which have never been cultured (Schloss *et al.*, *MBio* 2016). “Unculturability” is not an

intrinsic feature of these bacteria, but simply implies that our culturing techniques are still inadequate. Such “unculturability” does not therefore mean that they cannot be cultured, but instead suggests that we lack critical information on the biology of these species. This poses challenges, but also offers opportunities (Lagier *et al.*, Clin Microbiol Rev 2015). The study of “unculturable” bacteria could enable the investigation of the molecular aspects behind recalcitrant growth, enhancing our repertoire of microbiological techniques and allowing access to previously hidden metabolic diversity that will ultimately provide new natural products and reveal factors that contribute to ecological balance, nutrient cycles and/or host health.

The last decade has seen the development of several effective techniques for culturing these organisms. These “**culturomic**” approaches include:

(i) co-culturing with other (growth-promoting) bacteria, which has also revealed the molecular mechanisms of these beneficial effects;

(ii) bringing culture media and systems directly into the environment for in-situ cultivation of novel species; and

(iii) microscale cultivation in specific devices, which increases the isolation of “unculturable” bacteria by eliminating competition from bacteria sharing the same ecological niche.

With a large pool of new bacterial isolates potentially available for functional studies, it will be possible to elucidate the role of these highly diverse organisms in nature. Building modern strain storage infrastructures (biobanking) will be an essential step to preserve bacterial strain diversity (Bolan *et al.*, EMBO Rep 2016).

More importantly, successful cultivation of these novel bacterial species could have profound effects on human health. Screening of newly isolated strains could lead to the identification of new natural compounds that could ultimately support the antibiotic (and pharmaceutical) discovery pipeline, which has almost collapsed in recent decades. Culturomic approaches will also enable: the exploration of the close interactions between individual species and the host; insights into the dynamic relationships between the different components of ecosystems; investigation of the interactions between mutualistic bacteria, pathogens and host cells; the identification of inter-individual strain diversity and the status of ecosystems in different tissues and body districts; and clarification of the role of different strains in colonization resistance, as an important step in the development of prebiotics and probiotics (Lagier *et al.*, Nat Rev Microbiol 2018). Thus, in line with the One Health framework, the support of current and future “culturomic” efforts is expected to offer multiple benefits for health, ecology and science.

Resistome research

Antibiotic resistance (AR) is increasing alarmingly worldwide due to the indiscriminate use of antibiotics in human and veterinary medicine, farming and agriculture (O'Neill, AMR Report 2014). In Europe, AR is particularly strong in Italy, with a worryingly high frequency in both human and veterinary infections (ECDC; 2017; EFSA J, 2018). AR is natural in bacteria, for the simple reason that antibiotic-producing microorganisms must protect themselves against their own antibiotics in order to avoid suicide (Davies and Davies, Microbiol Mol Biol Rev 2010). All known bacterial pathogens have rapidly developed resistance to one or more antimicrobial compounds, suggesting the presence of a pre-existing pool of AR genes in ‘natural’ reservoirs and their transferability among microorganisms sharing the same habitat (Allen *et al.*, Nat Rev Microbiol 2010).

Although the downsides of AR are mainly clinical, this problem is not restricted to the clinic and should be addressed considering that the resistance gene flows both “from” and “to” natural environments, in line with the One Health framework. Most of the antibiotics given to humans are used in the household, and eventually end up in the sewage system.

More importantly, the use of antibiotics in agriculture and aquaculture leads to a significant increase in AR and to an accumulation of antibiotic-resistance genes (ARGs) in bacteria (Hu *et al.*, Front Med 2017). The environment is therefore the original source of AR and the reservoir of ARGs that form the antibiotic **resistome** (i.e. the collection of ARGs in a specific bacterium or ecological niche). To prevent the clinical spread of ARGs, it is necessary both to raise public awareness of the problem of AR and to trace reservoirs of resistance, in order to impede the dissemination of environmental ARGs (Laxminarayan *et al.*, Lancet Infect Dis 2013).

