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Relationship between the gut microbiome and diseases, including COVID-19

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Toussaint B., Raffael B. (co-authors), Petrillo M., Puertas Gallardo A., Munoz Pineiro A., Patak A., Querci M.

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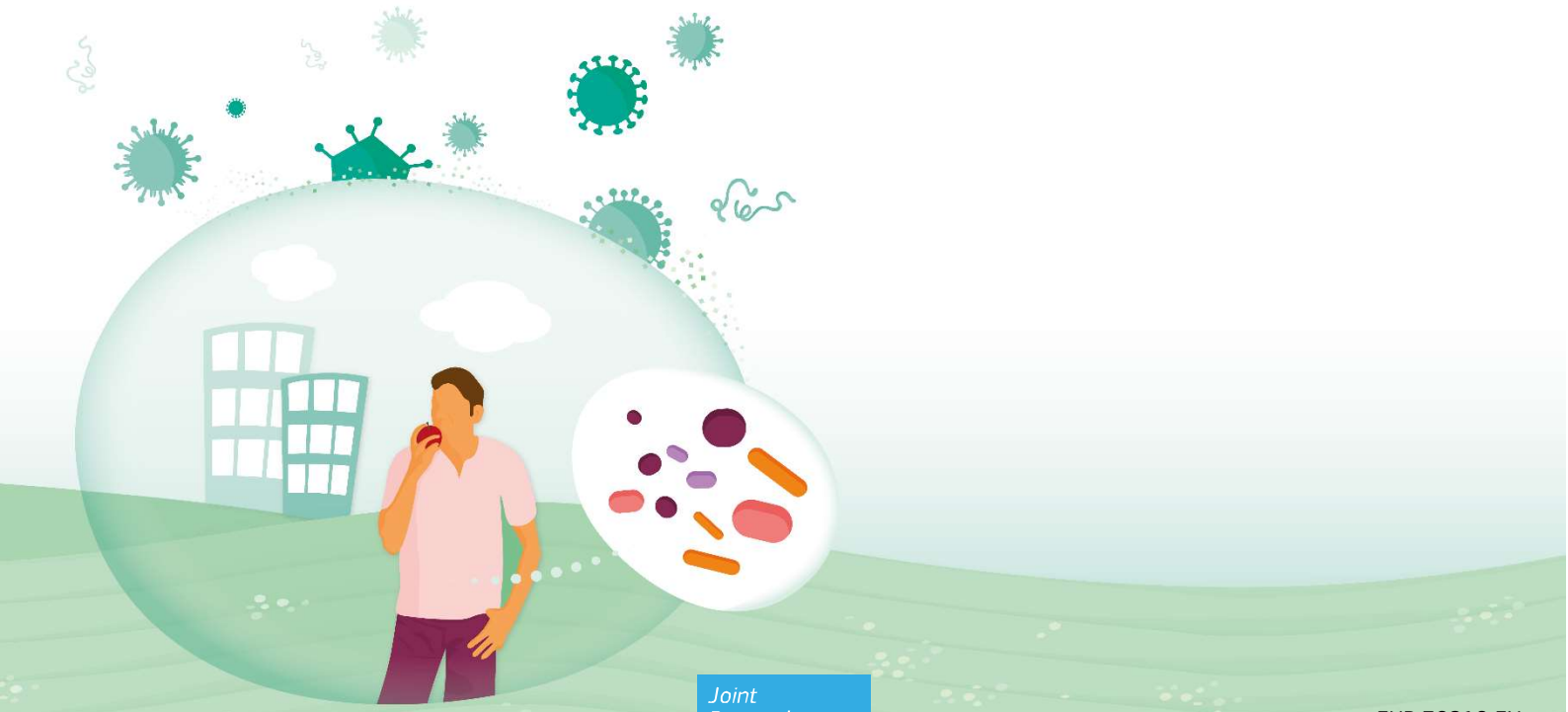

Joint Research Centre

EUR 30810 EN

Contact information
Name: Brigitte Toussaint
Address: Retieseweg 111, 2440 Geel, Belgium
Email: Brigitte.Toussaint@ec.europa.eu
Tel.: +32 14 571 339

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Contents

Foreword

In the last ten years the interest on the microbiome has been exponentially increasing. From mere presence into our body, the microbes that compose the microbiome have started to be seen as an important key factor for the wellbeing of their host. While more and more functions and roles of the microbiome are discovered, researchers started to study the relations and the effects that microbiome status has on human health. Still, the exact composition, the mechanisms of actions and all the implications of microbiome interactions with the human body are not well known and understood. Nevertheless, microbiome importance in some key roles deserves proper attention and exploration.

Acknowledgements

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Authors *(in alphabetical order)*

Amalia Munoz Pineiro, European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

Alex Patak, European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

Mauro Petrillo, Seidor Italy s.r.l. Past affiliation (until 15 June 2021): European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

Antonio Puertas Gallardo, European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

Maddalena Querci, European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

Barbara Raffael, European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

Brigitte Toussaint, European Commission Directorate General Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Knowledge for Health and Consumer Safety Unit

Abstract

Gut microbiome has often been named 'the second brain'. However, the recent outbreak of COVID-19 disease has reinforced the attention paid to this relatively mysterious 'soup' of beneficial microbes. The gastrointestinal symptoms associated with SARS-CoV-2 infection and the diagnosis of an associated gut dysbiosis along with long-term symptoms, have drawn the attention of the scientific community. The known communication axis between the gut microbiome and several organs of the human body have been related to the multi-organ disorders observed in severe COVID-19 infections. The perturbation of the immune system and the inflammatory cytokine storm are also put in perspective with the gut microbiome role on immunity and inflammation regulation.

This technical report tries to shed light on the roles, known and less known, of the gut microbiome and to reflect on the most recent scientific investigations by the review of more than 70 scientific papers since 2010. The question of the use of pre- and pro-biotics in our diet is also discussed as more scientists suggest that it could sustain, reinforce or restore the gut microbiome homeostasis and eventually help controlling the gastrointestinal symptoms of the COVID-19 disease. Many questions remain open, especially about the molecular mechanisms of the observed clinical effects. Both current and future clinical studies need to be deeply mined and pursued to bring the evidence behind the observations. A better knowledge on the role of microbiome during immune and inflammatory responses, and microbiome's impact, together with diet, on health are expected to be ultimate for the next-generation of co-treatment of respiratory diseases as well as immune and inflammation diseases.

