Microbiota and Probiotics: Chances and Challenges. A symposium report

Carrie H S Ruxton[1], Chiyuki Kajita[2], Paola Rocca[3], Bruno Pot[2]

[1] Nutrition Communications, Cupar, KY15 4HQ, UK

[2] Yakult Europe BV, Schutsluisweg 1, 1332 EN Almere, The Netherlands.

[3] Yakult Italia SrL, Via V. Monti 51, 20123 Milan, Italy.

Author Contributions

Conceptualization – P.R, C.K., B.P.; Project administration – P.R. and C.K.; Writing – Original Draft, C.H.S.R.; Writing – Review and Editing, B.P., C.H.S.R. and C.K.; Supervision – B.P.; Funding Acquisition – P.R.

This peer-reviewed article has been accepted for publication in Gut Microbiome but has not yet been copy-edited or typeset so may be subject to change during the production process. The article is considered published and may be cited using its DOI:

10.1017/gmb.2023.4

Gut Microbiome is co-published by Cambridge University Press and The Nutrition Society.

Abstract

The 10th International Yakult Symposium was held in Milan, Italy, on 13-14 October 2022. Two keynote lectures covered the crewed journey to space and its implications for the human microbiome, and how current regulatory systems can be adapted and updated to ensure the safety of microorganisms used as probiotics or food processing ingredients. The remaining lectures were split into sections entitled 'Chances' and 'Challenges'. The 'Chances' section related to opportunities for the science of probiotics and fermented foods to contribute to diverse areas of health such as irritable bowel syndrome, major depression, Parkinson's Disease, immune dysfunction, infant colic, intensive care, respiratory infections, and promoting healthy longevity. The 'Challenges' section included selecting appropriate clinical trial participants and methodologies to minimise heterogeneity in responses, how to view probiotics in the context of One Health, and understanding how substances of bacterial origin can cross the blood-brain barrier. The symposium provided evidence from cutting-edge research that gut eubiosis is vital for human health and, like space, the microbiota deserves further exploration of its vast potential.

Abbreviations: GM, gut microbiota; ISS, international space station; AMR, antimicrobial resistance; PD, Parkinson's Disease, SCFA, short-chain fatty acid; LPS, lipopolysaccharide; TLR, toll-like receptor; FMT, fecal microbiota transplant; MDD, major depressive disorder; HPA, hypothalamus-pituitary-adrenal; BDNF, brain-derived neurotrophic factor; LcS, *Lacticaseibacillus paracasei* strain Shirota; BBB, blood-brain barrier; CSF, cerebral spinal fluid; OMV, outer membrane vesicles; IBS, irritable bowel syndrome; T1D, type 1 diabetes; FF, fermented foods; LAB, lactic acid bacteria; RTI, respiratory tract infection; SRMA, systematic review and meta-analysis; RCT, randomised controlled trial; RA, risk assessment; QPS, the qualified presumption of safety; GRAS, generally recognized as safe; EFSA, European Food Safety Authority; Med, Mediterranean; NK, natural killer; TREC, T-cell receptor excision circles.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Introduction

Decades of research have revealed the remarkable extent to which the gut microbiota (GM) influences and interacts with many areas of the body beyond the large intestine. Slowly, a picture has emerged of the potential role of the GM in helping to modulate gut health, immune function, mineral absorption, metabolic balance, appetite, brain health, and aging.

This creates opportunities for the use of dietary or medical interventions which may impact the GM by promoting particular microbiological species, excluding others, or broadening microbiological diversity. It also poses challenges to understanding mechanisms, ideal intakes, appropriate health markers, and characteristics of responders, as well as how best to regulate products.

These were the topics considered by the 10th International Yakult Symposium held in Milan, Italy, on 13-14 October 2022. This report summarises the presentations given by a panel of international experts and invites reflection on the chances and challenges presented by the study of the GM and probiotics.

The crewed journey to space and its implications for the human microbiome

Space travel is a unique environment in which to study the human microbiome. Prof. Christine Moissl-Eichinger from the Medical University of Graz, Austria, outlined why a good understanding of the GM is essential for ensuring the success of crewed space missions, mainly as 8% of astronauts report gastrointestinal issues and access to medical interventions in space is limited.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Simulation experiments

Space training in closed systems provides opportunities to study changes in the GM and those microorganisms present in the environment (Kuehnast et al., 2022). One example is the Mars 500 experiment which saw six crew members spend 520 days in a terrestrial-based simulator to mimic a journey to Mars (Schwendner et al., 2017). During this time, samples at different time points were taken from the surfaces and air of the module, revealing that microbial communities followed the functions of humans and could also be altered by human activity (e.g., changing to a different cleaning product). This experiment also tracked the GM of the six crew members. Remarkably, given the constrained environment and similar diet, each person had their own signature GM which fluctuated over time but remained distinct from the GM of other crew members. Individual phyla, such as Pseudomonadota (formerly the Proteobacteria), Bacteroidota (formerly the Bacteriodetes), or Verrucomicrobiota (formerly Verrucomicrobia; Oren & Garrity, 2021) found in one person's GM could be completely missing in the GM of others. In three subjects, major fluctuations in microbial configurations occurred after 340 days (range 330-360 days) in the module, which could be related to stress, or the tasks being performed. These fluctuations were characterized by the depletion of *Faecalibacterium prausnitzii, Ruminococcus bromii, Blautia luti, Anaerostipes hadrus,* and *Roseburia faecis*.

Another Mars simulation model is the Hawai‘i Space Exploration Analog and Simulation (HI-SEAS) mission (Mahnert et al., 2021). This involved a team of astronauts spending 4-12 months in a 111 m^2 module, during which time samples were taken from different areas of the module and the crew's skin and feces. Some interesting patterns emerged. Firstly, the microbial diversity reflected the function of

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

the living area, e.g., the toilet and kitchen. There was a crossover in the human microbiota when interactions occurred, such as a higher number of pathogens on the skin of the crew on toilet cleaning duty. Secondly, while each person had their microbiota signature, there were evident crossovers of species between those astronauts who had the most interactions with other crew members. Thirdly, while the microbiome of the built environment remained relatively stable over time, the skin microbiome of the crew increased in diversity as it incorporated species from the environment. This was particularly the case during an episode where a technical failure of the toilet facilities forced individual crew members to carry out additional cleaning duties, providing more chance for them to come into contact with fecal bacteria, which was then reflected in their skin microbiome.

Experiments in space

Few studies have been conducted in space. In one of these, Mora et al. (2019) tested whether the unique conditions inside the International Space Station (ISS) altered the microorganisms found there. This is warranted since there is evidence that microgravity can influence the virulence of certain species (Rosenzweig et al, 2010), while technophilic microorganisms have been known to cause equipment to malfunction in space. The EXTREMOPHILES study involved sampling in several areas of the ISS over three months. The key learnings were:

- The diversity and composition of the ISS microbiome fluctuates in response to human activity reflecting the purpose of the different living areas but retaining a core group of stable species.
- The ISS microbiome is similar to indoor environments on Earth but has a greater prevalence of species that can make biofilms (for details, see Mora et

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

al., 2019). This is probably due to adaptation to thrive on the metal surfaces inside the ISS.

- While the ISS microbiome was mostly human-associated, it was reassuring that no evidence was found of selection for enhanced pathogenicity or antimicrobial resistance (AMR) (Mora et al., 2019).

Further studies have found that space travel disrupts the normal GM, probably due to the influence of stress. During one space mission, astronauts' skin, nose, and gut microbiomes changed markedly. The GM became more similar across crew members, primarily due to a drop in the abundance of several bacterial taxa mainly *Akkermansia*, *Ruminococcus*, *Pseudobutyrivibrio* and *Fusicatenibacter* (Voorhies et al., 2019). However, one longer-term study in twins (Garrett-Bakelman et al., 2019) found that the GM shifts back to the pre-flight pattern within 6 months of the astronaut returning to Earth.

To summarise, the microbiome of the built environment in space fluctuates around a set of core species but does not appear to present a particular risk to crew health in terms of pathogenicity, virulence, or antibiotic resistance. This is relevant as there are limited opportunities to treat microbial infections in space. While space travel disrupts humans' normal skin and gut microbiome, this effect is reversible. Future space experiments will help find the answers to essential questions such as how to control microbial outbreaks in space, how to treat microbial disease in space, whether there is a need for novel probiotics/prebiotics, and how the microbiome of space environments and crew can be monitored long term at vast distances from Earth.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Parkinson's Disease: evidence for the role of the gut

Initially viewed as a brain condition, there is growing evidence that the gut has a role in initiating Parkinson's Disease (PD), as discussed by Prof. Aletta Kraneveld from Utrecht University, The Netherlands.

PD affects 1% of older adults and is an incurable condition characterized by progressive tremors, muscle rigidity, postural instability, and intestinal dysfunction. This conflation of gut and brain symptoms implies two origins for the accumulation of α-synuclein (Lewy bodies) in the brain leading to neuro-inflammation and neurodegeneration (Horsager et al., 2020): either a direct central nervous system phenotype, or an indirect intestinal phenotype where leaky gut and endotoxemia lead to mucosal inflammation, microbiome changes and, eventually, α-synuclein accumulation (Scheperjans et al., 2018; Rietdijk et al., 2017).

Intestinal phenotype hypothesis

This is supported by more than 15 cohort studies which found correlations between neurological deterioration and gut dysbiosis characterized by reduced *Prevotella*, lower levels of fecal short-chain fatty acids (SCFAs), increased lipopolysaccharide- (LPS) producing bacteria, and increased pro-inflammatory Lactobacillaceae (Li et al., 2023). Murine models have built on this concept. Mice which genetically overexpress α-synuclein develop PD-related pathophysiology and motor dysfunction, but such changes do not occur if α-synuclein overexpression mice are bred germ-free. However, inoculating these germ-free mice with GM from PD patients induces the pathology to a greater extent than non-exposed α-synuclein overexpression mice,

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

proving that gut bacteria are essential to initiating the disease (Sampson et al., 2016).

Other studies corroborate gut-related mechanisms. Colonizing α-synuclein overexpression mice with *E. coli,* which produce curli fibers (pro-inflammatory proteins which mediate host cell adhesion and invasion), led to the further aggregation of α-synuclein in the gut and brain, and enhanced brain inflammation, gut problems, and motor dysfunction (Chapman et al., 2002). Another study (Matheoud et al., 2019) considered the role of the PINK1 gene, which is responsible for clearing mitochondria damaged during the progression of PD. Knocking out PINK1 would be expected to induce or exacerbate PD-like changes in animal models. However, this does not happen unless there is also an intestinal infection with LPS-producing bacteria.

A study of gut biopsies from PD patients revealed evidence of tight junction decline, leaky gut, and endotoxemia, and enhanced toll-like receptor (TLR) 4 expression, suggesting that PD is a TLR disease (Perez-Pardo et al., 2019). This hypothesis was tested using the pesticide, rotenone (an isoflavone molecule), which can initiate PD-like pathophysiology in animal models. Compared with wild-type mice which developed the expected pathogenic changes, oral exposure to rotenone for several weeks did not lead to gut dysbiosis or α-synuclein accumulation in TLR4 knock-out mice. In addition, the loss of dopamine-producing cells in the substantia nigra was less pronounced and there were fewer motor & cognitive problems. A similar disruption of the expected PD pathophysiology was seen following the use of TLR4 antagonists and TLR4 blocking antibodies, and when the vagus nerve was cut

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

suggesting that this is the likely route by which α-synuclein spreads, prion-like, to the brain (Kim et al., 2019).

Can GM modulation slow the progression of PD?

Fecal microbiota transplants (FMT) in murine models of PD reduce gut dysbiosis and neuroinflammation and result in fewer motor problems. Human trials are limited but demonstrate encouraging results for motor and non-motor symptoms in PD patients (Segal et al., 2021). Research on probiotics and synbiotics is more advanced and suggests that these are safe and effective, although further evidence is needed. *In vivo* studies report improved glucose metabolism, reduced inflammation, and neurodegeneration (Leta et al., 2021). In a review of eight clinical trials in PD patients given lactobacilli or bifidobacteria probiotics (Hong et al., 2022), constipation was significantly reduced, and modest anti-inflammatory effects were observed. A downside of using probiotics in PD is the potential for probiotic-drug interactions since bacterial decarboxylases may affect the bioavailability of L-dopamine, a Carbidopa component commonly used to manage PD neurological symptoms (van Kessel et al., 2019).

Murine PD models have been used to test the efficacy of diets containing precursors for neuronal membrane synthesis, such as long-chain omega-3 fatty acids, choline, uridine, vitamins, and minerals (Perez-Pardo et al., 2018a). Overall, the nutritional intervention was effective at partially alleviating the rotenone-induced neurological changes in mice. A further study tested an enhanced experimental diet containing the same nutrients as before plus prebiotic fibers but introduced it 28 days after rotenone exposure when adverse neurological changes had already occurred

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

(Perez-Pardo et al., 2017). Compared with the control diet, the enhanced prebiotic-rich diet was more effective at normalizing the mice's rotenone-induced motor and non-motor abnormalities. These findings suggest that dietary treatments can help reverse neurological changes in mice and that diets that modulate the GM appear to deliver more benefits than those providing nutritional support (Perez-Pardo et al., 2018b).

In summary, there is growing evidence for a gut-first model of PD. However, further robust human studies in target populations are needed to understand the gut-brain mechanisms involved and identify opportunities for early intervention.

Underlying mechanisms of depression and the modulating role of probiotics

Another condition potentially influenced by the gut–brain axis is major depressive disorder (MDD), which affects around 280 million people worldwide and is characterized by symptoms including depressed mood, anxiety, and insomnia. Dr. Kazunori Matsuda, from the Yakult Central Institute, Japan, proposed underlying mechanisms related to the GM and the therapeutic potential of probiotics.

Gut-brain axis

Bidirectional communication exists between the GM and the brain. The brain influences the gut via the autonomic nervous system, while the gut, including microbe-derived molecules, influences the brain via humoral and neuronal pathways (summarized in Suda & Matsuda 2022). The idea that the GM could be linked to depression arose from studies where mice receiving an FMT from MDD patients displayed depression-like behavior compared to control mice given an FMT from

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

healthy people (Zheng et al., 2016), which indicates the role of dysbiosis in MDD development.

Further evidence came from a systematic review of 17 studies characterizing the GM of MDD patients (Knudsen et al., 2021), which found reduced numbers of *Faecalibacterium*, a producer of butyrate, a SCFA linked to the maintenance of neurogenesis and anti-inflammatory effects. Other work reported that MDD patients have a lower abundance of bifidobacteria and lactobacilli than healthy controls (Aizawa et al., 2016). However, this is not a consistent pattern across studies, perhaps due to differences in subjects' backgrounds between the studies.