The recent development of high-throughput sequencing (HTS) tools that enable the culture-independent study of diverse environmental microbial communities has led to a relatively rapid growth in the cataloguing of ARGs (Wright, Nat Rev Microbiol 2007). However, HTS studies are often descriptive and need to be complemented by functional metagenomic approaches, to experimentally ascribe resistance functions to genes not previously identified as resistance determinants, or to discover resistance genes with novel sequences. Cryptic ARGs, i.e. those genes which are present but not expressed, as well as ARG precursors that encode for proteins conferring low-level resistance (i.e. capable of binding or modifying antibiotics with low affinity or specificity), should be identified through new algorithms, bioinformatic tools and appropriate databases. Cryptic genes and ARG precursors are probably the most dangerous determinants of resistance to next generation antibiotics, since they remain an unexplored source of new resistance mechanisms (Hu *et al.*, Front Med 2017; Garmedia *et al.*, Clin Microbiol Infect 2012). For future resistome studies, AR database harmonization, validation and accessibility are essential.

As research into AR expands, a proactive approach should be adopted to AR identification and surveillance and antibiotic therapy development. The antimicrobial development pipeline should include screening for existing environmental resistance threats to new drugs, before they spread.

Livestock production is a key area in the occurrence and diffusion of AR (Hu *et al.*, Front Med 2017; Marshall and Levy, Clin Microbiol Rev 2011), given the wide use of antibiotics since the 1950s as therapeutics, prophylactics, metaphylactics and growth promoters (AGPs). This issue is being addressed in Europe through prohibition of the use of AGPs (since 2006), and restriction of the uses of the antibiotic colistin (since 2015). ARGs can spread through animal handlers, the food chain and farm effluents (Forsberg *et al.*, Science 2012; McEachran *et al.*, Environ Health Perspect 2015; Noyes *et al.*, Sci Rep 2016). However, despite the body of evidence, the role played by livestock in resistome dispersal is still a matter of debate. The phylogenetic study of “resistomes” is therefore important to establish the boundaries of the ecological continuum of ARGs and to clarify the role of the livestock environment as an ecological corridor within these gene flows.

Another way to approach AR is to improve research into small bioactive molecules of microbial origin (the so-called *parvome*, according to Davies and Ryan, ACS Chemical Biol 2012). These molecules primarily include antibiotics, though several have a myriad of different targets and functions. Microbiome research should thus be combined with metabolomic investigations in order to assess the actual effect of this still elusive pool of microbial bioactive molecules, with the ultimate aim of developing new strategies to overcome AR.

4.2.2 Soils and sediments, sustainable agriculture and food processing

Microbiomes in terrestrial primary production

Due to the large heterogeneity of soil and environmental conditions across habitats and the lack of knowledge on soil microbe functions, the identification of specific links

between the composition of the **soil microbiome** and its functional capabilities is problematic. It is also difficult to predict the rates of any biogeochemical processes that might influence plant growth and nutritional properties. The use of simple markers such as bacterial-to-fungal ratios or phylum-level abundance is insufficient: there is a need to move beyond basic descriptions of community diversity and start identifying patterns in their complexity and recognizing when that complexity is important. While genomic data on the vast majority of undescribed soil microbial taxa are rapidly accumulating, there are still important gaps in our understanding of their functional attributes. Furthermore, the cultivation of many soil microbial taxa – particularly slow-growing ones – remains a difficult task, and this is adversely affecting the assessment of environmental tolerances, gene annotation, the quantification of enzyme kinetics and the identification of novel antibiotics or probiotics. Strategies are needed that use genomic data to identify conditions for the effective cultivation and isolation of difficult-to-grow microorganisms.

There is also a large knowledge gap in the roles and functions of the plant microbiome. It is now evident that plants adopt mechanisms for selective microbial recruitment from soil, hence sculpting the microbiome in their roots and, consequently, the rest of their organs (Lebeis *et al.*, Science 2015). Microorganisms are also known to contribute to immune function by alleviating stresses (Cherif *et al.*, Environ Microbiol Rep 2015) by means of specific mechanisms (Vigani *et al.*, Environ Microbiol 2018; Chialva *et al.*, New Phytol 2018). However, the study of such mechanisms is in its infancy and increased efforts are necessary to clarify the factors regulating the plant microbiome and its relationship with agricultural ecosystems.