The content of this report can also be visualised in two factsheets (short and long versions) entitled "Relationship between the gut microbiome and diseases, including COVID-19".

1 Introduction

Microbiome is a word that we hear more and more, and it is often exchanged with the word "microbiota".

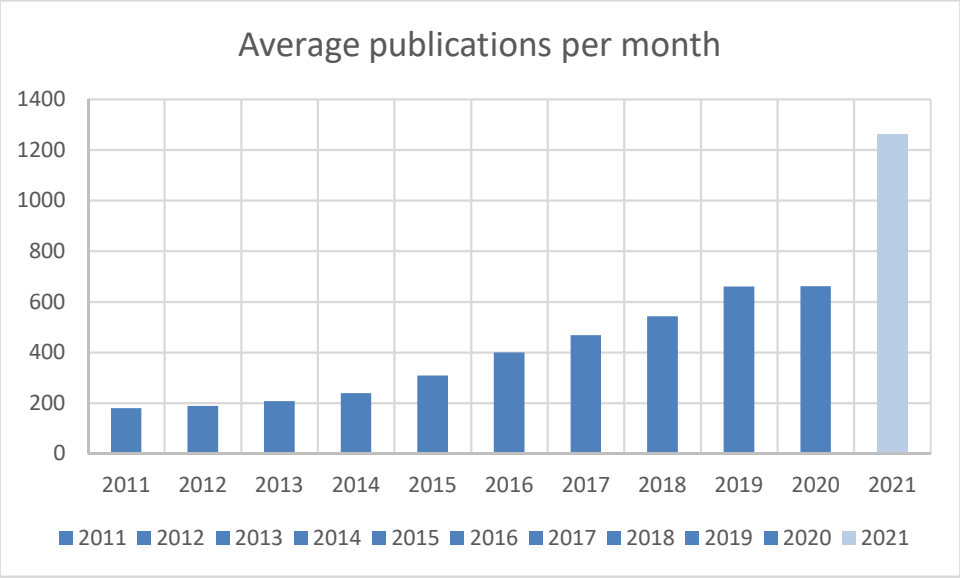
Although both terms, microbiome and microbiota, are used, sometimes interchangeably, there is a slight difference between the two:

- According to the Human Microbiome Project, the microbiome is "the collective genomes of the microbes (composed of bacteria, bacteriophages, fungi, protozoa and viruses) that live inside and on the human body" [**1**]. The microbiome is thus the whole environment comprising the microorganisms and their "theatre of activity" (structural elements, metabolites/signal molecules, and the surrounding environmental conditions)" [**2**].

- Whereas, the microbiota only "comprises all living members forming the microbiome" [**2**]. This refers to the taxonomy of the microorganisms, which always live in community and comprise very diverse species.

In the last 10 years the interest on the microbiome in the scientific community has exponentially grown, as the number of papers published on the topic. Analysing the scientific papers that can be found in PubMed and Scopus, 2011 saw the publication of 2,271 studies, while in 2020 the number of published papers on microbiome related topics rocketed up to 31,888, and in the first three month of 2021 already 10,450 studies have been published (Fig. 1).

Figure 1. Number of studies on microbiome over the last 10 years, reported as an average per month per year (2021 includes the months of January, February, March)



The consequence of all the scientific research behind these studies is that now more information about the microbiome, its roles in the human body, the effects of its perturbations, its links to several diseases and ways to promote its equilibrium have been discovered. Still, a lot is unknown, and many mechanisms of action are not completely understood.

1.1 Scope description

The relationships between the gut microbiome and diseases are more and more discussed in the scientific community, in particular in some severe outcome of the COVID-19 infection. A better knowledge on the molecular mechanisms of the gut microbiome and viruses and how a healthy gut microbiome can protect the

intestinal barrier and stimulate the immunity could have extremely high impact on potential co-treatment of virus infection, for example to limit multi-organ side effects and secondary infections.

Moreover, the use of pro- and prebiotics (and of a healthy diet) to reinforce the gut microbiome is discussed as a potential co-treatment for immune and inflammatory diseases. Clinical studies have shown a change in the composition of the gut microbiome in several cases of chronic diseases with inflammatory symptoms, such as obesity, diabetes, and asthma, which are extremely common in our European population. In the last years, there is a growing interest in that research area and still many gaps in understanding the mechanisms of interactions. But again, the potential impact of such co-treatments could be very high.

This document reports known data that are key to understand what the role of the microbiome in human health is, how it can be supported, and the still existing relevant/important gaps. We are convinced that within a short time, more clinical studies will bring essential information that should be carefully analysed.

1.2 Search strategy

This report is based on literature searches performed on the Scopus[1], ScienceDirect[2], EuropePMC[3] and National Centre for Biotechnology Information (NCBI)[4] repositories at the beginning of 2021.

The search string that was used is:

(("2019-nCoV" OR "2019nCoV" OR "COVID-19" OR "SARS-CoV-2" OR "COVID19" OR "COVID" OR "SARS-nCoV" OR ("wuhan" AND "coronavirus") OR "Coronavirus" OR "Corona virus" OR "corona-virus" OR "corona viruses" OR "coronaviruses" OR "SARS-CoV" OR "Orthocoronavirinae" OR "MERS-CoV" OR "Severe Acute Respiratory Syndrome" OR "Middle East Respiratory Syndrome" OR ("SARS" AND "virus") OR "soluble ACE2" OR ("ACE2" AND "virus") OR ("ARDS" AND "virus") OR ("angiotensin-converting enzyme 2" AND "virus")) AND "microbiome" AND "gut" AND "inflammation")

The search was limited to articles available in English. A total of 552 articles were collected, including review articles. From this collection, a selection (a short list) was made of relevant papers containing the name of at least one disease or one chemical compound described in "The Human Gut Microbiota: Overview and analysis of the current scientific knowledge and possible impact on healthcare and well-being" [**3**].

[1] https://www.scopus.com/
[2] https://www.sciencedirect.com/
[3] https://europepmc.org/
[4] https://www.ncbi.nlm.nih.gov/

2 The gut microbiome

Most of the human microbiome (95%) resides in the intestines, considered not only as component of the gastrointestinal (GI) tract, but also as the "largest human immune organ" [**4**].