Mechanisms

What are the likely mechanisms if gut dysbiosis were influential in the pathology of MDD? MDD is recognized as a multifactorial condition linked to abnormal stress response, reduced neurogenesis, and neuroinflammation, pathways where the GM may impact. Chronic stress is a risk factor for MDD onset, resulting in the hypothalamus-pituitary-adrenal (HPA) axis-mediated dysregulation of the stress response. The HPA axis is understood to be a key pathway of stress response through cortisol secretion. Normally, cortisol regulates its secretion via negative feedback through the HPA. However, in MDD patients, the feedback system is impaired, resulting in elevated blood cortisol, while brain exposure to high levels of cortisol induces chronic inflammation and reduced brain-derived neurotrophic factor (BDNF) protein – an important regulator of neuronal growth, survival, and plasticity.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Animal studies have found that the stress response is pronounced with a lack of GM when germ-free mice are exposed to physical restraint stress. However, when germ-free mice were inoculated with *Bifidobacterium infantis*, the exaggerated HPA stress response was reversed (Sudo et al., 2004). One signaling route from the gut to the brain is the vagus nerve, and some probiotic strains such as *Lacticaseibacillus paracasei* strain Shirota (LcS) have been shown to stimulate the activity of the gastric branch of the vagal afferent to suppress the stress-induced increase in blood corticosterone (Takada et al., 2016).

Reduced neurogenesis, another part of the pathophysiology of MDD, is believed to be caused by neuroinflammation and excessive stress, demonstrated by a smaller volume of certain brain regions in MDD patients (Treadway et al., 2015) and lower BDNF in cerebrospinal fluid (Mizui et al., 2019). This may have a GM link since germ-free mice have lower hippocampal levels of BDNF relative to specific pathogen-free mice (Sudo et al., 2004), and SCFAs can upregulate BDNF. Neuroinflammation, too, has a gut connection since the GM directly affects pro- and anti-inflammatory responses in the gut, and a leaky gut has been implicated in the pathogenesis of MDD. Increased gut permeability causes an influx of gut microbial components such as LPS, resulting in systemic inflammation and consequent neuroinflammation.

Could microbiome-based therapies help?

Studies suggest they can. FMT given to patients to treat symptoms of IBS has been found to have beneficial secondary effects on symptoms of depression (Huang et al., 2019), while a meta-analysis that pooled the results from 34 clinical trials concluded

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

that probiotics have modest beneficial effects on depression and anxiety (Liu et al., 2019). Two randomized, double-blind, placebo-controlled trials in this meta-analysis are explored in more detail. In the first study, 40 MDD patients were treated with a probiotic capsule (*Lactobacillus acidophilus* + *Lacticaseibacillus casei* + *Bifidobacterium bifidum*) or a placebo for 8 weeks. Significant improvements were seen in depressive symptoms, insulin resistance marker, and C-reactive protein in the probiotic group relative to controls (Akkasheh et al., 2016). In the second study, 81 MDD patients were given probiotics (*Lactobacillus helveticus* R0052 + *Bifidobacterium longum* R0175), prebiotics (galacto-oligosaccharide) or a placebo for 8 weeks, with symptoms of depression significantly improving only in the probiotic group relative to controls (Kazemi et al., 2019).

The latest research on LcS supported these observations; a 12-week open-label study of an LcS-fermented milk drink on patients with depression found improved depressive symptoms and sleep quality (Otaka et al., 2021). Another study revealed that 8-week of treatment with an LcS-fermented milk drink significantly attenuated the stress-induced rise in salivary cortisol in medical students under academic stress (Takada et al., 2016). These results suggest that some probiotic strains can modulate stress-induced activation of the HPA axis and the subsequent onset of depression.

In summary, the GM is likely involved in the pathophysiology of MDD via several pathways, and GM modulators, including probiotics and FMT, could be helpful adjunct therapies.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Overcoming the brain barrier: a challenge for bacteria?

Implicating the GM in the pathophysiology of brain diseases and conditions requires that bacterial substances can access brain tissues. How this might occur was the topic reviewed by Prof. Roosmarijn Vandenbroucke from the Flanders Institute for Biotechnology (VIB) and Ghent University, Belgium.

The brain is protected from the peripheral circulation by central nervous system barriers, which include the blood-brain barrier (BBB) and the lesser-known blood-cerebral spinal fluid (CSF) barrier, which sits within the brain ventricles. Both barriers are characterized by being selectively permeable and having several parts to their structure, including a layer of epithelial cells in the case of the blood-CSF barrier and endothelial cells in the case of the BBB; both possessing tight junctions which regulate access. The choroid plexus epithelial cells at the blood-CSF barrier share similarities with those in the gut and have microvilli at their apical side, enhancing the surface area.

Barrier functions

There is a difference in permeability between the two barriers since the capillaries which sit underneath the choroid plexus epithelial cells that form the blood-CSF barrier are fenestrated (i.e., leaky). This means no tight junction proteins connect the choroid plexus endothelial cells to one another (Vandenbroucke et al., 2016). The purpose of the choroid plexus is to remove waste products from the brain, act as its gatekeeper, and make CSF, a soup of different molecules, including nutrients, neurotrophins, and growth factors. The choroid plexus epithelial cells are in very close contact with the endothelial cells of the capillaries inside the choroid plexus.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

This enables them to respond to triggers from the peripheral circulation, such as cytokines, and consequently relay these peripheral signals to the brain, but how does this process occur?

One answer is via extracellular vesicles, cell-derived nanoparticles that transfer biological cargoes between cells and can cross the brain barriers bi-directionally, giving them a powerful influence across the body. Extracellular vesicles may originate from the body's cells or from bacteria, which potentially explains how the GM could have an impact on the brain. This was demonstrated in an animal experiment (Balusu et al., 2016a) where LPS was peripherally delivered, resulting in systemic inflammation and inflammation in brain cells. An extracellular vesicle inhibitor was then administered in the brain, which blocked the inflammatory signal to the brain, suggesting that extracellular vesicles act like a relay between the peripheral circulation and the brain. Animal studies suggest that a healthy gut microbiota is essential for the optimal development of the BBB since germ-free mice display increased BBB permeability compared with pathogen-free controls with normal gut microbiota (Braniste et al. 2014). There is also evidence that choroid plexus dysfunction via altered secretory, transport, immune. Barrier function plays a central role in aging and the risk of developing conditions such as Alzheimer's disease (Balusu et al., 2016b). Hence, targeting the GM composition, or administrating SCFAs might have therapeutic potential.

Bacterial extracellular vesicles and brain diseases

The discovery of extracellular vesicles, especially those derived from bacteria, has advanced understanding of how gut dysbiosis may influence the initiation and

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

progression of chronic progressive brain conditions. One example is the association between *Helicobacter pylori*, a gastrointestinal pathogen found in around half of adults, and an enhanced risk of Alzheimer's Disease. It has been hypothesized that bacterial-derived EVs, called outer membrane vesicles (OMV), if derived from Gram-negative bacteria, can cross the brain barriers, and initiate pathogenic changes, such as neuroinflammation or beta-amyloid plaque deposits (Xie et al., 2022). This was studied by loading *H. pylori* OMV with cre enzyme and feeding these to tdTomato reporter mice, genetically engineered mice whose cells turn red when cre is taken up. This study showed an apparent increase in red astrocytes, confirming that *H. pylori* OMV had traveled from the gut to the brain, crossing the brain barriers (Xie et al., 2022).

The impact of this was investigated by feeding wild type mice with *H. pylori*-derived OMV and studying the activity of cells in the brain (Xie et al.,2023). OMVs were found to overstimulate the microglia, leading to excessive synaptic pruning, evidenced by reduced dendrite length. Electrophysiological measurements then confirmed that *H. pylori*-derived OMV had detrimental effects on synaptic activity. To examine the clinical impact of OMV, a mouse model of Alzheimer's disease was treated with OMV for three weeks. The findings confirmed a significant effect on plaque deposition with more plaques and a larger plaque area than control mice. Hence, *H. pylori* OMV can access the brain and potentially accelerate pathogenic changes associated with Alzheimer's disease. At this stage, it is unclear how the OMV are crossing the blood-CSF barrier.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

In summary, a functioning blood-CSF barrier requires the presence of a GM and is strengthened by SCFA-producing taxa. Recent research shows that *H. pylori* OMV can enter the brain and accelerate changes associated with Alzheimer's disease, such as glial activation and plaque deposition.

IBS: is it all between the ears?

This was the intriguing question asked by Prof. Francisco Guarner from the Teknon Medical Centre, Spain. Irritable bowel syndrome (IBS) is characterized by chronic, relapsing diarrhea or constipation with no detectable cause. Bloating and pain are common symptoms (Lacy et al., 2017), often blamed on intestinal gas, but the symptoms could be due to heightened sensitivity to abdominal distention. This was demonstrated in an experiment (Barba et al., 2019) where patients who had reported reactions after eating lettuce were given an abdominal computer tomography scan before and after eating this trigger food. Average post-prandial girth increased by 35 mm, representing an 835 ml expansion of intra-abdominal volume, but only 40 ml of this was due to extra gas, which was within the normal range. It was concluded that patients felt bloated because consuming lettuce led to a conditioned response of diaphragm displacement, with computer tomography scans showing an average diaphragm descent of 7 ± 3 mm. Following behavioral training, patients reduced their anxiety-related response to trigger foods by learning to control their diaphragm movement.

<u>Is dietary restriction necessary for IBS?</u>

The low FODMAP diet, which restricts poorly absorbed short-chain carbohydrates including fructose, lactose, polyols, fructans, and galacto-oligosaccharides, is a

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

favored treatment for IBS and resolves symptoms in 50%-80% of patients (Staudacher et al., 2017). However, it entails short-to-medium term avoidance of certain foods, particularly plant-based foods, which may be neither convenient nor healthy for the patients. Hence, it may be better to employ cognitive behavioural therapy to condition a more positive response to trigger foods (Black et al., 2020). This has led to proposals that diet-induced symptoms in IBS are driven by dysregulation of the gut-brain axis since blinded interventions reveal similar increases in small bowel motility and colonic gas volume when IBS patients and healthy controls consume fructans (Wu et al., 2022).

Gut bacteria create intestinal gas by fermenting carbohydrates which begs the question: do IBS patients have a particular GM profile? In one study (Manichanh et al., 2014), patients complaining of flatus were compared with healthy controls before and after a 3-day challenge diet that was rich in plant foods. Even on the baseline 'usual' diet, patients reported more abdominal symptoms and gas than controls, which worsened in both groups following the challenge diet. Changes in the GM of patients mirrored the increased symptoms, with *Bilophila wadsworthia* correlating with the increased volume of gas expelled. However, the GM of patients reduced in diversity and changed more radically in response to the challenge diet compared with the controls, which remained relatively stable. Hence, the GM of IBS patients, whether due to their habitual diets or other lifestyle factors, appears to be less adapted to digesting a plant-based diet and more adapted to digesting protein. This may lead to a predominance of gas-producing taxa.

Implications for wider health

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

While it is difficult to differentiate people with and without gut dysbiosis simply by looking at their GM, there are associations between digestive symptoms and particular taxa. Pozuelo et al. (2015) found that patients with IBS had significantly lower microbial diversity and fewer microorganisms that produce butyrate and methane. Since these are responsible for disposing of hydrogen in the gut, their lower abundance in people with IBS could explain the excess of abdominal gas. *Prevotella* was more associated with healthier controls; interestingly, these taxa can digest vegetable matter. IBS is not the only condition characterized by microbial indicators since a study in 8,208 Dutch adults found that the GM of people with cancer, diabetes, cardiovascular disease, and neurological conditions share microbiome commonalities and could be differentiated from the GM of healthy people (Gacesa et al., 2022).

If the healthy GM profile favors those species adapted to ferment fiber-rich plant substrates, could a low FODMAP diet, which typically restricts these foods, drive unhelpful changes in the GM? This could be true, according to research that finds that a low FODMAP diet leads to atrophy of taxa adapted to digest vegetables (Halmos et al., 2015). Hence, alternative therapies are warranted to enable people with IBS to follow the recommended plant-rich diet for general health and disease prevention. Huaman et al. (2018) combined a Mediterranean (Med) diet with a prebiotic (galacto-oligosaccharide), which was tested against a low FODMAP diet in a randomized controlled trial. Similar reductions in gut symptoms were seen after 4 weeks on both diets, except flatus which was reduced only after the low FODMAP diet. However, some of these benefits were not sustained, as symptoms reappeared immediately after patients discontinued the low FODMAP diet. In contrast, the

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

benefits of the prebiotic-Med diet combination persisted during the 2-week follow-up when patients returned to their habitual diets. In addition, the diets had opposite effects on *Bifidobacterium* sp., with a decline seen after the low FODMAP diet versus an increase after the prebiotic-Med diet.

In summary, as plant-based diets are now widely recommended for health and disease prevention, it is important that people with IBS are supported to eat these by employing behavioral strategies, which condition a positive response to trigger foods rather than managing their symptoms with trigger food avoidance.

Gut microbial diversity: one health and probiotics

Taking his cue from One Health – the European program which recognizes the interconnectivity between the environment and human/animal health – Dr. Olaf Larsen from Vrije Universiteit, The Netherlands, discussed the role of microbial diversity, particularly of key taxa and guilds in promoting health.

The worldwide incidence of infectious diseases, including tuberculosis and measles, declined dramatically during 1950-2000 against a backdrop of rising autoimmune disorders, such as type 1 diabetes (T1D), Crohn's disease, and asthma (Bach 2002). This trend continues in a more recent analysis (Larsen et al., 2022). In particular, T1D incidence has risen steadily in Europe and the US over the past 40 years. However, as demonstrated by the SARS epidemics and the SARS-Covid-19 pandemic, infectious diseases are far from being eradicated.

Old friends

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

While the fall in infections is understandable, given vaccines and improved hygiene standards, the reason for the rise in autoimmune problems is less clear and may be related to the health of our microbiota. One theory is that humans, especially in early childhood, have limited exposure to beneficial microbes from food and the environment – referred to as 'old friends' – which leads to an overreactive immune system with the propensity to attack the body's own tissues as well as overacting to harmless microorganisms or antigens. Indeed, studies show that diminished exposure to microorganisms in early life correlates with an increased risk of atopic diseases (Von Mutius et al., 2000).

A deterioration in GM balance in Western countries has been cited as a reason for their greater burden of Covid-related mortality and higher rates of autoimmune and chronic non-communicable conditions. GM diversity correlates with risk (Dhar and Mohanty, 2020) and severity of Covid-19 (Yeoh et al., 2021). It may also influence the development of metabolic syndrome (Fan and Pedersen, 2021) which increased in prevalence from approximately 30% to 40% of the US adult population during 2000-2018, emphasizing the immense scale of this issue (Larsen et al., 2022). Completing the circle of disease risk, SARS-Covid-19 infection has been found to exacerbate metabolic disease (le Roux, 2021).