Another area where greater knowledge is required is how the microbiome contributes to its plant host's adaptation to abiotic stresses, such as ongoing climate change (East, Nature 2013), heatwaves, soil salinity, flooding events and nutritional imbalances, and to biotic stresses. Microbiome components are known to influence the modulation of plant hormone homeostasis and the architecture of the plant root system, favouring the plant's response to water and nutrients from the soil, but more evidence-based knowledge is needed. The impact of the plant microbiome on the quality of plant products is virtually unexplored, although conceptually, it is thought to affect the quality of the fruit and related products. Finally, the inheritability of the microbiome through the plant's seeds is another aspect requiring further research.

In relation to **animal microbiomes**, more research is needed into the factors affecting the host microbiota and cross-talk between the animal's system and microbiota. Progress in this area is limited by the absence of specific databases. Reference gene catalogues of the pig gut and rumen microbiome were published in 2016 and 2018 respectively (Xiao *et al.*, Nat Microbiol 2016; Seshadri *et al.*, Nature Biotechnol 2018), while a catalogue for poultry is being compiled.

A key need is the definition of a *National database of animal microbiomes/viromes*. This will require the application of innovative metagenomic approaches (e.g. ITS-based sequencing; Terabase sequencing and quantitative metagenomics) and the definition of longitudinal studies to characterize the gut microbiome, including the virome, at different production stages and synergistically associate it with a wide number of host descriptors (e.g. metabolome). Such a database would significantly speed up the identification of biological predictors and new beneficial microbes (new probiotics) able to improve animals' natural resistance to pathogenic infections and their feed efficiency in terms of nutrient utilization, thus reducing production costs and the environmental impact of livestock production. There is also an urgent need to define a strategy for the prediction and investigation of the biological functions of gut microbiome molecules in different conditions, such as the use of different dietary strategies (e.g. high vs. low dietary protein; different sources of fibre) or optimal and sub-optimal animal health. It would also reveal key molecules from co-regulated microbiota-host pathways as potential therapeutic targets, in

line with the EU strategy for replacing some of the antibiotics currently used in livestock with new therapeutic molecules. Finally, it would be useful to develop a national strategy for the definition, classification and exploitation of the biodiversity that characterizes Italian production, in order to uncover its specific microbiomes and how they can be used to improve the national production system.

Equally, studying the integrated biological systems of each species is essential to enable manipulation of the animal microbiome in order to improve animal health, feed efficiency and food quality and safety. In addition to the direct effect of the microbiota on the host and vice versa, the correlation between the animal microbiota and its technological effects are of great interest. For example, milk microbiota has long been investigated for its role in milk processing. These microorganisms enter milk from a variety of sources and can play a number of roles therein, such as facilitating fermentation, causing spoilage, promoting health or causing disease. There is also a concern that the presence of antibiotic residues in milk leads to the development of resistance, particularly in pathogenic bacteria (Quigley et al., FEMS Microbiol. Rev. 2013).

Finally insects - the biggest subphylum of multicellular Eukarya on Earth - should be taken into particular account, given their importance to human health (e.g. as disease vectors), ecosystems (e.g. pollination) and the economy (e.g. impact on agriculture).

Microbiomes in marine primary production. The microbiome of marine habitats is correlated with the health and productivity of their ecosystems. A holistic evaluation of this relationship requires investigation of the community as a whole, including temporal, geographic, taxonomic and functional information. A more comprehensive characterization of the genetic pool of the marine microbiome and the broader use of functional genes as indicators are also needed. These will enable an understanding of a wider range of processes that shape the composition of microbial communities and the more reliable prediction of the impacts of such processes on local bacterioplankton production, diversity and abundance and, in turn, on the health status of fish and other marine organisms. More efforts are needed to better assess the role of marine communities in the marine biosphere and in primary production.

The unravelling of intricate host-microbe symbioses and the identification of core microbiome functions will be key steps for understanding and exploiting the microbiome's potential in fish culture and marine vertebrate health (Tarnecki *et al.*, J Appl Microbiol 2017). Exploiting the microbial communities inhabiting and interacting with fisheries to improve their productivity, quality, safety and sustainability has also been proposed as a strategy to mitigate the disruptive changes in the sector. However, a better comprehension of the microbiome's role in fish biology will enable the implementation and validation of informed actions to improve the fish microbiome through the use of probiotics, prebiotics and feed supplements. The timing of these actions needs to be calibrated in order to optimise performance, in terms of improved fish health and food chain productivity/quality. They must also be integrated with interventions targeting microbiomes from wastewaters and sediments, thus increasing the overall environmental sustainability of the food system as well as delivering improved food quality, productivity and safety.