The total number of bacteria (composing 99.9% of the microbiome) in an average man (70 kg) is estimated to be $3.8x10^{13}$, the same order of magnitude as the human cells, and their total mass is about 0.2 kg [**5**]. However, these estimates don't take into consideration fungi, viruses and phages present in the body (< 0.1% of the microbiome). This little part of very diverse species of microbes is named the 'rare biosphere' [**6**]: Fungi are the major component of the rare biosphere and form the *mycobiome* (the mycobiome represents 0.01 – 0.1% of the gut microbiome [**7**], with *Candida albicans* being the most abundant [**8**]). Consequently, the number of total microorganisms found in the human GI tract has been predicted to exceed 10^{14}, which in terms of genome (microbiome) represents 100 times the human genome.

The exact number of microbial species present in the human body (gut, lung, skin) is difficult to estimate with accuracy and therefore only approximations are available. Among other reasons, this depends greatly on the DNA extraction methods used to identify the microbial species, which have an impact on their recovery [**9**].

With respect to bacteria, the human body would contains 500-1,000 different species [**10**]. The gut microbiome alone would contain approximately 160 species [**11**]. Firmicutes and Bacteroidetes are predominant in gut (they also preponderate in the lungs, together with Proteobacteria [**11**]). The most frequently found and abundant bacteria belong to the *Oscillospiraceae* family (which includes the *Faecalibacterium prausnitzii*), and of the *Lachnospiraceae* family. However, genome sequences of many species of the human gut microbiome remain unknown [**12**].

The gut microbiome composition is influenced by the diet, age, sex, environmental factors and genetics [**1**,**13**]. Therefore, there is up to now no standard profile for a healthy gut microbiome. Only relative changes in bacteria species presence and quantities, observed via faecal analysis, can be achieved.

3 Role of the gut microbiome in the human body

Important functions such as digestion, synthesis of vitamins and degradation of toxins are supported by the intestinal microbiome that forms the microbiome-gut axis [**4**]. But the gut microbiome is not only fundamental for these functions, as it also modulates:

- the gut permeability,
- the immune response,
- the inflammatory response,
- the inter-organ communication. [**4**,**14**,**15**,**16**,**17**].

If the gut permeability increases, the intestinal barrier becomes permissive allowing for example bacterial endotoxins produced in the gut to pass into the blood and potentially cause sepsis or multi-organ complications [**18**,**19**,**20**]. Changes in the lung microbiome with increase of bacteria normally found in the intestinal tract were also observed [**21**]. Moreover, a modified intestinal barrier can potentially allow viral infection spreads and microbes' translocations through the circulatory and lymphatic systems [**22**,**23**].

The gut microbiome also plays a role in modulating the immune system [**24**]. Several gut common bacteria are known immunomodulators (i.e. *Faecalibacterium prausnitzii, Eubacterium rectale* and bifidobacteria) [**16**]. Receptors of the small intestine membrane are involved in the recognition and tolerance of commensal bacteria versus pathogenic bacteria. This recognition seems to be part of a learning process during maturation of the immune system. Researchers have also described and reported the impact of the intestinal microbiota on the respiratory immunity, affecting the responses of respiratory epithelial cells and the exposure of antigens during respiratory virus attack [**25**,**26**,**27**].

Besides, the gut microbiome has an impact on the inflammatory response to infections. Although mechanisms are not fully elucidated, some of the metabolic sub products produced by the gut bacteria, called the Short-Chain Fatty Acids (SCFA) have anti-inflammatory properties [**28**]. In case of unbalanced microbiome and opportunistic infections, a cascade of inflammatory response has been observed [**11**]. Among the plethora of bacterial species present in the gut, eleven groups of similar ones (called microbial operational taxonomic units, OTUs) were observed as significantly associated with the increase of inflammatory cytokines [**29**]. This influence is suggested to be driven by faecal metabolites: in particular 45 faecal metabolites (mainly within those of the categories of amino acids, fatty acids and bile acids) showed significant associations with more than half of the selected microbial OTUs. It has been proposed that they might play a key role in mediating the effect of the core gut microbiota on host metabolism and inflammation [**29**].

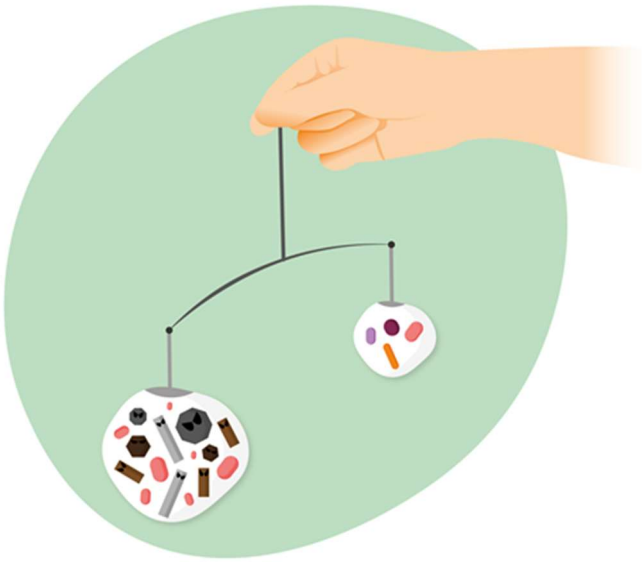
Finally, in the human body, organs communicate through cytokines, immunological, hormonal, and neuronal signals [**4**]. The routes of communications behind these signals are usually referred to as "communication axes". The gut microbiome has been reported as involved in the communication among different organs:

- The gut microbiome and the liver form the so-called "microbiome-gut-liver axis" [**30**,**31**].
- A reciprocal communication exists between the intestinal microbiome and the central nervous system through the "microbiome-gut-brain axis" [**31**].
- The "microbiome-gut-lung axis" is considerably discussed for bacterial and viral infections, as the intestinal microbiome amplifies the alveolar macrophage activity having a protective role in the host defence against pneumonia [**13**,**26**].
- A potential "microbiome-gut-heart axis" [**32**] and the molecular pathways on gut microbiome roles linking immunity, infection and cardiometabolic diseases are discussed [**33**].

4 Effects of gut microbiome perturbation

A *dysbiosis* occurs when the usual bacteria species presence and quantities in the GI tract are altered (e.g. when the number of harmless bacteria decreases and the number of opportunistic bacteria increases). This has an impact on the gastrointestinal function potentially causing GI symptoms such as nausea, vomiting and diarrhoea.