All of this indicates a need for Western populations to improve their exposure to 'old friends' and regain microbiota eubiosis – considered to be a state of balance in the GM between beneficial and harmful bacteria, which is normally associated with a disease-free host. The human gut loses a proportion of the conserved microbiome with each successive generation, possibly related to incomplete maternal-child

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

519 transmission (due to Caesarean births and lower than ideal breast-feeding rates) and
520 excessive antibiotic use, which has remained relatively stable despite concerns
521 about antibiotic resistance (Blaser and Falkow, 2009). New exposures do not
522 compensate for this decline in beneficial bacteria since society has adopted
523 unhelpful practices of indoor living and diets lacking in fermented foods. If these
524 ecosystem losses continue, a catastrophic collapse in the GM is hypothesised
525 leading to abrupt and possibly irreversible shifts between alternative ecosystem
526 states (Larsen and van de Burgwal, 2021). Increasing GM diversity increases
527 functionality, e.g., SCFA production, but only if the right species are introduced. If the
528 wrong diet and lifestyle are adopted, less favorable species could thrive, reducing
529 ecosystem resilience and creating functional redundancy.
530
531 Keystone taxa and guilds
532 A balanced GM includes keystone (core) taxa and guilds. Keystone taxa are: "highly
533 connected taxa that individually or in a guild exert a considerable influence on
534 microbiome structure and functioning, irrespective of their abundance, [hence] their
535 removal can cause a dramatic shift in microbiome structure and functioning."
536 (Banerjee et al., 2018). Guilds are small ecosystems where 2-10 taxa work together
537 as coherent functional units or exploit the same type of resources (Maurice and
538 Turnbaugh, 2018).
539
540 The absence of specific guilds has been linked with a greater risk of autoimmune
541 conditions and certain metabolic diseases, but these guilds can be restored with the
542 right interventions, which may include FMT, probiotics, or dietary fibers. In one
543 randomized controlled trial in people with type 2 diabetes, diets high in fiber

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

promoted SCFA-producing strains at the expense of strains that produced potentially detrimental compounds such as indole and hydrogen sulfide (Zhao et al., 2018). These GM changes in the high fiber group were associated with improved hemoglobin A1c levels. At present, the evidence is insufficient to determine whether single strain or multistrain probiotics are more effective at restoring eubiosis (McFarland, 2021) although the theoretical research suggests a higher diversity in microbial guilds leads to a more efficient system. Hence, the choice of an appropriate probiotic should be based not on the number of strains in the product but on evidence-based efficacy trials. There is also an issue with non-responders which implies that a personalized approach is needed to determine the correct keystone taxa and guilds.

In summary, to avoid the risk of catastrophic collapse in the GM, we need to take a One Health approach to promote microbiota eubiosis. This includes greater biodiversity and exposure to 'old friends', appropriate substrates from high-fiber and plant-rich diets, as well as limiting antibiotic use and excessive hygiene.

Opportunities relating to fermented foods

One source of ‘old friends’ is traditional fermented foods (FF), according to Prof. Paul Cotter, from the Teagasc Food Research Centre and APC Microbiome Ireland, Ireland, who reviewed some recent research on this topic.

FF are “foods made through desired microbial growth and enzymatic conversions of food components” (Marco et al., 2021). Examples include kefir, sourdough bread, yogurt, kimchi, and kombucha. The different microbes used to make FF determine

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

the fermentation process, flavor, nutrients/bioactive compounds, and potential health benefits, including nutritive alteration of the ingredients, presence of bioactive compounds that affect intestinal and systemic function or modulation of the immune system. However, not all FF work as probiotics and referring to FF microorganisms as probiotics is misleading unless backed by evidence from human studies.

Used as a means to preserve foods, FF have a long history of use in nations around the world (Gänzle, 2022; Jimenez et al., 2022). The expansion of modern research techniques has helped investigate the microbiota of FF, highlighting differences across foods and, indeed, different versions of the same food type. This inherent variability has complicated standardization, an issue further complicated by different standards and regulations between countries (Mukherjee et al., 2022). As an example, the term 'kefir' is reserved for dairy in some jurisdictions, e.g., Germany, and cannot be applied to water kefirs.

Fermented food research

A global initiative was set up to apply shotgun metagenomic sequencing to a diverse range of FF, eventually sourcing 58 international artisan products (Leech et al., 2020). Food type, e.g., dairy, brine- or sugar-based, was the primary driver of microbial composition, and foods within these clusters had more similar microbiomes than those from other clusters. Several FF did not fit with any cluster, including coconut kefir and soya-based foods for which there are relatively little data. Multiple potentially novel microbial species were identified, which could represent untapped functionality resources.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Further work (Pasolli et al., 2020) has mapped lactic acid bacteria (LAB) species found in FF with those present in the human GM, finding that, for some species, closely related LAB strains occur in both food and gut environments. This provides new evidence that FF can be a source of LAB for the gut microbiome. The next phase will look at African FF as these have been under-researched. Africa contains a wealth of FF examples that contain microbes that differ significantly from those found in FF from other continents.

Microbiome Applications for Sustainable food systems through Technologies and Enterprise (MASTER) is a new initiative that applies analytical techniques to FF typically used to study the human GM. One MASTER study (Cotter, personal communication) found specific clusters of microbial genes associated with colonization, gut survival, modulation pathways, and human health within FF microbes. Indeed, FF contained significantly more health-associated gene clusters than non-fermented substrates, indicating the transformative influence of adding microbes to foods. The work could be used to identify which FF are worth testing further in human clinical trials.

The example of water and milk kefir

Kefir grains contain a consortium of bacteria and yeasts, although the specific microorganisms in water and milk kefir grains are very different. Water kefir is a fermented beverage made by inoculating water kefir grains into a sugar (sucrose)-rich solution supplemented with fruits. Often made in a household setting, the mixture is left to stand at room temperature for 1 to 3 days, after which the grains are filtered out to obtain the final drink. A recent study (Mortensen, personal

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

communication) sourced water kefir grains from around the globe and fermented them with the same substrate. Heat maps based on microbial taxonomy revealed differences in α-diversity across countries and at least 10 clusters of microbial communities which could be important for flavor, shelf life, or health. This work could help define international standards for water kefirs, which tend to differ from country to country regarding their microbiome.

Milk kefir is made by fermenting milk with milk kefir grains. Research has identified specific microbes linked to volatile compounds which could help develop optimal flavor profiles for new products, for example adding *Lactobacillus kefiranofaciens* NCFB 2797 to increase fruitiness (Walsh et al., 2016). This work is being expanded to 64 international milk kefir samples to determine theoretically which microbes could indicate potential health attributes. This is important as while milk kefir has been linked with several health benefits, including cholesterol reduction and antimicrobial activity, the quality of evidence is often poor (Bourrie et al., 2016). Notably, some animal studies evaluating the impact of kefir on obesity, dyslipidemia, and metabolic diseases suggest that the health-promoting attributes of kefir depend on specific microbes, which could explain why some kefirs do not produce any health effects (Bourrie et al., 2020). Indeed, a study to deconstruct the microbes in artisan kefirs found that *Lactobacillus* and yeast were essential components for lowering plasma cholesterol in mice (Bourrie et al., 2021).

Ultimately, understanding which microbes in FF are important for health could help inform standards for commercial products and may eventually lead to population recommendations for specific microorganisms to be consumed through the diet.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

644

645 **Living foods: safe salvation for health**

646 Continuing the theme of fermented foods, Prof. Lorenzo Morelli, from the Catholic
647 University of the Sacred Heart, Italy, described how modern research techniques can
648 improve the understanding of traditional production methods.

649

650 Since around 7000 BC, humans have preserved protein-rich foods using different
651 tools, including salt, smoke, and fermentation. The term fermentation comes from the
652 Latin verb 'fervere', which means 'to boil', possibly referring to the bubbles seen
653 when liquids are fermented. An example of a traditional FF is Parmesan cheese
654 which is still made only with raw milk in copper pots and using the previous day's
655 culture – called 'back slopping'. Commercially available bacteria cultures are not
656 permitted, and the cheese must be ripened for more than a year for safety. There is
657 good genetic evidence that these traditional methods have selected a sub-population
658 of LAB whose chromosomes are adapted to making Parmesan cheese since they
659 can grow at 51 °C, which usually is high for such bacteria.

660

661 <u>New methods to solve old problems</u>

662 However, a weak point of traditional back slopping is the undefined age and viability
663 of the bacterial cells, given that cheese-making requires the correct balance of lactic
664 acid and viable cells. Older bacteria produce too much lactic acid which eventually
665 kills the culture. Uncertainty can also be introduced by raw milk, whose composition
666 and bacterial profile are influenced by different seasons and pastures. Newer
667 research technologies can be used to address these traditional problems. In one
668 study (Bellassi et al., 2021), researchers used metabolomics and genomics to

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

669 discriminate between milk produced by cows fed hay and milk from cows fed hay
670 and fresh vegetables.
671
672 The bacteria used to make FF are multifunctional, transforming raw ingredients'
673 aroma, flavor, taste, and durability. It has been found that sourdough cultures are
674 essential for flavor and leavening and act as natural preservatives (Bourdichon et al.,
675 2021). Biopreservation refers to enhanced food safety and extended shelf life of
676 foods by indigenous and/or intentionally added microbiota, inhibiting the growth of
677 pathogenic and spoilage organisms due to microbiological competition and
678 production of antimicrobial metabolites (Shi and Maktabdar, 2022). This is an
679 important attribute as consumers want foods to have a longer shelf life yet remain
680 concerned about chemical preservatives and plastic packaging. There is a potential
681 role for LAB against fungal spoilage of foods (Siedler et al., 2019), as demonstrated
682 by an experiment that found that breads inoculated with mold were better preserved
683 after 7 days when made with LAB compared with regular yeast. Modern techniques
684 could be used to leverage these hitherto unknown benefits of cultures. The
685 antimicrobial characteristics of several microorganisms are already recognized by
686 GRAS [generally recognized as safe].
687
688 Human impact
689 LAB also interact with our bodies, as first recognized by Russian zoologist and Nobel
690 laureate Élie Metchnikoff (Mackowiak, 2013), who hypothesized in the early 20th
691 century that ‘intestinal putrefaction’ shortens life but that lactic acid could be an
692 antidote. This led him to be the earliest advocate of LAB as therapeutic agents and
693 he is often considered the 'father' of probiotics. While Metchnikoff's original

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

694 experiments could be described as hazardous – for example, injecting himself with
695 pathogens or feeding lactic acid to volunteers – he went on to advocate the use of
696 LAB in fermented foods, stating in 1907: '*Dependence of the intestinal microbes on*
697 *the food makes it possible to adopt measures to modify the flora in our bodies and to*
698 *replace the harmful microbes by useful microbes'*.
699
700 Metchnikoff incorrectly assumed that colonic bacteria could be modulated using
701 supplemental lactic acid. Still, it is reasonable to assume that the GM could be
702 influenced by a range of LAB by-products found in FF, including bioactive peptides
703 (Ali et al., 2022). These have been associated with anti-hypertensive, angiotensin-
704 converting enzyme (ACE) inhibitory, antioxidant, anti-inflammatory, and
705 immunomodulatory effects, which could deliver health benefits (Raveschot et al.,
706 2018; Beltrán-Barrientos et al., 2016). Bioactive peptides may also improve mineral
707 bioavailability (Tenenbaum et al., 2022), which could support healthy aging and the
708 prevention of osteoporosis. Since the neurotransmitter γ-aminobutyric acid (GABA)
709 is one of the by-products of LAB metabolism, it has been hypothesised that
710 fermented foods could influence the brain. The potential anti-hypertensive effects of
711 reduced sodium sourdough, made with *Levilactobacillus brevis* CECT 8183, were
712 investigated in a laboratory study (Peñas et al., 2015). The results showed
713 significantly increased total antioxidant activity, GABA levels, and ACE inhibitory
714 effects compared with the control bread, suggesting that innovative breads could be
715 developed to reduce blood pressure.
716

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Hence in summary, while there is a long history of humans using bacteria to preserve nutrients through fermentation, their interactions in our bodies and potential impact on health are only beginning to be understood.

Living drugs: a solution with many benefits

This narrative was continued by Prof. Stephan C. Bischoff, from the University of Hohenheim in Stuttgart, Germany, who described how FF evolved first into functional foods and supplement products, then medical applications. These require different approaches to safety assessment, regulation and methodologies to establish evidence of efficacy. This is because the purpose of probiotics has evolved from health maintenance to the prevention, management, or treatment of diseases and abnormal conditions.

Oral microbiota therapy can include probiotics, prebiotics, and postbiotics; the latter being inanimate microorganisms and/or their components that confer a health benefit on the host (Salminen et al., 2021). For probiotic medical trials, it is crucial to consider strain, dosage, target population, disease type, and progression. Understanding mechanisms is also vital to support medical claims and ensure that the right probiotics are targeted at the right population of patients (Daliri et al., 2021). Given recent advances in knowledge, relevant pathways of action include the gut-brain axis and the gut-liver axis, with the potential for probiotics to modulate a range of metabolic, inflammatory, and neurological conditions. So, where is the evidence currently?

<u>Respiratory tract infections (RTI)</u>

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

742 Cochrane reviews are a gold standard of independent systematic review and meta-
743 analysis (SRMA). In one of these, probiotics were found to lower the incidence but
744 not the duration of RTI (OR 0.58; 95% CI 0.36 - 0.92) and reduced antibiotic
745 prescriptions (0.67; 95% CI 0.45 - 0.98) (Hao et al., 2011). These conclusions were
746 confirmed in updated reviews of studies on adults and children (Hao et al., 2015;
747 Quick, 2015). Other SRMAs have concluded that probiotics and prebiotics effectively
748 improved response to the influenza vaccine (Lei et al., 2017), while fermented dairy
749 products protected against RTI (Rashidi et al., 2021).
750
751 However, one issue with SRMAs is the heterogeneous approach to probiotic strains,
752 i.e., dosage and duration of the administration, which can create inconsistencies that
753 make null conclusions more likely, as already mentioned by Dr Larsen. Another
754 issue is that SRMAs can be based on several small pilot trials subject to publication
755 bias. Hence, there is a need to consider well-conducted large randomized controlled
756 trials (RCTs), of which several now exist:

757 • A 6-week trial of three probiotics on common cold symptoms in 581 college
758 students found that *B. bifidum* increased illness-free days (Langkamp-Henken
759 et al., 2015).
760 • A 6-month trial in 171 children found that a probiotic plus vitamin C reduced
761 coughing, absenteeism, and antibiotic usage (Garaiova et al., 2021).
762 • Two 12-week trials of fermented milk with *Lacticaseibacillus paracasei* strain
763 Shirota found prevention of the common cold and influenza in 96 office
764 workers (Shida et al., 2017), and reduced risk of acute upper RTI in 1003
765 children (Mai et al., 2021).
766

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Moving to the hospital environment, the severe condition of ventilator-associated pneumonia is a common issue for intensive care patients. Here, too, SRMAs have confirmed that probiotics have a therapeutic role in this condition, as there is robust evidence for a 30% reduction (Bo et al., 2014; Ji et al., 2021; Sharif et al., 2022). A large RCT backs this using a 4-strain preparation (*L. acidophilus*, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* subsp. *lactis,* and *Saccharomyces cerevisiae* var boulardii) in 112 trauma patients (Tsilika et al., 2022). However, another large RCT (n=2653) found no significant benefit *of Lacticaseibacillus rhamnosus* GG for ventilator-associated pneumonia (Johnstone et al., 2021).