The in situ stimulation of indigenous marine bacteria from the marine microbiomes (including microbiomes from marine holobionts, such as fishes, shellfish, corals and sponges) is a promising new approach. These bacteria can degrade a wide range of natural organic compounds, such as lipids, proteins and carbohydrates. In this scenario, the "wild" microbiomes will be used both as bioindicators of environmental health and as new sources for next-generation applications of blue-technology approaches, such as the bioprospecting of marine microbiomes with biodegrading potential and their use in microbiome bio-activators to enhance the degradation of organic matter, hence mitigating its environmental impact and improving sustainability.

Microbiomes in food production and nutrition. In foods and beverages, even minor alterations to species/biotype diversity or numbers may result in significantly different products and variations in their safety, nutritional, functional and organoleptic properties and shelf life. A microbiome composition that is temporally and spatially stable and resilient thus provides the consistent fermentation and process conditions that are needed to produce high quality foods and beverages (Marco et al. 2017. *Current Opinion Biotechnol.*). The establishment, assembly and shaping of the core microbiome in foods and beverages is only possible through an extraordinary access to complementary genetic information based on omics techniques that reveal their overall composition and functionality. Omics-based approaches provide detailed insights into the food microbiome and its relationship with safety and quality attributes as a whole. Their use will reveal not only the genomic essence of a microbiome, but also if a food production facility eco-niche will influence the core microbiome and whether the overall core microbiome forges food production (Notebaart, et al. 2018. *Current Opinion Biotechnol.*). Omics platforms dedicated to the establishment, assembly and shaping of the food core microbiome will be fundamental tools for the improvement and standardization of protocols for the production of foods and beverages, with a focus on biotechnology transfer to small and medium enterprises (Magnúsdóttir et al., 2017. *Nature Biotechnol.*). The role of the food microbiome in health and in progression to chronic degenerative diseases requires further investigation, and should include the contribution of overall lifestyle and eating patterns to the final outcomes. This implies that the microbiome resulting from the eating pattern as a whole must be studied and this, in turn, requires new approaches for data collection and analysis.

4.3 Actions required to boost microbiome application in Italy

There is a major need to standardize the approaches used to analyse microbiomes, as poor reproducibility is often a critical issue. Improved and standardized methods are required for:

- i) experimental design,
- ii) selection of molecular analysis techniques,
- iii) data analysis and the integration of multiple omics data sets.

These would minimize noise and bias, enable more robust conclusions to be reached on the impact of the gut microbiome on human health and enable the development and implementation of more robust microbiome-tailored applications both in the clinical sector and throughout the food system.

Italy also needs improved technological tools for the characterization of microbiomes. While the compositional structure of microbiomes can be investigated with cost-effective 16S rRNA sequencing (for bacterial components) or ITS-based sequencing (for fungi), metabolomic approaches are essential for the comprehension of microbiome functionality, metagenomics, metatranscriptomics and metaproteomics. These require terabase sequencing, to explore phylogenetic resolution down to strain level, and quantitative metagenomics, to quantify microbiome variation. Microbiome studies also require robust data banking, bioinformatics and multidimensional statistics as well as new infrastructures, repositories and tools for the storage, organization, sharing, extraction and use of data and specific information from microbiome studies across the globe.

The sector would therefore greatly benefit from the establishment of a national network of experts, research centres and infrastructures. This would also benefit the health sector, where it would allow researchers to:

- collect and provide data on the baseline microbiota profiling of healthy Italian subjects of different ages, with different lifestyles and in different body districts,;
- identify new microbiome biomarkers of disease and the development of dedicated diagnostic tools;
- test any proposed new microbiome biomarker in the context of multimorbidity, to ensure that it retains its diagnostic and discriminative properties in real-life, often elderly patients with multimorbidity;
- develop new therapeutic approaches involving microbiomes;
- establish metagenomics/metaproteomics/metabolomics-based drug discovery campaigns;
- identify previously neglected lifestyle, dietary and environmental drivers of dysbiosis;
- build a comprehensive demographic database (dataset) obtained by processing samples using standardized methods and parameters for biostatistics;
- define, characterize and provide data on nutritional protocols and exercise programmes that can improve the microbiota profile;
- assess Mediterranean diet factors promoting a healthy microbiome structure;
- standardize the storage of microbiota profiling data, to be made available to clinicians, microbiologists and omics microbiota scientists;
- analyse the entire host virome, in order to characterize currently undefined viruses and their role in determining the host's immunological status;
- assess the impact of probiotic formulation on gut microbiota eubiosis and host health;
- characterize next generation probiotics;
- evaluate nutritional properties in novel and traditional foods according to the changes they induce in gut microbiota taxa and/or functions;
- analyse the role of the microbiome in drug metabolism, efficacy and side effects;
- further investigate the impact of microbiomes on cancer immunotherapy;
- monitor antibiotic resistance in the microbiome and the diffusion of antibiotic resistance in Italy, to be prioritized at least in critical settings.