Exposure to environmental factors, including diet, toxins, drugs, and pathogens can cause dysbiosis or at least a flattening of microbiome diversity [**34**]. The microbiome diversity also decreases with ageing [**35**].



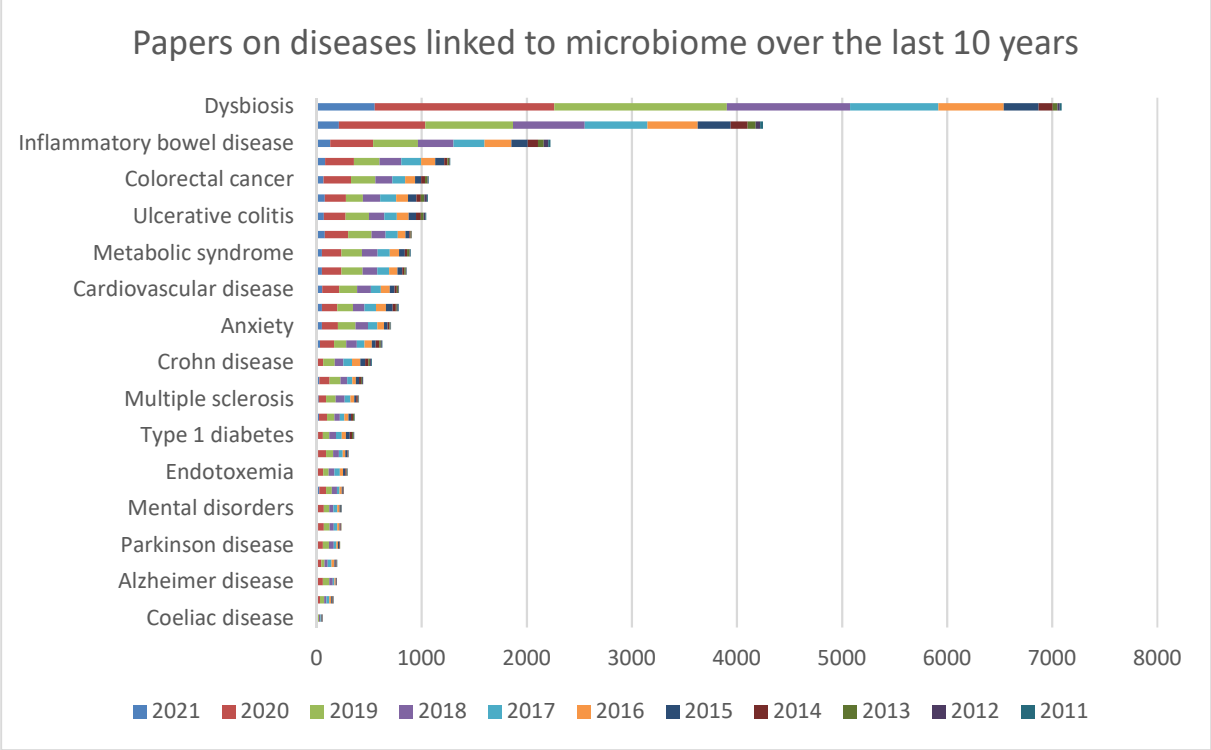
In turn, dysbiosis could favour the appearance of disease states such as infections, immune-mediated diseases such as allergy and auto-immune disorders [**13**]. Dysbiosis could also worsen underlying diseases (e.g. rheumatoid arthritis, different types of cancer, diabetes mellitus, obesity, cardiovascular diseases – through the gut-heart axis -, asthma – through the gut-lungs axis -, and metabolic, autoimmune, and neurodegenerative disorders, such as Parkinson's disease – through the gut-brain axis). [**4**,**36**,**37**,**38**,**39**,**40**,**41**,**42**,**43**,**44**]. In the case of obesity, visceral fat, which is already in a pro-inflammatory state in patients with dysmetabolic syndrome, acts as an enhancer of inflammation, being an uncoupling link between the intestine and systemic and pulmonary inflammation through the mesenteric lymphatics [**45**].

Altered gut microbiome composition has also been associated to autism spectrum disorders severity [**46**].

The diseases that are most associated to the status of microbiome, according to scientific studies, are reported in Fig. 2, where the growing interest over the past 10 years is also indicated.

It was demonstrated that the microbiome can be the site of viral replication, i.e. Hepatitis E virus [**47**], and scientific literature now converges on assuming that also severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can exist and replicate in the guts (including in the faeces) [**4**,**16**,**17**,**48**,**49**,**50**,**51**,**52**] even after disappearance of the respiratory symptoms or in the absence of GI symptoms.

Figure 2. Diseases that are most frequently associated to microbiome in scientific studies, over the last 10 years (in 2021, only the three first months)



4.1 Gut microbiome and COVID-19

Several studies assume an important role of the gut microbiome in the response of the immune system towards respiratory viral infections [**14**] and many recent ones now link the status of microbiome to COVID-19. From a search in Scopus[5] and PubMed[6], it is possible to see that, in 2019, 5 papers on microbiome and SARS-CoV-2 or COVID-19 have been published, in 2020 the number of publications increased to 1,232 and only in the first three months of 2021 the number of published papers reached 836.

4.1.1 SARS-CoV-2 and dysbiosis

Many studies tend to confirm that links exist between SARS-CoV-2 infection and gut dysbiosis. Understanding whether COVID-19 severity is primary cause, secondary cause, concomitant cause or consequence of gut dysbiosis is still under debate. In the case of SARS-CoV-2 infection, dysbiosis and GI symptoms have been observed in most patients during hospitalisation, since the infection until recovery [**48**,**49**,**53**]. In the case of COVID-19, the relative abundances of bacteria in the gut microbiome have thus been compared in patients suffering from SARS-CoV-2 infection and in healthy individuals. Pilot studies report that the faecal microbiome of infected patients was significantly modified compared with controls, with an enrichment of opportunistic pathogens and a depletion of beneficial bacteria, at all times of hospitalization [**16**,**17**,**48**]. Those alterations persisted even after clearance of SARS-CoV-2 (determined from nasopharyngeal swabs) and resolution of respiratory symptoms. Moreover, the gut alteration showed different levels of intensity that

The faecal analysis of COVID-19 patients showed that microbiome changes persisted up to 30 days after disease resolution [**16**]. In addition, the clinical state of COVID-19 patients deteriorates significantly more in presence of GI symptoms [**48**,**55**]. On the other hand, dysbiosis (or at least a decrease in microbiome diversity) can occur due to other factors, such as age, diabetes, obesity, cardiovascular diseases, etc. Interestingly, those factors seem also to lead to a more severe COVID-19 outcome [**56**].