Gastro-intestinal disorders

A major indication for probiotics is antibiotic-associated diarrhea. The evidence for *S. cerevisiae* var boulardii and *Lactoballicus* sp. is so well established, with a risk reduction of more than 50% (Szajewska and Kołodziej 2015a; Szajewska and Kołodziej 2015b) that further data are unnecessary.

Probiotics are also recommended in the German IBS guidelines since few effective drug treatments exist for this condition (Layer et al., 2021). However, the opposite is true for inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease, where probiotics offer weak beneficial effects that are inferior to drugs (Kaur et al., 2020). Small intestine bacterial overload results from gut dysbiosis and is characterized by bloating, pain, and post-prandial diarrhea. A SRMA by Zhong et al. (2017) found that probiotics could not prevent small intestine bacterial overload but lowered gut hydrogen levels and improved treatment efficacy, including for

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

abdominal pain. German guidelines indicate that it is best practice to use probiotics alongside antibiotics and a low FODMAP diet (Layer et al., 2021).

In medicine, probiotics are most effective for RTI and gastrointestinal conditions. In the future, probiotics could treat other types of conditions such as metabolic syndrome, obesity, and neurological diseases but, to do this, new probiotics need to be discovered and tested in clinical trials. In a recent trial (Gutiérrez-Castrellón et al., 2022) a new patented 4-strain probiotic improved remission rates and viral load in patients with SARS-Covid-19. Further research and product development are required to deliver the advantages of living drugs to all parts of the body.

Safety of microorganisms used as probiotics

Before being included in the food system, microorganisms must be risk assessed to ensure consumer safety. Is the current system fit for this purpose? This was discussed by Prof. Pier-Sandro Cocconcelli from the Università Cattolica del Sacro Cuore, Italy, who identified four trends in risk assessment (RA).

Microorganisms are deliberately introduced into the food chain to assist food production (e.g., to create FF) and to benefit animal and human health. RA involves hazard identification and characterization, exposure assessment, and risk characterization, but this system was designed with pathogens, not probiotics, in mind. Hence, adjustment is needed to enable the system to provide adequate assessment, for example, using dosage data from intervention studies rather than population exposure.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

816 Trend #1: the process of RA is rapidly evolving

817 Guidance on regulating microorganisms in food and feed has been rapidly evolving
818 in Europe since 2005 due to the evolution of methodologies which have become
819 increasingly complex since the advent of genomics.

820

821 Trend #2: increased complexity of microbial RA

822 The RA system for microorganisms combines taxonomy, genomics, a qualified
823 presumption of safety (QPS), AMR, virulence, and end-use. QPS is a fast-track
824 approach that reduces unnecessary extensive safety testing by utilizing the body of
825 knowledge on the species plus a safety decision tree. It differs from the US system
826 of GRAS, which is generally limited to a specific application made following a safety
827 assessment (Franz et al., 2011).

828

829 More than 100 microorganisms have been granted QPS status in Europe, but their
830 evidence is still updated bi-annually to ensure safety. For new microorganisms, the
831 decision tree is followed and if QPS is not given, a full safety assessment is required.
832 Even for QPS microbes, evidence of acquired AMR means that no approval will be
833 given since the food system should not add to the burden of AMR and enable these
834 genes to be mobilized in the human or animal gut. In contrast, intrinsic AMR is not
835 considered a safety concern if this is inherent to wild-type bacterial species.

836

837 Trend #3: genomics is fundamentally changing RA approaches

838 Some microorganisms have multiple characteristics ranging from pathogen to food
839 culture, which taxonomy alone does not recognize; hence, genomic methods are
840 needed. One example is *Enterococcus faecium* which can be a pathogenic,

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

commensal, food culture, or probiotic organism, depending on the clade. While EFSA has produced guidance on genomics (European Food Safety Authority [EFSA] 2021), it refers to methods rather than purpose. In contrast, microbial RA is concerned with identification, genetic modification, and finding AMR genes, which suggests that the guidance on genomics needs updating.

Genomic techniques provide precise information on microbial phylogenesis but add complexity, making combining old and new data harder. In the case of *E. faecium,* gene sequencing can enable specific AMR genes to be identified. However, it can also overturn previous taxonomy, as a study (Belloso Daza et al., 2021) concluded that clade B of *E. faecium* should be reassigned as *Enterococcus lactis.* Yet, while genomics may be suitable for identification, it still cannot tell us if microorganisms are safe. To do this, RA requires phenotypic testing based on determining a minimum inhibitory concentration of the potentially resistant gene and whole-genome sequencing to search for known AMR genes. In the example of *E. faecium,* whole-gene sequencing found mobilizable AMR genes in a sample taken from ready-to-eat sausages (Belloso Daza et al., 2022), highlighting the need for constant vigilance.

Yet, there remain shortcomings in using genomics to determine pathogenicity since genes for successful gut colonization could act to promote virulence in a pathogen or survivability in a probiotic. Also, the definition and application of “intrinsic resistance” are not absolute, and there is a non-alignment between international regulatory bodies. Hence, an evolving approach to RA is needed.

Trend #4: new products and applications

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

This impacts RA because it extends the continuum from natural to synthetic microorganisms. Synthetic biology is the application of science, technology, and engineering to facilitate and accelerate the design, manufacture, and/or modification of genetic materials in living organisms (Scientific Committee on Emerging and Newly Identified Health Risks et al., 2014). As new microorganisms could be potentially indistinguishable from non-genetically modified versions, RA should be based on the nature of the final strain and not on the methodology used to get there. The EU is already considering how to regulate this area since genetically modified microorganisms are already present in non-EU markets. Another consideration is the risk assessment of non-viable cells used in the food supply, such as postbiotics, which could be treated like biomasses or novel foods.

In summary, the regulatory system is still evolving to ensure proper RA of potentially useful microorganisms, aided by advancements in methodologies.

The importance of the responder/non-responder issue for clinical trials

RA and authorization of health claims depend on high-quality evidence. Yet, the gold standard RCT may not be the most appropriate for nutrition research, including trials of probiotics, argues Prof. Robert Jan Brummer from Örebro University, Sweden.

In the hierarchy of medical evidence, the RCT is near the top, only surpassed by systematic reviews and meta-analyses of RCTs. While these types of studies undoubtedly work for medicine where drug compounds are standardized, relatively constant, and produce a large signal-to-noise ratio (i.e., the effect of the intervention compared with the effect of interpersonal variations), they may not be appropriate for

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

other health interventions which are not standardized, e.g., because they are natural foods or ingredients, or where subtle changes in health are seen in the long term. Hence, the RCT model only works effectively to provide evidence of efficacy where certain assumptions can be made. These are:

- External validity – being able to generalize the findings of RCTs to a defined population;
- Independence of effects – where the observed effect is most likely due to the intervention and not a confounding variable;
- Adequate characterization of the intervention and placebo (Zeilstra et al., 2018).

These assumptions may not always be valid for nutritional interventions, such as dietary interventions or probiotics, which can often yield inconsistent results in RCTs, which are then amplified in meta-analyses.

External validity

To be clinically useful, nutritional interventions must work in a definable group of people (age, sex, health status, nutritional status) in a particular public health or hospital setting. Lack of external validity is one explanation for the widespread underuse in the routine practice of many treatments that were shown beneficial in trials and are recommended in guidelines (Rothwell 2006). Inter-individual variation in participant response is a barrier to external validity because, unlike pharmaceuticals, nutritional interventions often have subtle effects which can be overwhelmed by the background 'noise' created by many individual variations in clinical response. A larger sample size does not help since this often increases the

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

heterogeneity of the study population and inter-individual variation. One example is a hypertensive drug which would be expected to deliver a fall in systolic blood pressure of 10-15 mmHg (Paz et al., 2016), considerably greater in magnitude than the anticipated 4 mmHg fall from a 4g reduction in salt intake (He et al., 2013) which would be a significant dietary shift for the target population. Hence, in the presence of non-compliance and intra-individual variation, the dietary intervention must work harder than a pharmaceutical treatment to achieve a statistically significant result.

Independence of effects

It would be illogical to combine all brands of hypertensive drugs into one RCT. Yet, trials of probiotics often mix species and strains into one intervention, reducing the chances of a clear, unambiguous result. This is then compounded by systematic reviews and meta-analyses that pool studies using various strains. Different strains of probiotics have different clinical effects, making it necessary to understand the mode of action to select the correct outcome variable and patient group. It is also essential to control the potential for bias, particularly from the rest of the diet.

Adequate characterization

It is a fundamental error to assume that they are standardized because probiotics can be put into capsules like drugs. Probiotics are living organisms that evolve once they reach the recipient's colon, depending on the available substrates provided by the diet, e.g., the amount and types of fermentable carbohydrates and proteins. This means that the same product does not imply the same treatment in every individual recipient; thus, in the case of probiotics, the idea that the treatment is well-defined may be questionable (Zeilstra et al., 2018).

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

941

942 Way forward

943 Three concepts may be considered to address the issues of inter- and intra-
944 individual variation. Firstly, by considering responsive nutrition, which aims to target
945 interventions by identifying likely responders through machine learning analysis of
946 health, genetic, drug, and dietary data. This could create a phenotype for optimal
947 responsiveness, which could help target probiotic interventions to those most likely
948 to respond. Responsive nutrition differs from personal nutrition. The latter focuses on
949 providing the best dietary intervention on an individual basis. Secondly, by trying to
950 limit intra-individual variation as far as possible. This could be done by conducting
951 many trials, on fewer people with a stable background pattern of the primary
952 outcome measure, rather than one trial on many people with unspecified intra-
953 individual variation (Larsen et al., 2020). Thirdly, surrogate biomarkers can show
954 short-term changes predictive of a health effect instead of medium-term disease
955 markers, which other lifestyle factors may influence. One example is functional brain
956 imaging which, in a 4-week RCT of probiotics (Rode et al., 2022), demonstrated
957 significant changes in brain morphology and resting-state brain function linked to
958 stress management of the brain.

959

960 In summary, non-response and intra-individual variation hamper a clear
961 understanding of the efficacy of probiotics, and we need to look beyond the classic
962 RCT design to overcome this challenge.

963

964 **Development of the infant microbiota**

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Turning from foods back to the human body, Prof. Christoph Lacroix, from ETH Zurich, Switzerland, described the acquisition of the microbiome in infanthood and discussed how different lifestyle and environmental factors can influence which taxa thrive, hence, which functions are expressed.

From the sterile environment of the womb, the infant's gut is rapidly colonized by pioneer microorganisms (Khan et al., 2015), evolving in terms of taxa and diversity over the first few years. This remains relatively stable until old age, when diversity declines. Modern techniques like metagenomics enable us to look at microbial function over the life course, which is more important than taxonomy.

GM acquisition

Initially dominated by LAB, the infant gut microbiome changes most rapidly between the ages of one and six months with the cessation of breast-feeding, rather than the introduction of solid food, correlating with maturation into an adult-type microbiota (Bäckhed et al., 2015). Building on this research, Roswall et al. (2021) conducted a longitudinal cohort of 471 healthy Swedish children to track the development of the GM from birth to five years, noting four discrete trajectories for different microbes. The greatest changes occurred in the first year of life, and by the age of 3-5 years, the child GM was closest to that of adults, although still evolving.

Roswall et al. (2021) identified four major trajectories for individual genera in the developing gut microbiota of infants and young children, with some genera peaking at 4-12 months, others increasing rapidly between 4-12 months before stabilizing by 3 years, and a final group increasing in relative abundance after 12 months and

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

990 continuing to increase until five years. These shifts were linked to the cessation of
991 breast feeding, the introduction of solids, increased socialization outside the family
992 and increased diet diversity.
993
994 Both vertical (from the mother) and horizontal (from the birth environment)
995 transmission determine which pioneer species colonize the post-natal gut. Factors
996 include maternal diet and health, vaginal vs. Caesarean birth, skin-to-skin contact,
997 breast or bottle feeding, and antibiotic use (Marques et al., 2010). Molecular
998 methods have revealed the presence of more microorganisms in human milk than
999 previously believed, such as skin bacteria, Bacteroidota phylum, and clostridia
1000 (Selma-Royo et al., 2022). Indeed, the bacterial diversity of human milk may even
1001 exceed that of neonatal feces (Jost et al., 2013). However, this could be explained
1002 by different population densities and structures and the limited resolution of the
1003 sequencing methods. There is also evidence of bacterial translocation through the
1004 entero-mammary pathway since similar strict anaerobe species and strains have
1005 been found in maternal feces, breast milk, and infant feces (Perez et al., 2007).
1006
1007 Beneficial role of microbes
1008 A comprehensive study tracked the impact of breastfeeding on GM changes in
1009 seven healthy neonates aged 4 to 30 days (Jost et al., 2012). Neonate feces were
1010 dominated either by *Bifidobacterium* or *Bacteroides* sp. Strict anaerobes
1011 outnumbered facultative anaerobes within the first days, which was earlier than
1012 assumed, but major adult-type butyrate producers, such as *Roseburia* and
1013 *Faecalibacterium*, were not detected. While most infant gut bacteria are lactate
1014 producers from the main dietary carbohydrate lactose, some species must

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

metabolize lactate, potentially toxic if allowed to accumulate, mainly into propionate (Chassard et al., 2014). Sulfate-reducing bacteria can remove hydrogen, a secondary metabolite produced by different taxa of the infant gut such as clostridia and *Veillonella* that may be linked to bloating and colic.

Such findings have led to the hypothesis that infants with colic may have more bacteria producing hydrogen and/or fewer bacteria that can metabolize lactate and hydrogen. This was demonstrated in a 2-year prospective cohort study of 40 infants, including 8 with colic, which also found that peak lactate production occurred when infants were 2-3 months (Pham et al., 2017). Further research revealed a switch between the lactate-utilizer, hydrogen-producer *Veillonella* in the first year of life to the lactate-utilizer butyrate-producer, *Anaerobutyricum hallii*, in the second year of life, which was associated with weaning (Pham et al., 2022).

This was tested further in a gnotobiotic model (Rocha et al., 2022) where rats were inoculated with feces from healthy infants or those with colic. After milk formula feeding, rats with colic-associated microbiota produced significantly more hydrogen in feces and had a higher abundance of *Veillonella* than healthy controls. Supplementation of the lactate-utilizer and propionate-producer *Cutibacterium avidum* P279 to rats with the colic-associated microbiota reduced gut hydrogen levels compared with animals receiving a placebo. The results confirm the benefit of cross-feeding between bacteria in the infant's gut and suggest that targeted probiotics could help manage colic in human infants.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

In summary, these studies suggest a broad window of opportunity for dietary interventions tailored to support the evolving infant GM. A good example is the promotion of taxa involved in lactate and hydrogen cross-feeding to help address infant colic. However, more research is needed to understand better the mechanisms and functions of the infant GM, particularly from low- and middle-income countries.

Microbiota composition from 1 till 100

Beyond infanthood, the GM continues to change, with implications for long-term health, as discussed by Prof. Gaspar Pérez Martínez from the Institute of Agrochemistry and Food Technology (CSIC), Spain.