A national microbiome network would also help boost microbiome-based terrestrial and aquatic primary production and the food system, based on local natural assets and higher added value crops, livestock products and foods in Italy. This requires:

- The development of research-driven understanding of how soil, marine sediment, water, plant and animal microbiome systems protect Italian primary production against nutritional, biotic and abiotic stresses and of applications to improve the sustainability, quality and nutritional and health-giving properties of its products and the food and feed originating therefrom;
- The evaluation of how plant, livestock, fish and food microbiomes contribute to the “typicity” of Italian primary productions and foods, and the championship of healthy, balanced Italian microbiome structures, in order to promote their added value in international markets.

The creation of this national network of experts and infrastructures would also help considerably in interconnecting the different pillars of the microbiome food system, exploiting its potential and capitalizing on the strengths and skills found in Italy.

Finally, an interdisciplinary network on microbiome knowledge and potential would also be essential to:

- Create public awareness of the importance of microbiome-based medicine, primary production and food products and the opportunities they offer. This could also involve the main Italian and European scientific societies;
- Educate and train researchers as well as support their careers, mobility, and development of skills in relevant microbiome sectors, taking into account ethical and regulatory issues, to ensure the highly qualified workforce needed to underpin an advanced, robust industry based on knowledge of microbiomes.

5. Conclusions

Microbiome research has delivered many interesting basic findings and advanced techniques, enabling this discipline to become a beacon of hope with great social and economic potential in Europe and further afield. To date, the European Commission has funded 216 projects under FP7 and Horizon2020 to advance knowledge of and the exploitation of microbiomes, but Italy's role has so far been minor. This is mostly due to a lack of coordination among the main national experts and institutes active in the sector and a lack of joint initiatives. Following the recommendations of the International Bioeconomy Forum, the next FP Horizon Europe is very likely to fund the transition from current microbiome research to the microbiome economy.

A national initiative coordinating and leveraging on existing expertise and facilities in the wide microbiome domain will increase Italy's competitiveness in both basic science and translational applications, its ability to attract forthcoming European and international funding opportunities, and its prospects in terms of jobs and the country's sustainable and competitive growth.

The initiative presented herein proposes a coordinated national initiative to assess the areas in which Italy's existing knowledge, resources and biobanks might give it an advantage over other countries. This would involve the following actions:

- a) establishing a national network of experts and public and private organizations working in the sector in order to pool the existing national data, knowledge, infrastructures and skills in relation to the microbiomes and viromes of humans, foods, plants, terrestrial and aquatic animals;
- b) fostering their cooperation with the private sectors within the frame of the Italian health and agri-food technology clusters;
- c) developing and implementing joint R&I initiatives and policies by promoting investment in R&I and spin offs/start-ups in the sector, and facilitating their interconnection with international networks to encourage the application of microbiome know-how as well as to promote best practices, standards and consistent protocols;
- d) promoting researcher careers, training and mobility, and the development of skills in the different microbiome domains; and
- e) exchanging knowledge across the scientific and political community and ensuring the efficient use of the available resources, while advocating public understanding of the value of microbiomes for the health of the planet and all the humans, plants and animals that live on it.

This initiative should also help promote knowledge of microbiomes and their potential in the Mediterranean area through the PRIMA (<https://ec.europa.eu/research/environment/index.cfm?pg=prima>) and BLUEMED (<http://www.bluemed-initiative.eu/>) initiatives, both coordinated by Italy, for a more

sustainable and productive bioeconomy, greater social cohesion and greater political stability in this region.

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