[5] https://www.scopus.com/search/form.uri?display=basic#basic
[6] https://pubmed.ncbi.nlm.nih.gov/

4.1.2 SARS-CoV-2 effect on intestinal barrier

Using an intestinal SARS-CoV-2 infection model on a chip, researchers found that SARS-CoV-2 is potentially able to compromise the intestinal barrier, and that levels of bacterial endotoxins increase in patients with excessive inflammation and comorbidities [**20**]. The disruption of the gut barrier integrity linked to dysbiosis could possibly lead to translocation of SARS-CoV-2 from the lung to the intestine via the circulatory and lymphatic systems [**57**]. Inversely, lung microbiome was found to be enriched with bacteria from the intestinal tract and correlated with long-term symptoms [**21**].

Dysbiosis associated to COVID-19 and increasing gut permeability, could allow toxins produced in the gut to pass into the blood and potentially cause multi-organ complications [**18**,**19**] or increase existing liver, pancreatic, cardiac and neurological disorders (such as Parkinson's disease [**37**]). Indeed, multi-organ failures were observed in COVID-19 patients, such as liver, kidney and pancreatic injuries [**30**,**58**]. Also, COVID-19 patients with cardiac involvement had elevated markers of gut leakage and inflammation activation [**32**].

4.1.3 SARS-CoV-2 and inflammation

The highest SARS-CoV-2 mortality and morbidity have been reported in older patients and in those with underlying chronic diseases that are associated with inflammation, (such as hypertension, obesity, diabetes mellitus, and coronary artery disease) [**28**,**36**,**48**,**56**]. The visceral fat, which is already in a pro-inflammatory state in patients with dysmetabolic syndrome, acts as an enhancer of inflammation, being an uncoupling link between the intestine and systemic and pulmonary inflammation through the mesenteric lymphatics [**45**].

The gut alteration showed different levels of intensity that correspond to faecal levels of SARS-CoV-2 and to the severity of the infection [**16**,**29**,**48**] and to elevated blood concentration of inflammatory markers [**16**,**29**]. Bacteria species from the Bacteroidetes phylum (*B. dorei*, *B. thetaiotaomicron*, *B. massiliensis*, and *B. ovatus*) decreased in the faeces during SARS-CoV-2 infection, sometimes even in the proportions of COVID-19 severity [**48**]. *Faecalibacterium prausnitzii* (an anti-inflammatory bacterium) also decreased [**48**]. Inversely, bacteria such as *Coprobacillus*, *Clostridium ramosum*, and *Clostridium hathewayi*, increased with COVID-19 severity [**48**]. Notably, Bacteroidetes also decreased in patients negative to COVID-19 but with chronic diseases associated with inflammation, such as hypertension, obesity, diabetes mellitus and coronary artery disease, as well as in the older patients [**48**]. As if COVID-19 and chronic inflammatory diseases had cumulative effects, the highest SARS-CoV-2 mortality and morbidity have been reported in patients suffering from both [**28**,**35**,**48**].

The molecular mechanisms of the inflammation in relation with the gut microbiome have been investigated. In a recent clinical study, the presence of 20 blood proteomic biomarkers has been used to establish a Proteomic Risk Score (PRS) which predicts the progression to severe COVID-19 in 31 infected patients. In a set of 990 individuals without infection, this proteomic risk score was positively associated with proinflammatory cytokines mainly among older, but not younger, individuals. Furthermore, those 20 blood proteomic biomarkers have been correlated to the presence of a core set of 20 groups of gut bacteria (called microbial operational taxonomic units, OTUs) in 301 individuals. These OTUs were mainly assigned to *Bacteroides* genus, *Streptococcus* genus, *Lactobacillus* genus, *Ruminococcaceae* family, *Lachnospiraceae* family and *Clostridiales* order. Eleven of those OTUs were in turn significantly associated with the increase of 10 inflammatory cytokines in another set of 366 individuals. This study might suggest an implication of the gut microbiome in the predisposition of older patients towards a severe outcome of the COVID-19 disease [**29**]. This influence is probably driven by faecal metabolites. In particular, 45 faecal metabolites (mainly within the categories of amino acids, fatty acids and bile acids) showed significant associations with more than half of the selected microbial OTUs. It has been proposed that they might play a key role in mediating the effect of the core gut microbiota on host metabolism and inflammation [**29**]. Among the 31 COVID-19 patients, 10 % increment in the protein risk score (calculated based on the 20 protein biomarkers) was associated a 57% higher risk of progressing to clinically severe phase [**29**]. In another study, faecal microbiota alterations were also associated with faecal levels of SARS-CoV-2 and COVID-19 severity [**48**]. Moreover, in a study on the composition of the gut microbiota of COVID-19 patients, the concentration of the proinflammatory factor IL-18 was found to be higher in COVID-19 patients (but not in seasonal flu patients), and the microbiota pattern in COVID-19 patients seemed to be positively correlated with a high expression of IL-18 [**19**,**54**].

4.1.4 SARS-CoV-2 and immunity

The impact of the gut microbiome on the global immunity is also being discussed intensively. Gut microbiota harbours a multi species community with a strong impact on host immune homeostasis. However, our knowledge about this gut microbiota and its symbiotic relationship with immune activation in association with SARS-CoV-2 has not yet fully exploited. For example, it seems that there is a fragile equilibrium between T cell-mediating immunity becoming either victim of SARS-CoV-2 or actor of the systemic cytokine storm caused by SARS-CoV-2, especially in adults [**59**]. Those immunity mechanisms during the starting phase of SARS-CoV-2 infection deserve future research efforts.

In a small clinical study, several gut bacteria with known immunomodulatory effect, such as *Faecalibacterium prausnitzii*, *Eubacterium rectale* and bacteria of *Bifidobacterium* order, were underrepresented in stool samples collected from 27 Covid-19 patients. Those lower levels persisted up to 30 days after disease resolution. Moreover, the gut composition was perturbed up to certain degrees that correspond with the disease severity and with elevated concentrations of inflammatory cytokines and blood markers (C reactive protein, lactate dehydrogenase, aspartate aminotransferase and gamma-glutamyl transferase). Therefore, the study suggests that the gut microbiome is involved in the magnitude of COVID-19 severity possibly by modulation of the host immune responses. Furthermore, the gut microbiota dysbiosis after disease resolution could contribute to persistent symptoms, highlighting a need to understand how gut microorganisms are involved in inflammation and COVID-19 [**16**].