The microbiome clock

While the GM of infants and adults differ in species, diversity, and functionality, a quantitative theory of intestinal aging remains elusive because there are no recognised step changes in GM during adulthood. Some older adults have a GM similar to younger people, and there is an overlap between clusters of signature species linked to decades of life.

In a study of 367 healthy Japanese volunteers (Odamaki et al., 2016) from infanthood to very old age, bifidobacteria dominated in early life, but the relative abundance of Actinomycetota (formerly the Actinobacteria) substantially declined after weaning and was progressively replaced by Bacillota (formerly the Firmicutes). A further change occurred around 70 years when increases were seen in the relative abundance of Bacteroidota and Pseudomonadota while Bacillota declined. Using

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

samples from 1165 adults, a machine learning model could predict a healthy person's age from their GM to an accuracy of fewer than 6 years. However, this did not work for patients with T1D who exhibited microbiome age acceleration (Galkin et al., 2020). In a different study (Bian et al., 2017) with 1000 healthy Chinese volunteers, GM patterns showed remarkable similarities between healthy aged and younger adults for overall GM composition, a fact observed in previous studies (Odamaki et al., 2016). In this case, health was a better predictor of GM aging than years of life. Interestingly, this study also revealed a stable diversity across all age categories, with a shift in GM profiles around 19-24 years of age which could reflect changes in hormones or lifestyles, e.g., going to university or the army.

Factors affecting GM composition across life

Five factors were outlined: environment, diet, genetics, antibiotics, and health.

Environment: Children exposed to less urbanized environments have a lower risk of autoimmune conditions. Studies in Finnish and German children (Kirjavainen et al., 2019) found a reduced incidence of asthma in farm-raised children, with the indoor dust of farmhouses having a lower abundance of Streptococcaceae. Asthma risk in children who did not live on farms decreased as their home microbiota composition became more like farm homes. Studies on tribal people have found a distinct and far richer GM diversity compared to industrialized populations (Clemente et al., 2015; Conteville et al., 2019), which could reflect the absence of antibiotics and differences in physical activity, diet, and exposure to outdoor microorganisms. People who exercise have a greater alpha diversity than sedentary people but few differences in taxa. The largest difference is in the metabolomics profile since regular exercisers

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

have higher fecal SCFAs and harbor a greater proportion of phyla that break down carbohydrates, probably reflecting their habitual diets.

Diet: the GM responds to diet as it determines available substrates. A multi-center metagenomics study (Arumugam et al., 2011) found three distinct clusters of GM composition associated with substrates rather than nationality. Subsequent studies collapsed these into two distinctive groups correlated with animal fat consumption: protein and simple sugars (*Bacteroides* group) or vegetables, complex carbohydrates, and fiber (*Prevotella* group). This was seen in practice when the GM was studied in people with different diets (De Filippis et al., 2016). Prevotellaceae were more abundant with plant-based diets, while Bacteroidota were more abundant in vegans and vegetarians than in omnivores. However, higher fecal SCFAs were seen with high dietary compliance, even in omnivores, when split by adherence to the Med diet. Changing the diet from meat-based to vegetarian, or vice-versa, can alter the GM, but only while the diet is maintained. Habitual vegetarians return more quickly to their baseline GM after resuming their usual diets (David et al., 2014). Consuming a functional drink based on *Cyperus esculentus L*. (tiger nuts) also shifted the GM pattern towards SCFA producers, but this depended on the baseline microbiome of each individual (Selma-Royo et al., 2022).

Genetics: A study of UK twins (Goodrich et al., 2016) uncovered familial hereditary lineages with greater similarities within the Ruminococcaceae and Lachnospiraceae families for monozygotic compared to dizygotic twins. An analysis of fecal samples from 71 individuals found that the diversity and composition of bifidobacteria were strongly associated with the histo-blood group ABH secretor/non-secretor status,

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

which appears to be one of the host genetic determinants for GM composition (Wacklin et al., 2011).

Antibiotics: While having an overall positive influence on human health, antibiotics nevertheless inflict ecological disaster on the GM, wiping out helpful species alongside pathogens. The GM does regrow but typically does not achieve the same balance of species, particularly in people taking repeated antibiotic courses. Some individuals never recover their baseline GM (Chng et al., 2020). A SRMA (Duong et al., 2022) of observational studies found an increased long-term risk of auto-immune conditions and obesity in children given multiple antibiotic courses.

Health: Certain conditions have an impact on the GM. Coeliac disease changes the balance of GM species and increases diversity, while the time window between seroconversion and T1D in genetically susceptible children is characterized by reduced alpha diversity and a higher prevalence of species linked to inflammation (Kostic et al., 2015). These observations fit with the broader theory of gut dysbiosis affecting the etiology of several chronic diseases, which could also be bi-directional, as demonstrated by the finding that sepsis induces low-grade inflammation and oxidative stress in the gut via such as TNF-α and interleukin-1β. This adversely changes GM balance since Reactive Oxygen Species have selective antibacterial effects (Cernada et al., 2016). At the other end of the age spectrum, there are associations between GM changes and the initiation of immunosenescence (Candore et al., 2008).

Gut microbiota changes in the young and old

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Continuing the theme of looking at society's oldest people, Prof. Patrizia Brigidi, from the University of Bologna, Italy, discussed the GM of centenarians using data on individuals from four distinct age groups (young, elderly, centenarians, and semi-supercentenarians) living in the same geographical area of Italy (Biagi et al., 2016).

Age is a key variable that impacts GM composition and function and represents an adaptive trajectory across the human lifecycle (Rampelli et al., 2020). GM changes provide the host with ecological services calibrated to each stage of life. For example, the relative importance of vitamin biosynthesis, fermentation, RNA degradation, and bile salt metabolism varies with age (Lynch & Pedersen 2016). In particular, age-related changes in lifestyle and nutritional behavior, prescribed drug use, changes in gut physiology and functionality, i.e., reduced intestinal motility and increased intestinal permeability, impact on the GM composition and its crosstalk with the host, nurturing inflammageing, a chronic low-grade inflammatory status characteristic of the old age, immunosenescence and metabolic disorders.

Healthy semi-super centenarians, aged 105-109 years, represent a good model for studying healthy aging as they have survived for 20 years longer than their demographic cohort and have somehow escaped the major chronic age-related disorders and causes of mortality. The GM of this age group was compared with three other sub-groups with mean ages of 100, 72.5, and 30.5 years based on 16S rRNA amplicon sequencing analyses (Biagi et al., 2016). The GM composition in the youngest and oldest groups could be clearly differentiated, with the middle age groups having some overlap and biodiversity declining with age. A core of highly prevalent bacteria, mostly belonging to Ruminococcaceae, Lachnospiraceae, and

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Bacteroidaceae families were detected whose abundance decreased during aging, leaving space for the growth of subdominant species.

Further research has observed that the GM of long-lived individuals is characterized by a rearrangement in the Bacillota population, with a decline in *Faecalibacterium prausnitzii* and enrichment in facultative anaerobes, notably pathobionts, which correlates with an increase of the inflammatory status (Lynch & Pedersen 2016). Similar findings have been reported from other longevity areas of the world (Ren et al., 2021; Kim et al., 2019). However, the GM of the semi-supercentenarians had greater enrichment of health-associated groups (e.g., *Akkermansia*, *Bifidobacterium*, and Christensenellaceae); a key difference from the GM of centenarians.

Metagenomics has been used to examine the functions of bacteria in the GM of older people (Lynch & Pedersen 2016). This has revealed a rearrangement in metabolic pathways related to carbohydrate and amino acid metabolism in agreement with the loss of *Eubacterium* and *Faecalibacterium* and the increase of Pseudomonadota sp. This shift from a saccharolytic to a proteolytic profile induces a marked decrease in SCFA production and availability of tryptophan and an increase in indolic metabolites, which correlate with cognitive impairment, inflammation, and cancer. The aged GM was also enriched in microorganisms capable of generating unique secondary bile acids, which could be involved in reducing the risk of infection with pathobionts (Yuko Sato et al., 2021). Interestingly, compared with younger individuals, the GM of the Italian elderly over 100 years had more genes for xenobiotic metabolism, particularly for chemicals deriving from the industrial manufacturing of many indoor products, such as synthetic fibers, resins, and

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

synthetic leather (Lynch & Pedersen 2016). This could reflect an adaptive response to increased exposure to these anthropic pollutants over a lifetime.

Looking specifically at GM characteristics that could be a marker of longevity, Christensenellaceae is worthy of further study as it is more abundant in long-lived people independent of their culture, diet, and lifestyle (Kong et al., 2016; Tuikhar et al., 2019). Research in different age groups has revealed that a greater abundance of Christensenellaceae is associated with lower body mass index, visceral adipose tissue and inflammation, more favorable lipid traits (lower total cholesterol, Apo B levels, triglycerides), and higher levels of fecal SCFAs (Waters et al., 2019). Hence Christensenellaceae could be a future candidate as probiotic.

Dietary modification could also encourage the acquisition of beneficial species for healthier aging. In the NU-AGE study, Ghosh et al. (2020) recruited 1250 healthy, pre-frail adults aged 65-79 from five European countries and randomized them to a 12-month nutritional intervention consisting of a Med diet with vitamin D supplementation versus a control diet. The GM was analyzed in 612 participants before and after the intervention. Adherence to the intervention diet enriched specific GM taxa that were positively associated with cognitive function markers and negatively associated with frailty and inflammatory markers, including C-reactive protein and interleukin-17. The diet-modulated GM changes were also associated with increased SCFAs and lower production of secondary bile acids.

In summary, age group separation of the GM composition is evident, and longevity adaptation seems linked to the enrichment of health-associated GM species,

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

including *Akkermansia*, Christensenellaceae, *Bifidobacterium*, and Odoribacteraceae, involved in the establishment of new homeostasis. These bacterial taxa could be promoted using dietary interventions to improve the 'health span' of the elderly.

Probiotics and the aging immune system

The final presentation of the Yakult International Symposium was given by Dr. Caroline Childs at the University of Southampton, UK, who examined the role of the GM in immunosenescence.

How does the immune system age?

The thymus is responsible for manufacturing immune cells, such as T-cells, but this ability declines sharply with age after the peak thymus activity in childhood. By age 50, active thymus tissue is significantly replaced with adipose cells, resulting in lower production of naïve immune cells and a higher proportion of memory T-cells with a low functional capacity. The function of immune cells *in vitro* correlates with clinical outcomes, so it is no surprise that the coronavirus pandemic – representing a novel immune challenge – disproportionately affected older populations. Aging is characterized by chronic, low-level inflammation (inflammageing) and a greater risk of morbidity and mortality. Older people are more likely to get infections, and their immune system responds less effectively to these and vaccinations, e.g., only 30-50% of elderly adults gain protection from influenza vaccinations (Demicheli et al., 2018).

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

T-cells fall into two categories; cytotoxic T-cells, which fight infections, and helper T-cells which act like project managers. However, T-cell aging is not automatically linked to chronological age. Some 70-year-olds may have the T-cell functionality of 30-year-olds, and vice versa (Kaczorowski et al., 2017), which correlates with the findings noted by previous speakers describing the overlap in GM composition for different age groups. Building on this point, a study of 178 older adults (Claesson et al., 2012) found that the fecal microbiota composition clustered by diet and with participants residing in care homes or the community. The care home GM was less diverse and correlated significantly with measures of frailty, co-morbidity, and inflammatory markers of inflammation. Interestingly, moving from the community to a care setting changed the diet immediately, but it took around a year for the GM to respond (O'Toole & Jeffery 2015).

One key change in the aging GM is the shift away from Bifidobacterium (Arboleya et al., 2016), a genus associated with immuno-modulatory properties. An *in vitro* study (You & Yaqoob, 2012) found that exposure of human mononuclear cells to probiotics from bifidobacteria and lactobacilli strains produced immunomodulatory effects, but the response was also significantly influenced by the age of the volunteer.

How can immune ageing be measured?

Flow cytometry can measure and differentiate immune cells from human samples and determine immune age by looking at the relative proportions of naïve cells and different types of memory cells, i.e., central, effector or terminally differentiated effector. Accumulation of T EMRA cells is characteristic of aging. Other biomarkers of cell ageing include the CD28 marker on T-cells, which helps to stabilise their

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

interaction with B cells, which produce antibodies. CD28 is progressively lost with aging while, in contrast, the CF57 marker, which is linked to the immune response to viruses and cancer cells, appears on the T-cells and natural killer (NK) cells of older adults. This is thought to indicate cell exhaustion. A study (Tae et al., 2015) in patients the morning after having a myocardial infarction revealed that the frequency of CD57 in their CD8 T-cell population positively correlated with cardiovascular mortality 6 months later. In other research, CD57 was a marker of a poor NK cell response to influenza vaccination in older subjects which could not be offset by supplementation with a synbiotic containing *B. longum* (Przemska-Kosicka et al., 2018).

Another marker of immune aging is T-cell receptor excision circles (TRECs). These circles of DNA form when T-cells are created in the thymus and are exported to the cell cytosol. TRECs decline in concentration with each round of cell division as T-cells replicate and mature (Lang et al., 2011). Hence, one may see more TRECs in the T cells of younger people and those with younger immune systems than in older or immunosenescent people (Mitchell et al., 2010). Seropositivity to viruses which disrupt immune function, such as cytomegalovirus or even SARS-Covid-19, is also a helpful marker.

Probiotics are beneficial for immune function as they lower the burden of certain infections and reduce antibiotic use (Hao 2015), potentially saving health systems millions of Euros (Lenoir-Wijnkoop et al., 2015). However, the data have a high level of heterogeneity, lowering the overall evidence quality. A review of the impact of probiotics, prebiotics, and synbiotics on immune response in older adults found

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

evidence of improved vaccine responsiveness, NK cell activity and phagocytosis, and a reduced incidence of infections (Childs & Calder, 2017). However, only two studies used specific markers of immunosenescence, reporting increases in naïve T cells and TRECs after probiotics, and a third of the studies were not randomized controlled trials.

A SRMA of six eligible trials (Gui et al., 2020) found that probiotic use ranging from 3 to 12 weeks significantly increased NK cell activity in healthy older adults but concluded that the overall results were insufficiently convincing given the small sample sizes and very large heterogeneity. A systematic review (Chenhuichen et al., 2022) of nine RCTs and one secondary analysis assessed a broader range of parameters relating to immunity, metabolic health, GM, and cognitive function, finding overall benefits for probiotics and prebiotics, although the risk of bias in studies was considered high. Further studies should take account of immunological age at baseline to reduce heterogeneity and utilize markers of immune cell aging and function.

Conclusions

The evidence for the role of the GM in acute and chronic human health is now substantial, with indications that the influence of our microorganisms goes well beyond the gut to include the immune system, metabolism, and brain. While aging and genetics impact on the composition and diversity of the GM, it is nevertheless clear that modifiable factors, such as diet, antibiotic use, exercise, and exposure to outdoor-type microbes, may be more important for achieving microbiota eubiosis. This provides people with the chance to adopt more gut-friendly lifestyles. Still, it also

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

raises several challenges including gathering the proper evidence to ascertain which microbiota interventions are right for which population groups, understanding the mechanisms involved, developing effective probiotic and prebiotic products, and ensuring that these are appropriately regulated. As outlined in this fascinating symposium and summarised in Figure 1, there is now a tantalising opportunity to find ways to live in harmony with our GM, which could offer widespread human health benefits.