5 Can we protect the gut microbiome homeostasis?

As there is no standard composition of a healthy microbiome, relative comparisons from faecal analyses can help defining sets of bacteria and of their metabolites commonly and abundantly present in healthy or sick individuals.

Many scientists agreed that targeted modulation of the gut microbiota might have a therapeutic use [**48**] and that the idea of using probiotics as complement in the treatment of COVID-19 should be discussed and evaluated [**14**].

What are probiotics and prebiotics?

Probiotics are live microorganisms (mainly bacteria) that, when administered in adequate amounts, confer a health benefit on the host [**60**].

Prebiotics are non-digestible food ingredients stimulating the activity and growth of probiotics after colon fermentation. They are nutrients for the gut microbiome and their degradation products are short chain fatty acids (SCFAs) [**61**,**62**,**63**].

Synbiotics are mixtures of pre- and pro-biotics [**62**].

Over the years, a growing interest on the effects of probiotics on the health of gut microbiome is reflected in the growing amount of scientific literature published on the topic: from the 94 papers that can be found in PubMed and Scopus in 2011, as many as 2,744 publications can be retrieved in 2020.

The pre- and pro-biotics mechanisms of gut modulation might be explained either via direct interaction with the intestinal immune and epithelial cells or via indirect modulation by the intestinal microbiome [**62**]. For example, probiotics would enhance the intestinal epithelial barrier, compete with pathogens for nutrients and adhere to the intestinal epithelium, producing antimicrobial substances and modulating the host immune system (both innate and adaptive) [**62**].

Nowadays, stronger data are collected in favour of their clinical use in the prevention of gastrointestinal disorders, antibiotic-associated diarrhoea and reduction of allergy and respiratory infection symptoms [**31**,**51**,**64**]. For example, beneficial microorganisms such as *Lactobacillus rhamnosus CRL1505* are involved in the improvement of respiratory antiviral defenses [**27**]. More and more scientists suggest the beneficial role of probiotics in gut and lung immunity and mental health through modulation of the gut-lung and gut-brain axis and underline the need for further investigation [**31**,**64**,**65**]. Some prebiotics like galacto-oligosaccharides can also favour persistence of bifidobacteria in older patients and increase anti-inflammatory IL-10 while reducing pro-inflammatory cytokines (including IL-6, IL-1β and TNF-α) [**51**].

The use of antibiotics in clinical treatment of various infections is often essential in patients. However, it is now well recognised that antibiotics (particularly broad-spectrum antibiotics) can adversely affect the balance of the resident gut microflora resulting in dysbiosis, or microflora imbalance, of the gastrointestinal tract and can therefore act as an immune suppressor. For this reason, it is common use to prescribe probiotics when strong antibiotic treatment is given.

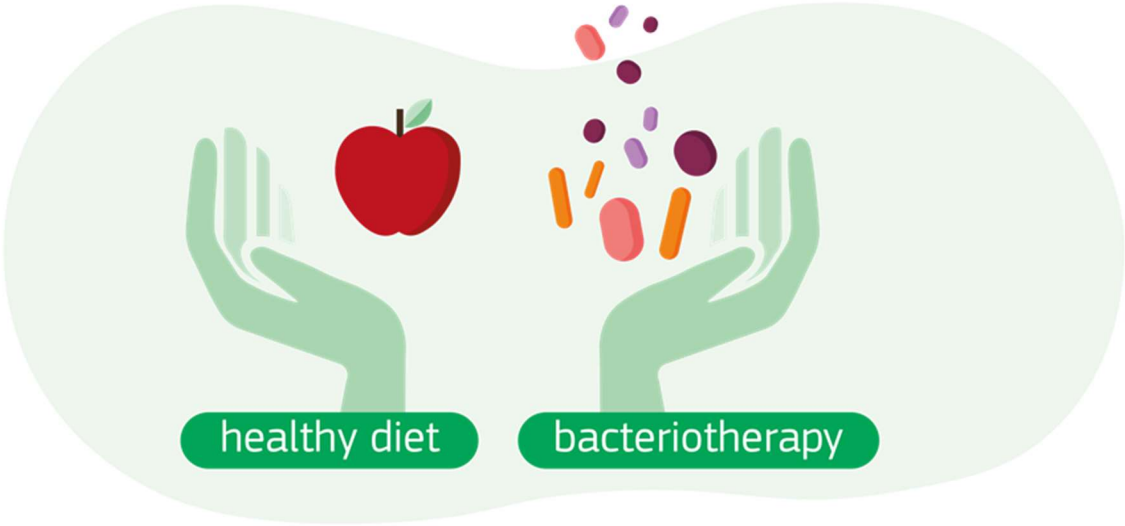
As the gut microbiota can be modulated by diet, a personalized diet could be implemented as a complement to current therapies. This diet would include fermented foods and pre- and pro-biotics which will play an important role in strengthening the overall immune response, especially for older and immune-compromised people [**28**,**35**,**49**,**65**,**66**,**67**].

Even if the mechanisms involved in the action of the gut microorganisms on the inflammatory response and with the virus are still not fully understood and would need further studies [**14**,**16**,**29**,**35**,**48**,**68**], scientists suggest the use of pre- and pro-biotics (the term "bacteriotherapy" is sometimes used) to help maintaining or restoring a healthy gut microbiome, not in the aim to cure COVID-19 but to help the microbiome to play its usual regulation role and reduce GI symptoms and, eventually, to prevent secondary infections [**13**,**28**,**35**,**49**,**51**,**66**,**69**,**70**]. The use of pre- and pro-biotics is not at all presented as curative against COVID-19 but their inclusion into our diet could help reducing gut inflammation, support mucosal immunity and possibly reduce the severity or the duration of the infection [**65**].

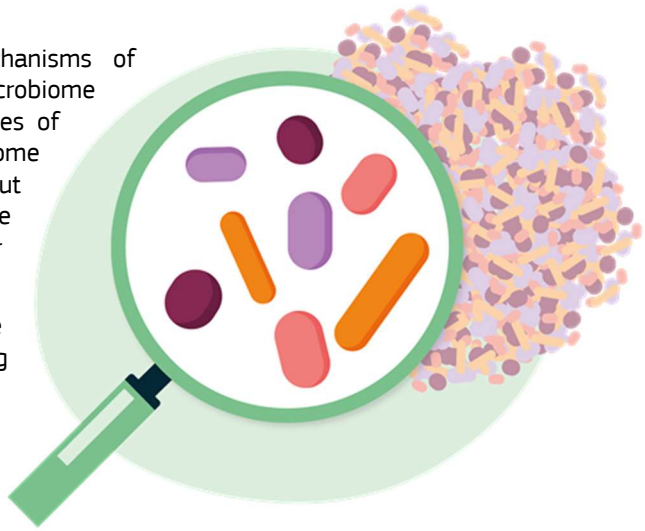
Besides, some dietary changes to promote a healthy microbiome are also investigated. A healthy diet should procure an appropriate daily intake of Vitamin A, C, D and E, B6, B12, folate which are recommended to

reinforce the immune system, as well as zinc, copper, selenium, iron, omega-3 fatty acids and amino acids [**48**,**71**]. Sufficient protein intake is also crucial for optimal antibody production [**61**]. Moreover, phytochemicals (such as carotenoids and polyphenols), dietary fibres [**61**,**72**] and omega-3 fatty acids [**28**] interact positively with the microbiome. In particular, dietary fibres have clear anti-inflammatory effects [**61**,**72**]. They are fermented by certain gut bacteria in the colon (*Bacteroides spp., Bifidobacterium spp. and Prevotella spp*., but also *Streptococcus spp., Firmicutes, Clostridium spp.* and many others) and metabolised into SCFAs [**61**]. SCFAs, including acetate, propionate and butyrate have been observed to regulate host metabolism, immune system, and cell proliferation [**4**,**28**,**61**,**71**,**73**]. It has been hypothesised that they play a key role in neuro-immunoendocrine regulation. However, the underlying mechanisms have not been fully elucidated [**73**]. Dietary seaweeds also contain numerous components that can exert antioxidant, anti-inflammatory, and antiviral effects, directly and indirectly, by improving the gut microbiota [**74**].

Unhealthy diet has often been correlated with chronic inflammation, and this can influence the immune system. On the contrary, a healthy diet rich in cereals, whole grains, legumes, fruits and vegetables helps the gut microbiome to fulfil its functions [**72**,**75**].



6 Research and knowledge gaps



Knowledge gaps still exist concerning the mechanisms of interaction between pathogenic viruses and the microbiome and vice versa, as well as on the diverse influences of environmental and individual factors on the microbiome composition. Communications between the gut microbiome and other organs are suggested but the exact influence of bacteria species and their metabolites is still under investigations.

The main gaps that could be extrapolated from the analysed papers are summarised in the following paragraphs, with a focus on COVID-19.

6.1 Diagnosis of viral infection

Some scientists proposed to use the change in microbiome profile occurring during SARS-CoV-2 infection as a signature to distinguish COVID-19 from influenza or for early diagnostic, as gastrointestinal symptoms can precede respiratory symptoms, and even for prediction of a severe evolution. They discuss the "potential value of the gut microbiota as a diagnostic biomarker and therapeutic target for COVID-19" [**68**]. They propose a personalised medicine where "clinical trials may characterize baseline individual microflora and their genetic pattern of responses upon probiotic introduction, therefore revealing the potency of probiotic application in human disease prevention and treatment" [**13**]. However, the large variations of microbiota composition among different populations makes it extremely difficult to standardise a 'healthy microbiota' and further validations studies are needed [**68**,**69**]. The potential value of the gut microbiota as a diagnostic biomarker and therapeutic target for COVID-19, was suggested (but further validation is needed) [**68**].

6.2 Mechanisms of microbiome interaction

One of the first questions to elucidate is how the intestinal bacteria interact in response to SARS-CoV-2 infection [**28**]. To answer this question, it is necessary to further determine the gut microbial composition (the microbiota), its metabolites and its role in the COVID-19 susceptible and asymptomatic populations [**76**]. The mechanisms that lead to dysbiosis are not accurately known. And inversely, the gut microbiota effect on the disease severity is being explored in a few studies.

Then, it is necessary to identify the different phases of the infection and progression of the disease, from the intestinal symptoms and modification of the gut barrier permeability to the dysregulation of the immune system and the multiorgan disorders. Pathogenesis of SARS-CoV-2 infection shares significant similarities to those of some immune-mediated diseases, such as inflammatory bowel diseases or rheumatoid arthritis, leading to the hypothesis that targeted therapies used for the treatment of immune-mediated diseases could be effective to treat (and possibly prevent) the main complications of COVID-19 [**77**]. Deeper investigations into innate, humoral, and T cell-mediated immunity during the critical first weeks upon SARS-CoV-2 infection are necessary [**59**].

If the mechanisms of entry of SARS-CoV-2 into the intestinal tract, and the disease progression are elucidated, it is possible then to design new treatments targeting gut microbiota that might modify the evolution of COVID-19 disease [**28**]. Likewise, a deeper understanding of how to modulate the gut microbiome (eventually through the diet, the use of prebiotics and probiotics, or faecal transplants) is fundamental [**28**]. Better comprehension of the mechanisms of the probiotic efficiency in respiratory diseases for example could favour their use as prophylactic or field therapy [**31**]. The use of modified or engineered intestinal bacteria or metabolites is also a proposed alternative.

6.3 Personalised medicine

Due to the population genetic diversity, scientists even propose that a region-based, microbiome genome-based, personalised food or engineered bacteria or even metabolites of bacteria could be proposed in parallel to other current treatments, to help restoring a healthy microbiome, with anti-viral and anti-inflammatory effects. Indeed, the genomics data suggest that a specific variant of SARS-CoV-2 gets enriched with the specific demographic region. Overall, demographic data suggests that host influences mutation and expression of the virus [**13**,**28**,**67**]. Additionally, "managing the patients" intestinal leakage and microbiota dysbiosis may be also relevant, namely using nutritional measures with prebiotic and/or probiotic activity, including bioengineered probiotic strategies able to deliver pharmacological agents [**51**].