INSERT FIGURE 1

Acknowledgments

The authors would like to thank the speakers for reading and approving the summaries of their presentations.

Financial Support

The speakers CM-E, AK, RV, FG, PC, LM, SCB, R-JB, CL, GP-M, PB and CC received an honorarium for their contribution and their travel costs were covered by Yakult Europe BV. Travel costs of OL and KM were covered by Yakult Europe BV. C.H.S.R. received financial support (to cover travel and time) from Yakult Europe to attend the symposium and to draft this report.

Conflicts of interest

BP and CK are employees of Yakult Europe BV; PR is an employee of Yakult Italy SrL; OL is an employee of Yakult Nederland; KM is an employee of the Yakult Central Institute, Yakult Honsha Co., Ltd., Japan.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Publishing ethics statement

This manuscript is our own original work and does not duplicate any previously published work. This manuscript has been submitted only to this journal – it is not under consideration, accepted for publication or in press elsewhere. All listed authors know of and agree to the manuscript being submitted to the journal. This manuscript contains nothing that is abusive, defamatory, fraudulent, illegal, libellous, or obscene.

References

Aizawa, E., Tsuji, H., Asahara, T., Takahashi, T., Teraishi, T., Yoshida, S., Ota, M., Koga, N., Hattori, K., & Kunugi, H. (2016). Possible association of *Bifidobacterium* and *Lactobacillus* in the gut microbiota of patients with major depressive disorder. *Journal of Affective Disorders, 202*, 254-257.

Akkasheh, G., Kashani-Poor, Z., Tajabadi-Ebrahimi, M., Jafari, P., Akbari, H., Taghizadeh, M., Memarzadeh, M.R., Asemi, Z., & Esmaillzadeh, A. (2016). Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. *Nutrition, 32*(3), 315-320.

Ali, A., Kamal, M.M., Rahman, M.H., Siddiqui, M.N., Haque, M.A., Saha, K.K., & Rahman, M.A. (2022). Functional dairy products as a source of bioactive peptides and probiotics: Current trends and future prospectives. *Journal of Food Science & Technology*, *59*(4),1263-1279.

Arboleya, S., Watkins, C., Stanton, C., & Ross, R.P. (2016). Gut Bifidobacteria populations in human health and aging. *Frontiers in Microbiology*, *7*,1204.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

1362 Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R.,
1363 Fernandes, G.R., Tap, J., Bruls,T., Batto, J.M., Bertalan, M., Borruel, N., Casellas,
1364 F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M.,
1365 … Bork, P. (2011) Enterotypes of the human gut microbiome. *Nature, 473*(7346),
1366 174-180.

1367 Bach, J.F. (2002). The effect of infections on susceptibility to autoimmune and
1368 allergic diseases. *New England Journal of Medicine, 19*(12),911-920.

1369 Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Li,
1370 Y., Xia, Y., Xie, H., Zhong, H., Khan, M. T., Zhang, J., Li, J., Xiao, L., Al-Aama, J.,
1371 Zhang, D., Lee, Y.S., Kotowska, D., Colding, C., … Wang, J. (2015). Dynamics and
1372 stabilization of the human gut microbiome during the first year of life. *Cell Host &*
1373 *Microbe, 17*(5), 690-703.

1374 Balusu, S., Brkic, M., Libert, C., & Vandenbroucke, R. E. (2016b). The choroid
1375 plexus-cerebrospinal fluid interface in Alzheimer's disease: more than just a barrier.
1376 *Neural Regeneration Research, 11*(4), 534-537.

1377 Balusu, S., Van Wonterghem, E., De Rycke, R., Raemdonck, K., Stremersch, S.,
1378 Gevaert, K., Brkic, M., Demeestere, D., Vanhooren, V., Hendrix, A., Libert, C., &
1379 Vandenbroucke, R. E. (2016a). Identification of a novel mechanism of blood-brain
1380 communication during peripheral inflammation via choroid plexus-derived
1381 extracellular vesicles. *EMBO Molecular Medicine, 4*(10),1162-1183.

1382 Banerjee, S., Schlaeppi, K., & van der Heijden, M. G. A. (2018). Keystone taxa as
1383 drivers of microbiome structure and functioning. *Nature Reviews Microbiology, 16*,
1384 567–576.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

1385 Barba, E., Sánchez, B., Burri, E., Accarino, A., Monclus, E., Navazo, I., Guarner, F.,
1386 Margolles, A., & Azpiroz, F. (2019). Abdominal distension after eating lettuce: The
1387 role of intestinal gas evaluated in vitro and by abdominal CT imaging.
1388 *Neurogastroenterology & Motility, 31*(12), e13703.

1389 Bellassi, P., Rocchetti, G., Nocetti, M., Lucini, L., Masoero, F., & Morelli, L. (2021). A
1390 combined metabolomic and metagenomic approach to discriminate raw milk for the
1391 production of hard cheese. *Foods, 6*(1), 109.

1392 Belloso Daza, M. V., Cortimiglia, C., Bassi D., & Cocconcelli P. S. (2021). Genome-
1393 based studies indicate that the *Enterococcus faecium* Clade B strains belong to
1394 Enterococcus lactis species and lack of the hospital infection associated markers.
1395 *International Journal of Systematic and Evolutionary Microbiology, 71,* doi:
1396 10.1099/ijsem.0.004948.

1397 Belloso Daza, M. V., Milani, G., Cortimiglia, C., Pietta, E., Bassi, D., & Cocconcelli,
1398 P. S. (2022). Genomic insights of *Enterococcus faecium* UC7251, a multi-drug
1399 resistant strain from ready-to-eat food, highlight the risk of antimicrobial resistance in
1400 the food chain. *Frontiers in Microbiology, 13*, 894241.

1401 Beltrán-Barrientos, L. M., Hernández-Mendoza, A., Torres-Llanez, M. J., González-
1402 Córdova, A. F., & Vallejo-Córdoba, B. (2016). Invited review: Fermented milk as
1403 antihypertensive functional food. *Journal of Dairy Science, 99*(6), 4099-4110.

1404 Biagi, E., Franceschi, C., Rampelli, S., Severgnini, M., Ostan, R., Turroni, S.,
1405 Consolandi, C., Quercia, S., Scurti, M., Monti, D., Capri, M., Brigidi, P., & Candela,
1406 M. (2016). Gut microbiota and extreme longevity. *Current Biology, 6*(11),1480-1485.

1407 Bian, G., Gloor, G. B., Gong, A., Jia, C., Zhang, W., Hu, J., Zhang, H., Zhang, Y.,
1408 Zhou, Z., Zhang, J., Burton, J. P., Reid, G., Xiao, Y., Zeng, Q., Yang, K., & Li, J.

(2017). The gut microbiota of healthy aged Chinese is similar to that of the healthy young. *mSphere*, *27*(5), e00327-17.

Black, C. J., Thakur, E. R., Houghton, L.A., Quigley, E. M. M., Moayyedi, P., & Ford, A. C. (2020). Efficacy of psychological therapies for irritable bowel syndrome: systematic review and network meta-analysis. *Gut, 69*(8), 1441-1451.

Blaser, M. J., & Falkow, S. (2009). What are the consequences of the disappearing human microbiota? *Nature Reviews Microbiology, 7*, 887–894.

Bo, L., Li, J., Tao, T., Bai, Y., Ye, X., Hotchkiss, R. S., Kollef, M. H., Crooks, N.H., & Deng, X. (2014). Probiotics for preventing ventilator-associated pneumonia. *Cochrane Database Systematic Reviews*, *10*(10), CD009066.

Bourdichon, F., Arias, E., Babuchowski, A., Bückle, A., Bello, F. D., Dubois, A., Fontana, A., Fritz, D., Kemperman, R., Laulund, S., McAuliffe, O., Miks, M. H., Papademas, P., Patrone, V., Sharma, D. K., Sliwinski, E., Stanton, C., Von Ah, U., Yao, S., & Morelli, L. (2021). The forgotten role of food cultures. *FEMS Microbiology Letters, 368*(14), fnab085.

Bourrie, B. C., Willing, B. P., Cotter, P.D. (2016). The microbiota and health promoting characteristics of the fermented beverage kefir. *Frontiers in Microbiology, 7*, 647.

Bourrie, B. C. T., Ju, T., Fouhse, J. M., Forgie, A. J., Sergi, C., Cotter, P. D., & Willing, B. P. (2021). Kefir microbial composition is a deciding factor in the physiological impact of kefir in a mouse model of obesity. *British Journal of Nutrition, 125*(2),129-138.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

1431 Bourrie, B. C. T., Richard, C., & Willing, B. P. (2020) Kefir in the prevention and
1432 treatment of obesity and metabolic disorders. *Current Nutrition Reports, 9*(3),184-
1433 192.

1434 Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Tóth, M., Korecka,
1435 A., Bakocevic, N., Ng, L.G., Kundu, P., Gulyás, B., Halldin, C., Hultenby, K., Nilsson,
1436 H., Hebert, H., Volpe, B.T., Diamond, B., Pettersson, S. (2014) The gut microbiota
1437 influences blood-brain barrier permeability in mice. *Science Translational Medicine,*
1438 *6*(263), 263ra158.

1439 Candore, G., Balistreri, C. R., Colonna-Romano, G., Grimaldi, M. P., Lio, D., Listi', F.,
1440 Scola, L., Vasto, S., & Caruso, C. (2008). Immunosenescence and anti-
1441 immunosenescence therapies: the case of probiotics. *Rejuvenation Research, 11*(2),
1442 425-432.

1443 Cernada, M., Bäuerl, C., Serna, E., Collado, M. C., Martínez, G. P., & Vento, M.
1444 (2016). Sepsis in preterm infants causes alterations in mucosal gene expression and
1445 microbiota profiles compared to non-septic twins. *Scientific Reports, 6(*6), 25497.

1446 Chapman, M. R., Robinson, L. S., Pinkner, J. S., Roth, R., Heuser, J., Hammar, M,
1447 Normark, S., & Hultgren, S. J. (2002). Role of *Escherichia coli* curli operons in
1448 directing amyloid fiber formation. *Science, 295*(5556), 851-855.

1449 Chassard, C., de Wouters, T., & Lacroix, C. (2014). Probiotics tailored to the infant: a
1450 window of opportunity. *Current Opinion in Biotechnology, 26,* 141-147.

1451 Chenhuichen, C., Cabello-Olmo, M., Barajas, M., Izquierdo, M., Ramírez-Vélez, R.,
1452 Zambom-Ferraresi, F., & Martínez-Velilla, N. (2022). Impact of probiotics and
1453 prebiotics in the modulation of the major events of the aging process: A systematic
1454 review of randomized controlled trials. *Experimental Gerontology, 164*,111809.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

1455 Childs, C. E., & Calder, P. C. (2017). Modifying the Gut Microbiome Through Diet:
1456 Effects on the Immune System of Elderly Subjects. In: Handbook of
1457 Immunosenescence. Eds. Fulop T, Franceschi C, Hirokawa K, Pawelec G. Springer.

1458 Chng, K. R., Ghosh, T. S., Tan, Y. H., Nandi, T., Lee, I. R., Ng, A. H. Q., Li, C.,
1459 Ravikrishnan, A., Lim, K. M., Lye, D., Barkham, T., Raman, K., Chen, S. L., Chai, L.,
1460 Young, B., Gan, Y. H., & Nagarajan, N. (2020). Metagenome-wide association
1461 analysis identifies microbial determinants of post-antibiotic ecological recovery in the
1462 gut. *Nature Ecology & Evolution, 4*(9), 1256-1267.

1463 Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'Connor, E. M., Cusack, S.,
1464 Harris, H. M., Coakley, M., Lakshminarayanan, B., O'Sullivan, O., Fitzgerald, G.F.,
1465 Deane, J., O'Connor, M., Harnedy, N., O'Connor, K., O'Mahony, D., van Sinderen,
1466 D., Wallace, M., Brennan, L., … O'Toole, P. W. (2012). Gut microbiota composition
1467 correlates with diet and health in the elderly. *Nature, 488*(7410), 178-184.

1468 Clemente, J. C., Pehrsson, E. C., Blaser, M. J., Sandhu, K., Gao, Z., Wang, B.,
1469 Magris, M., Hidalgo, G., Contreras, M., Noya-Alarcón, Ó., Lander, O., McDonald, J.,
1470 Cox, M., Walter, J., Oh, P. L., Ruiz, J. F., Rodriguez, S., Shen, N., Song, S.J., …
1471 Dominguez-Bello, M. G. (2015). The microbiome of uncontacted Amerindians.
1472 *Science Advances, 1*(3), e1500183.

1473 Conteville, L.C., Oliveira-Ferreira, J., & Vicente, A. C. P. (2019). Gut microbiome
1474 biomarkers and functional diversity within an Amazonian semi-nomadic hunter-
1475 gatherer group. *Frontiers in Microbiology, 10*, 1743.

1476 Daliri, E. B., Ofosu, F. K., Xiuqin, C., Chelliah, R., & Oh, D. H. (2021). Probiotic
1477 effector compounds: Current knowledge and future perspectives. *Frontiers in*
1478 *Microbiology, 12,* 655705.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

David, L. A, Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe B. E., Ling, A. V., Devlin, A. S., Varma, Y., Fischbach, M. A., Biddinger, S. B., Dutton R. J., & Turnbaugh. P. J., (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature, 505,* 559-563.

De Filippis, F., Pellegrini, N., Vannini, L., Jeffery, I.B., La Storia, A., Laghi, L., Serrazanetti, D. I., Di Cagno, R., Ferrocino, I., Lazzi, C., Turroni, S., Cocolin, L., Brigidi, P., Neviani, E., Gobbetti, M., O'Toole, P. W. & Ercolini, D. (2016). High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut, 65*(11), 1812-1821.

Demicheli, V., Jefferson, T., Di Pietrantonj, C., Ferroni, E., Thorning, S., Thomas, R. E., & Rivetti, A. (2018). Vaccines for preventing influenza in the elderly. *Cochrane Database Systematic Reviews, 2*(2), D004876.

Dhar, D., & Mohanty, A. (2020). Gut microbiota and Covid-19-possible link and implications. *Virus Research, 285,* 198018.

Duong, Q. A., Pittet, L. F., Curtis, N., & Zimmerman, P. (2022). Antibiotic exposure and adverse long-term health outcomes in children: A systematic review and meta-analysis. *Journal of Infection, 85*(3), 213-300.

European Food Safety Authority [EFSA]. (2021). EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. *EFSA Journal, 19*(7) 6506.

Fan, Y., & Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. *Nature Reviews Microbiology, 19*, 55–71.