7 Evolution of policies and other initiatives

In 2014, European Parliament member Sergio Paolo Francesco Silvestris addressed a question to the European Commission about the relationship between gut microbiota and diseases such as diabetes, Crohn's disease, cirrhosis and obesity. The Commission's answer mentioned some evidence of a role of gut microbiota on obesity in rodents but underlined the lack of understanding of the difference in gut microbiota of obese and non-obese individuals and a lack of understanding of the impact of the diet on the gut microbiome composition. The Commission even mentioned a lack of evidence of cause-effect relationship and of clinical relevance to humans [**78**].

Obviously, since that time, more clinical studies brought evidence of a cause-effect relationship between diet and gut microbiome as well as between gut microbiome and several diseases in humans. Still the mechanisms and the interactions are not well understood but are actively investigated in the scientific community.

In May 2021, a STOA online workshop entitled "Health and economic benefits of microbiomes" was organised by the EPRS (European Parliamentary Research Service) Scientific Foresight Unit [**79**]. The meeting highlighted the importance of microbiomes in human, animal and environmental health, including possible mitigation effect on pollution and climate change. The related threats of antimicrobial resistance (AMR) and healthcare-associated infections (HAIs) were also mentioned. Microbiomes have also an important role to play in economics, in particular by improving livestock animal health and soils composition, thus reducing the use of antimicrobials and pesticides.

The World Economic Forum (WEF), the Food and Agriculture Organization (FAO) and the Organisation for Economic Co-operation and Development (OECD), have recognised the potential applications into circular bio-economy. Microbiomes can substantially contribute to Sustainable Development Goals and EU Green Deal ambitions.

There is still a need to better understand the mechanisms of interactions of those multiple influences, to conduct clinical studies and produce robust data on large-scale. These requirements correspond with the One-Health approach and with the concept of personalised medicine that take into account the complexity of individual health history, environmental exposure, and sometimes microbiome modifications at different lifetimes.

However, there is currently a lack of EU legislation directly applied to microbiomes and microbiomes are not subject to the EU food law risk assessment requirements.

In this perspective, EFSA is mandated to assess risks to human, animal and environmental health from substances linked to food and feed production. EFSA is currently working on new scientific information, looking for cause-effect relationships and molecular mechanisms [**79**]. It will also publish the research questions the EU and Member State levels need to address from a regulatory perspective, such as the link between microbiomes and diet/toxicology. EFSA launched a thematic grant in March 2020 on this topic to collaborate with EU Member States [**80**]. It will allow planning future EU research with risk assessment perspectives.

Other challenges include a standard description of a healthy microbiome, design and validation of standard analytical procedures, agreed criteria to establish causal pathways and data sharing.

The U. S. Food and Drug Administration (FDA) has issued a guidance document for industry about the Chemistry, Manufacturing and Controls (CMC) of Investigational New Drug Applications (INDs) among which are Live Biotherapeutic Products (LBPs) [**81**]. It has also worked on the risks assessment of Fecal Microbiota Transplantation (FMT) [**82**]. FMT consists in implanting a liquid suspension of intestinal microbiota extracted from the stool of a healthy donor into the gastrointestinal tract of the recipient. This treatment restores the recipient's intestinal flora and increases bacterial diversity, helping to achieve an optimal function of the intestinal system. The mechanism of action is not completely understood and even causes confusion for a possible standardisation. Indeed, FMT is considered as a medicinal product in UK, as a biological product in North America, and as a human cell/tissue product in Europe [**83**].

Othmar Karas, Vice-President of the European Parliament (PPE, Austria) and STOA Panel member, pointed out that despite a number of tools such as the circular economy action plan, the Farm to Fork Strategy, and the EU4Health programme, a lack of EU regulation persists around microbiomes and these gaps must be filled: 'Now we need to act' [**79**].

8 Conclusions and future perspectives

Many knowledge gaps concerning the mechanisms of interaction between viruses, including SARS-CoV-2, and the microbiome and vice versa, as well as on the diverse influences of environmental and individual factors on the microbiome composition still exist. Communications between the gut microbiome and other organs are suggested but the exact influence of bacteria species and their metabolites is still under investigations.

Gut microbiome effects on COVID-19 severity and mortality rate and, furthermore, the therapeutic effect of a proper diet on COVID-19 infection are a 'good' hypothesis. Further research and clinical trials are recommended.

Due to the population genetic diversity and region-specific microbiome variation, some scientists propose that a region-based, microbiome genome-based, personalized food or bacteria or even metabolites of bacteria in parallel to other current treatments, could help restoring a healthy microbiome, with anti-viral and anti-inflammatory effects [**13**,**35**,**67**]. Overall, demographic data suggest that host influences mutation and expression of the SARS-CoV-2 virus [**13**,**28**,**67**].

Adopting nutritional habits and lifestyle that facilitate a healthy status of the gut microbiome will have positive effects on the anti-inflammatory processes and should help protecting the intestinal epithelium barrier, the immune capacity of the microbiome and the communication along the gut-lung-brain-heart axis. A healthy balanced diet (especially important for older and immune-compromised people) includes fruits, vegetables, wholegrains, plant oils and fish, providing adequate levels of vitamins A, C, D, E, B6, B12, folate, Zn, Cu, Se, Fe, omega-3 fatty acids, proteins, carotenoids, polyphenols, dietary fibres. It should foresee low consumption of alcohol, high-saturated fat foods, refined sugar and sugar-containing beverages. Even if there is no formal approval by food authorities of western countries, like FDA and EFSA, on the use of probiotics for preventing or treating health issues, a personalized diet including pre- and pro-biotics is even suggested as a complement to current therapies in several scientific articles [**28**,**35**,**48**,**49**,**66**,**67**,**72**].

The effects and mechanisms of action of diet on microbiome and on related diseases should be further investigated, particularly in the context of the COVID-19 pandemic, to provide more precise information of the nutrients and doses needed to obtain each effect. Given the increasing number of scientific publications in the field of pre- and probiotics benefits and on immunity-related microbiome, new clinical studies are to be expected to shed light on those mechanisms.

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List of abbreviations and definitions

ACE2	Angiotensin-Converting Enzyme 2
Cu	Copper
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
Fe	Iron
GI	Gastrointestinal
IL	Interleukin
IFN-γ	Interferon gamma
NCBI	National Centre for Biotechnology Information
OTU	Operational Taxonomic Unit
PRS	Proteomic Risk Score
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SCFA	Short-Chain Fatty Acids
Se	Selenium
Spp.	Species
TNR	Tumor Necrosis Factor
Zn	Zinc

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