Franz, C., Huch, M., Abriouel, H. & Gálvez A. (2011). *Enterococcus* and Safety. pp.431-447. In: Qualified Presumption of Safety (QPS) and Generally Recognised as

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

1503 Safe (GRAS) Concepts. Ed: Semedo-Lemsaddek T, Barreto-Crespo MT, Tenreiro R.
1504 Nova Editors.

1505 Gacesa, R., Kurilshikov, A., Vich Vila, A., Sinha, T., Klaassen, M. A. Y., Bolte, L.A.,
1506 Andreu-Sánchez, S., Chen, L., Collij, V., Hu, S., Dekens, J. A. M., Lenters, V. C.,
1507 Björk, J. R., Swarte, J. C., Swertz, M. A., Jansen, B.H., Gelderloos-Arends, J.,
1508 Jankipersadsing, S., Hofker, M., … Weersma, R. K. (2022). Environmental factors
1509 shaping the gut microbiome in a Dutch population. *Nature, 604*(7907), 732-739.

1510 Galkin, F., Mamoshina, P., Aliper, A., Putin, E., Moskalev, V., Gladyshev, V. N., &
1511 Zhavoronkov, A. (2020). Human Gut Microbiome Aging Clock Based on Taxonomic
1512 Profiling and Deep Learning. *iScience, 23*(6), 101199.

1513 Gänzle, M. (2022). The periodic table of fermented foods: limitations and
1514 opportunities. *Applied Microbiology and Biotechnology, 106*(8), 2815-2826.

1515 Garaiova, I., Paduchová, Z., Nagyová, Z., Wang, D., Michael, D. R., Plummer, S. F.,
1516 Marchesi, J. R., Ďuračková, Z., & Muchová, J. (2021). Probiotics with vitamin C for
1517 the prevention of upper respiratory tract symptoms in children aged 3-10 years:
1518 randomised controlled trial, *Beneficial Microbes. 12*(5), 431-440.

1519 Garrett-Bakelman, F. E., Darshi, M., Green, S. J., Gur, R. C., Lin, L., Macias, B. R.,
1520 McKenna, M. J., Meydan, C., Mishra, T., Nasrini, J., Piening, B. D., Rizzardi, L. F.,
1521 Sharma, K., Siamwala, J. H., Taylor, L., Vitaterna, M. H., Afkarian, M., Afshinnekoo,
1522 E., Ahadi, S., … Turek, F. W. (2019) The NASA Twins Study: A multidimensional
1523 analysis of a year-long human spaceflight. *Science, 364*(6436): eaau8650.

1524 Ghosh, T. S., Rampelli, S., Jeffery, I. B., Santoro, A., Neto, M., Capri, M., Giampieri,
1525 E., Jennings, A., Candela, M., Turroni, S., Zoetendal, E. G., Hermes, G. D. A.,
1526 Elodie, C,, Meunier, N., Brugere, C. M., Pujos-Guillot, E., Berendsen, A. M., De

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

1527 Groot, L. C. P. G. M., Feskins, E. J. M., … O'Toole, P. .W. (2020). Mediterranean
1528 diet intervention alters the gut microbiome in older people reducing frailty and
1529 improving health status: the NU-AGE 1-year dietary intervention across five
1530 European countries. *Gut, 69*(7), 1218-1228.

1531 Goodrich, J. K., Davenport, E. R., Beaumont, M., Jackson, M. A., Knight, R., Ober,
1532 C., Spector, T. D., Bell, J. T., Clark, A. G., Ley, R. E. (2016). Genetic Determinants
1533 of the Gut Microbiome in UK Twins. *Cell Host & Microbe,19*(5), 731-743.

1534 Gui, Q., Wang, A., Zhao, X., Huang, S., Tan, Z., Xiao, B., & Yang, Y. (2020). Effects
1535 of probiotic supplementation on natural killer cell function in healthy elderly
1536 individuals: a meta-analysis of randomized controlled trials. *European Journal of*
1537 *Clinical Nutrition, 74*(12), 1630-1637.

1538 Gutiérrez-Castrellón, P., Gandara-Martí, T., Abreu, Y., Abreu, A. T., Nieto-Rufino, C.
1539 D., López-Orduña, E., Jiménez-Escobar, I., Jiménez-Gutiérrez, C., López-
1540 Velazquez, G., Espadaler-Mazo, J. (2022). Probiotic improves symptomatic and viral
1541 clearance in Covid19 outpatients: a randomized, quadruple-blinded, placebo-
1542 controlled trial, *Gut Microbes, 14*(1), 2018899.

1543 Halmos, E. P., Christophersen, C. T., Bird, A. R., Shepherd, S. J., Gibson, P. R.,
1544 Muir, J. G. (2015). Diets that differ in their FODMAP content alter the colonic luminal
1545 microenvironment. *Gut, 64*(1), 93-100.

1546 Hao, Q., Dong, B. R., Wu, T. (2015). Probiotics for preventing acute upper
1547 respiratory tract infections. *Cochrane Database Systematic Reviews*, Feb 3;(2),
1548 CD006895.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Hao, Q., Lu, Z., Dong, B. R., Huang, C. Q., Wu, T. (2011). Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Systematic Reviews*, Sep 7(9), CD006895.

He, F. J., Li, J. & Macgregor, G. A. (2013). Effect of longer-term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. *British Medical Journal, 346*, f1325.

Hong, C. T., Chen, J. H., Huang, T. W. (2022). Probiotics treatment for Parkinson disease: a systematic review and meta-analysis of clinical trials. *Aging (Albany NY), 14*(17), 7014-7025.

Horsager, J., Andersen, K. B., Knudsen, K., Skjærbæk, C., Fedorova, T. D., Okkels, N., Schaeffer, E., Bonkat, S. K., Geday, J., Otto, M., Sommerauer, M., Danielsen, E. H., Bech, E., Kraft, J., Munk, O. L., Hansen, S. D., Pavese, N., Göder, R., Brooks, D.J. … Borghammer, P. (2020). Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. *Brain, 143*(10), 3077-3088.

Huaman, J. W., Mego, M., Manichanh, C., Cañellas, N., Cañueto, D., Segurola, H., Jansana, M., Malagelada, C., Accarino, A., Vulevic, J., Tzortzis, G., Gibson, G., Saperas, E., Guarner, F., Azpiroz, F. (2018). Effects of prebiotics vs a diet low in FODMAPs in patients with functional gut disorders. *Gastroenterology, 155*(4), 1004-1007.

Huang, H. L., Chen, H. T., Luo, Q. L., Xu, H. M., He, J., Li,.Y. Q., Zhou, Y.L., Yao, F., Nie, Y. Q., Zhou, Y. J. (2019). Relief of irritable bowel syndrome by fecal microbiota transplantation is associated with changes in diversity and composition of the gut microbiota. *Journal of Digestive Disorders, 20*(8), 401-408.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Ji, T., Zhu, X., Shang, F., Zhang, X. (2021). Preventive Effect of Probiotics on Ventilator-Associated Pneumonia: A Meta-analysis of 2428 Patients. *Annals of Pharmacotherapy, 55*(8), 949-962.

Jimenez, M. E., O'Donovan, C. M., de Ullivarri, M. F., Cotter, P. D. (2022). Microorganisms present in artisanal fermented food from South America. *Frontiers in Microbiology, 13,* 941866.

Johnstone, J., Meade, M., Lauzier, F., Marshall, J., Duan, E., Dionne, J., Arabi, Y. M., Heels-Ansdell, D., Thabane, L., Lamarche, D., Surette, M., Zytaruk, N., Mehta, S., Dodek, P., McIntyre, L., English, S., Rochwerg, B., Karachi, T., Henderson, W. … Cook, D. (2021). Prevention of Severe Pneumonia and Endotracheal Colonization Trial (PROSPECT) Investigators and the Canadian Critical Care Trials Group. Effect of Probiotics on Incident Ventilator-Associated Pneumonia in Critically Ill Patients: A Randomized Clinical Trial, *Journal of the American Medical Association, 326*(11), 1024-1033.

Jost, T., Lacroix, C., Braegger, C., Chassard, C. (2013). Assessment of bacterial diversity in breast milk using culture-dependent and culture-independent approaches. *British Journal of Nutrition, 110*(7), 1253-1262.

Jost, T., Lacroix, C., Braegger, C., Chassard, C. (2012). New insights in gut microbiota establishment in healthy breast-fed neonates. *PLoS One, 7*(8), e44595.

Kaczorowski, K.J., Shekhar, K., Nkulikiyimfura, D., Dekker, C. L., Maecker, H., Davis, M. M., Chakraborty, A. K., Brodin, P. (2017). Continuous immunotypes describe human immune variation and predict diverse responses. *Proceedings of the National Academy of Science, 114*(30), E6097-E6106.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

1595 Kaur, L., Gordon, M., Baines, P. A., Iheozor-Ejiofor, Z., Sinopoulou, V., Akobeng, A.
1596 K. (2020). Probiotics for induction of remission in ulcerative colitis. *Cochrane*
1597 *Database Systematic Reviews, 3*(3), CD005573.

1598 Kazemi, A., Noorbala, A. A., Azam, K., Eskandari, M. H., Djafarian, K. *(2019).* Effect
1599 of probiotic and prebiotic vs placebo on psychological outcomes in patients with
1600 major depressive disorder: A randomized clinical trial. *Clinical Nutrition, 38*(2), 522-
1601 528.

1602 Khan, S., Hansen, R., Scott, K., Martin, J., Berry, S., Stevenson, M., Okapapi, A.,
1603 Hold, G., & Munro, M. (2015). The human gut is probably sterile at birth. *Archives of*
1604 *Disease in Childhood, 100,* A50-A51.

1605 Kim, B. S., Choi, C. W., Shin, H., Jin, S. P., Bae, J. S., Han, M., Seo, E. Y., Chun, J.,
1606 Chung, J. H. (2019). Comparison of the Gut Microbiota of Centenarians in Longevity
1607 Villages of South Korea with Those of Other Age Groups. *Journal of Microbiology &*
1608 *Biotechnology, 29*(3), 429-440.

1609 Kim, S., Kwon, S. H., Kam, T. I., Panicker, N., Karuppagounder, S. S., Lee, S., Lee,
1610 J. H., Kim, W. R., Kook, M., Foss, C. A., Shen, C., Lee, H., Kulkarni, S., Pasricha,
1611 P.J., Lee, G., Pomper, M. G., Dawson, V. L., Dawson, T. M., Ko, H. S. (2019).
1612 Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain
1613 Models Parkinson's Disease. *Neuron, 103*(4), 627-641.

1614 Kirjavainen, P. V., Karvonen, A. M., Adams, R. I., Täubel, M., Roponen, M.,
1615 Tuoresmäki, P., Loss, G., Jayaprakash, B., Depner, M., Ege, M. J., Renz, H.,
1616 Pfefferle, P. I., Schaub, B., Lauener, R., Hyvärinen, A., Knight, R., Heederik, D. J. J.,
1617 von Mutius, E., Pekkanen, J. (2019). Farm-like indoor microbiota in non-farm homes
1618 protects children from asthma development. *Nature Medicine, 25*(7), 1089-1095.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Knudsen, J. K., Bundgaard-Nielsen, C., Hjerrild, S., Nielsen, R. E., Leutscher, P., Sørensen, S. (2021). Gut microbiota variations in patients diagnosed with major depressive disorder-A systematic review. *Brain and Behavior, 11*(7), e02177.

Kong, F., Hua, Y., Zeng, B., Ning, R., Li, Y., Zhao, J. (2016). Gut microbiota signatures of longevity. *Current Biology, 26*(18), R832-R833.

Kostic, A. D., Gevers, D., Siljander, H., Vatanen, T., Hyötyläinen, T., Hämäläinen, A. M., Peet, A., Tillmann, V., Pöhö, P., Mattila, I., Lähdesmäki, H., Franzosa, E. A., Vaarala, O., de Goffau, M., Harmsen, H., Ilonen, J., Virtanen, S. M., Clish, C. B., Orešič, M. … Xavier, R. J. (2015). The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host & Microbe, 17*(2), 260-273.

Kuehnast, T., Abbott, C., Pausan, M. R., Pearce, D. A., Moissl-Eichinger, C., Mahnert, A. (2022). The crewed journey to Mars and its implications for the human microbiome. *Microbiome, 10*(1), 26.

Lacy, B. E. & Patel, N. K. (2017). Rome criteria and a diagnostic approach to irritable bowel syndrome. *Journal of Clinical Medicine, 6*(11), 99.

Lang, P. O., Mitchell, W. A., Govind, S., Aspinall, R. (2011). Real time-PCR assay estimating the naive T-cell pool in whole blood and dried blood spot samples: pilot study in young adults. *Journal of Immunological Methods, 369*(1-2), 133-140.

Langkamp-Henken, B., Rowe, C. C., Ford, A. L., Christman, M. C., Nieves, C. Jr, Khouri, L., Specht, G. J., Girard, S. A., Spaiser, S. J., Dahl, W. J. (2015). *Bifidobacterium bifidum* R0071 results in a greater proportion of healthy days and a lower percentage of academically stressed students reporting a day of cold/flu: a

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

randomised, double-blind, placebo-controlled study. *British Journal of Nutrition, 113*(3), 426-434.

Larsen, O. F. A., Claassen, E., Brummer, R. J. (2020). On the importance of intraindividual variation in nutritional research. *Beneficial Microbes, 11*(6), 511-517.

Larsen, O. F. A. & van de Burgwal, L. H. M. (2021). On the verge of a catastrophic collapse? The need for a multi-ecosystem approach to microbiome studies. *Frontiers in Microbiology, 12*, 784797.

Larsen, O. F. A., van der Grint, M., Wiegers, C. and van de Burgwal LHM (2022). The gut microbiota: Master of puppets connecting the epidemiology of infectious, autoimmune, and metabolic disease. *Frontiers in Microbiology, 13,* 902106.

Layer, P., Andresen, V., Allescher, H., Bischoff, S. C., Claßen, M., Elsenbruch, S., Freitag, M., Frieling, T., Gebhard, M., Goebel-Stengel, M., Häuser, W., Holtmann, G., Keller, J., Kreis, M. E., Kruis, W., Langhorst, J., Jansen, P. L., Madisch, A., Mönnikes, H. … Wedel T. (2021) Update S3-Leitlinie Reizdarmsyndrom: Definition, Pathophysiologie, Diagnostik und Therapie. Gemeinsame Leitlinie der Deutschen Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten (DGVS) und der Deutschen Gesellschaft für Neurogastroenterologie und Motilität (DGNM) AWMF-Registriernummer: 021/016. *Zeitschrift für Gastroenterologie, 59*(12), 1323-1415.

le Roux, C. W. (2021). COVID-19 alters thinking and management in metabolic diseases. Nature Reviews Endocrinology, 17, 71–72.

Leech, J., Cabrera-Rubio, R., Walsh, A. M., Macori, G., Walsh, C. J., Barton, W., Finnegan, L., Crispie, F., O'Sullivan, O., Claesson, M. J., Cotter, P. D. (2020). Fermented-Food Metagenomics Reveals Substrate-Associated Differences in

Taxonomy and Health-Associated and Antibiotic Resistance Determinants. *mSystems, 5*(6), e00522-20.

Lei, W. T., Shih, P. C., Liu,.S. J., Lin, C. Y., Yeh, T. L. (2017). Effect of Probiotics and Prebiotics on Immune Response to Influenza Vaccination in Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients, 9*(11), 1175.

Lenoir-Wijnkoop, I., Gerlier, L., Bresson, J. L., Le Pen, C., & Berdeaux, G. (2015). Public health and budget impact of probiotics on common respiratory tract infections: a modelling study. *PLoS One, 10*(4), e0122765.

Leta, V., Ray Chaudhuri, K., Milner, O., Chung-Faye, G., Metta, V., Pariante, C. M., Borsini, A. (2021). Neurogenic and anti-inflammatory effects of probiotics in Parkinson's disease: A systematic review of preclinical and clinical evidence. *Brain, Behavior, and Immunity, 98*, 59-73.

Li Z, Liang H, Hu Y, Lu L, Zheng C, Fan Y, Wu B, Zou T, Luo X, Zhang X, Zeng Y, Liu Z, Zhou Z, Yue Z, Ren Y, Li Z, Su Q, Xu P. Gut bacterial profiles in Parkinson's disease: A systematic review. CNS Neuroscience & Therapeutics, 2023 Jan;29(1):140-157.

Liu, R. T., Walsh, R. F. L., & Sheehan, A. E. (2019). Prebiotics and probiotics for depression and anxiety: A systematic review and meta-analysis of controlled clinical trials. *Neuroscience & Biobehavioral Reviews, 102*, 13-23.

Lynch, S. V., & Pedersen, O. (2016). The Human Intestinal Microbiome in Health and Disease. *New England Journal of Medicine, 375*(24), 2369-2379.

Mackowiak, P. A. (2013). Recycling Metchnikoff: probiotics, the intestinal microbiome and the quest for long life. *Frontiers in Public Health, Nov 1*, 52.

1690 Mahnert, A., Verseux, C., Schwendner, P., Koskinen, K., Kumpitsch, C., Blohs, M.,
1691 Wink, L., Brunner, D., Goessler, T., Billi, D., Moissl-Eichinger, C. (2021). Microbiome
1692 dynamics during the HI-SEAS IV mission, and implications for future crewed
1693 missions beyond Earth. *Microbiome, 9*, 27.

1694 Mai, T. T., Thi Thu, P., Thi Hang, H., Trang, T. T. T., Yui, S., Shigehisa, A., Tien,
1695 V.T., Dung, T. V., Nga, P. B., Hung, N. T., & Tuyen. L. D. (2021). Efficacy of
1696 probiotics on digestive disorders and acute respiratory infections: a controlled clinical
1697 trial in young Vietnamese children. *European Journal of Clinical Nutrition, 75*(3), 513-
1698 520.

1699 Manichanh, C., Eck, A., Varela, E., Roca, J., Clemente, J. C., González, A., Knights,
1700 D., Knight, R., Estrella, S., Hernandez, C., Guyonnet, D., Accarino, A., Santos, J.,
1701 Malagelada, J. R., Guarner, F., & Azpiroz, F. (2014) Anal gas evacuation and colonic
1702 microbiota in patients with flatulence: effect of diet. *Gut, 63*(3),401-408.

1703 Marco, M. L., Sanders, M. E., Gänzle, M., Arrieta, M. C., Cotter, P. D., De Vuyst, L.,
1704 Hill, C., Holzapfel, W., Lebeer, S., Merenstein, D., Reid, G., Wolfe, B. E., & Hutkins,
1705 R. (2021). The International Scientific Association for Probiotics and Prebiotics
1706 (ISAPP) consensus statement on fermented foods. *Nature Reviews*
1707 *Gastroenterology & Hepatology, 18*(3), 196-208.

1708 Marques, T. M., Wall, R., Ross, R. P., Fitzgerald, G. F., Ryan, C. A., & Stanton, C.
1709 (2010). Programming infant gut microbiota: influence of dietary and environmental
1710 factors. *Current Opinion in Biotechnology, 21*(2), 49-56.

1711 Matheoud, D., Cannon, T., Voisin, A., Penttinen, A. M., Ramet, L., Fahmy, A. M.,
1712 Ducrot, C., Laplante, A., Bourque, M. J., Zhu, L., Cayrol, R., Le Campion, A.,
1713 McBride, H. M., Gruenheid, S., Trudeau, L. E., & Desjardins, M. (2019). Intestinal

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

infection triggers Parkinson's disease-like symptoms in Pink1-/- mice. *Nature, 571*(7766), 565-569.

Maurice, C. F., & Turnbaugh, P. J. (2018). Quantifying the metabolic activities of human-associated microbial communities across multiple ecological scales. *FEMS Microbiology Reviews, 37*, 830–848.

McFarland, L. V. (2021). Efficacy of Single-Strain Probiotics Versus Multi-Strain Mixtures: Systematic Review of Strain and Disease Specificity. *Digestive Diseases and Sciences, 66(*3), 694-704.

Mitchell, W. A., Lang, P. O., & Aspinall, R. (2010). Tracing thymic output in older individuals. *Clinical and Experimental Immunology, 161*(3), 497-503.

Mizui, T., Hattori, K., Ishiwata, S., Hidese, S., Yoshida, S., Kunugi, H., & Kojima, M. (2019). Cerebrospinal fluid BDNF pro-peptide levels in major depressive disorder and schizophrenia. *Journal of Psychiatry Research, 113*, 190-198.

Mora, M., Wink, L., Kögler, I., Mahnert, A., Rettberg, P., Schwendner, P., Demets, R., Cockell, C., Alekhova, T., Klingl, A., Krause, R., Zolotariof, A., Alexandrova, A., & Moissl-Eichinger, C. (2019). Space Station conditions are selective but do not alter microbial characteristics relevant to human health. *Nature Communications, 10*(1), 3990.

Mortensen S., Coakley M., Kilcawley K, Cotter PD. The pan-metagenome investigation of the water kefir microbiome. In preparation.

Mukherjee, A., Gómez-Sala, B., O'Connor, E. M., Kenny, J. G., & Cotter, P. D. (2022). Global regulatory frameworks for fermented foods: A review. *Frontiers in Nutrition, May 9*, 902642.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

1737 Odamaki, T., Kato, K., Sugahara, H., Hashikura, N., Takahashi, S., Xiao, J. Z., Abe,
1738 F., & Osawa, R. (2016). Age-related changes in gut microbiota composition from
1739 newborn to centenarian: a cross-sectional study. *BMC Microbiology, 16*, 90.

1740 Oren, A, & Garrity, G.M. (2021). Valid publication of the names of forty-two phyla of
1741 prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*,
1742 *71*(10), 005056.

1743 Otaka, M., Kikuchi-Hayakawa, H., Ogura, J., Ishikawa, H., Yomogida, Y., Ota, M.,
1744 Hidese, S., Ishida, I., Aida, M., Matsuda, K., Kawai, M., Yoshida, S., & Kunugi, H.
1745 (2021) Effect of *Lacticaseibacillus paracasei* strain Shirota on improvement in
1746 depressive symptoms, and its association with abundance of actinobacteria in gut
1747 microbiota. *Microorganisms, 9*(5), 1026.

1748 O'Toole, P. W., & Jeffery, I. B. (2015). Gut microbiota and aging. *Science,*
1749 *350*(6265), 1214-1215.

1750 Pasolli, E., De Filippis, F., Mauriello, I. E., Cumbo, F., Walsh, A. M., Leech, J.,
1751 Cotter, P. D., Segata, N., & Ercolini, D. (2020). Large-scale genome-wide analysis
1752 links lactic acid bacteria from food with the gut microbiome. *Nature Communications,*
1753 *11*(1), 2610.

1754 Paz, M. A., de-La-Sierra, A., Sáez,.M., Barceló, M. A., Rodríguez, J. J., Castro, S.,
1755 Lagarón, C., Garrido, J. M., Vera, P., & Coll-de-Tuero, G. (2016). Treatment efficacy
1756 of anti-hypertensive drugs in monotherapy or combination: ATOM systematic review
1757 and meta-analysis of randomized clinical trials according to PRISMA statement.
1758 *Medicine (Baltimore), 95*(30), e4071.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Peñas, E., Diana, M., Frias, J., Quílez, J., & Martínez-Villaluenga, C. (2015). A multistrategic approach in the development of sourdough bread targeted towards blood pressure reduction. *Plant Foods for Human Nutrition, 70*(1), 97-103.

Perez, P. F., Doré, J., Leclerc, M., Levenez, F., Benyacoub, J., Serrant, P., Segura-Roggero, I., Schiffrin, E. J., & Donnet-Hughes, A. (2007). Bacterial imprinting of the neonatal immune system: lessons from maternal cells? *Pediatrics, 119*(3), e724-32.

Perez-Pardo, P., Broersen, L. M., Kliest, T., van Wijk, N., Attali, A., Garssen, J., Kraneveld, A. D. (2018b). Additive effects of levodopa and a neurorestorative diet in a mouse model of Parkinson's disease. *Frontiers in Aging Neuroscience, 10*, 237.

Perez-Pardo, P., de Jong EM, Broersen, L. M., Kliest, T., van Wijk, N., Attali, A., Garssen, J., & Kraneveld, A. D. (2017). Promising effects of neurorestorative diets on motor, cognitive, and gastrointestinal dysfunction after symptom development in a mouse model of Parkinson's disease. *Frontiers in Aging Neuroscience, 9*, 57.

Perez-Pardo, P., Dodiya, H. B., Broersen, L. M., Douna, H., van Wijk, N., Lopes da Silva, S., Garssen, J., Keshavarzian, A., Kraneveld, A. D. (2018a). Gut-brain and brain-gut axis in Parkinson's disease models: Effects of a uridine and fish oil diet. *Nutritional Neuroscience, 21*(6), 391-402.

Perez-Pardo, P., Dodiya, H. B., Engen, P. A., Forsyth, C. B., Huschens, A. M., Shaikh, M., Voigt, R. M., Naqib, A., Green, S. J., Kordower, J. H., Shannon, K. M., Garssen, J., Kraneveld, A. D., & Keshavarzian, A. (2019). Role of TLR4 in the gut-brain axis in Parkinson's disease: a translational study from men to mice. *Gut, 68*(5), 829-843.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

1781 Pham, V. T., Greppi, A., Chassard, C., Braegger, C., & Lacroix, C. (2022). Stepwise
1782 establishment of functional microbial groups in the infant gut between 6 months and
1783 2 years: A prospective cohort study. *Frontiers in Nutrition, 9*, 948131.

1784 Pham, V. T., Lacroix, C., Braegger, C. P., & Chassard, C. (2017). Lactate-utilizing
1785 community is associated with gut microbiota dysbiosis in colicky infants. *Scientific*
1786 *Reports, 7*(1), 11176.

1787 Pozuelo, M., Panda, S., Santiago, A., Mendez, S., Accarino, A., Santos, J., Guarner,
1788 F., Azpiroz, F., & Manichanh, C. (2015). Reduction of butyrate- and methane-
1789 producing microorganisms in patients with irritable bowel syndrome. *Scientific*
1790 *Reports, 5*: 12693.

1791 Przemska-Kosicka, A., Childs, C. E., Maidens, C., Dong, H., Todd, S., Gosney, M.
1792 A., Tuohy, K. M., & Yaqoob, P. (2018). Age-related changes in the natural killer cell
1793 response to seasonal influenza vaccination are not influenced by a synbiotic: a
1794 randomised controlled trial. *Frontiers in Immunology, 9*, 591.

1795 Quick, M. (2015). Cochrane Commentary: Probiotics for prevention of acute upper
1796 respiratory infection. *Explore (NY), 11*(5), 418-420.

1797 Rampelli, S., Soverini, M., D'Amico, F., Barone, M., Tavella, T., Monti, D., Capri, M.,
1798 Astolfi, A., Brigidi, P., Biagi, E., Franceschi, C., Turroni, S., & Candela, M. (2020).
1799 Shotgun metagenomics of gut microbiota in humans with up to extreme longevity
1800 and the increasing role of xenobiotic degradation. *mSystems, 5*(2), e00124-20.

1801 Rashidi, K., Razi, B., Darand, M., Dehghani, A., Janmohammadi, P., & Alizadeh, S.
1802 (2021). Effect of probiotic fermented dairy products on incidence of respiratory tract
1803 infections: a systematic review and meta-analysis of randomized clinical trials.
1804 *Nutrition Journal, 20*(1), 61.

Raveschot, C., Cudennec, B., Coutte, F., Flahaut, C., Fremont, M., Drider, D., & Dhulster, P. (2018). Production of bioactive peptides by *Lactobacillus* species: From gene to application. *Frontiers in Microbiology, 9*, 2354.

Ren, M., Li, H., Fu, Z., & Li, Q. (2021). Succession analysis of gut microbiota structure of participants from long-lived families in Hechi, Guangxi, China. *Microorganisms, 9*(12), 2524.

Rietdijk, C. D., Perez-Pardo, P., Garssen, J., van Wezel, R. J., & Kraneveld, A. D. (2017). Exploring Braak's hypothesis of Parkinson's disease. *Frontiers in Neurology, 8,* 37.

Rocha Martin, V. N., Del'Homme, C., Chassard, C., Schwab, C., Braegger, C., Bernalier-Donadille, A., & Lacroix, C. (2022). A proof-of-concept infant-microbiota associated rat model for studying the role of gut microbiota and alleviation potential of *Cutibacterium avidum* in infant colic. *Frontiers in Nutrition,9*, 902159.

Rode, J., Edebol Carlman, H. M.T., König, J., Hutchinson, A. N., Thunberg, P., Persson, J., & Brummer, R. J. (2022). Multi-strain probiotic mixture affects brain morphology and resting state brain function in healthy subjects: An RCT. *Cells, 11*(18), 2922.

Rosenzweig, J. A., Abogunde, O., Thomas, K., Lawal, A., Nguyen, Y. U., Sodipe, A., & Jejelowo, O. (2010). Spaceflight and modeled microgravity effects on microbial growth and virulence. Applied microbiology and biotechnology, 85(4), 885–891. https://doi.org/10.1007/s00253-009-2237-8

Roswall, J., Olsson, L. M., Kovatcheva-Datchary, P., Nilsson, S., Tremaroli, V., Simon, M. C., Kiilerich, P., Akrami, R., Krämer, M., Uhlén, M., Gummesson, A., Kristiansen, K., Dahlgren, J., & Bäckhed, F. (2021). Developmental trajectory of the

healthy human gut microbiota during the first 5 years of life. *Cell Host & Microbe, 29*(5), 765-776.

Rothwell, P. M. (2006). Factors that can affect the external validity of randomised controlled trials. *PLoS Clinical Trials, 1*(1), e9.

Salminen, S., Collado, M.C., Endo, A., Hill, C., Lebeer, S., Quigley, E. M. M., Sanders, M.E., Shamir, R., Swann, J.R., Szajewska, H. & Vinderola, G. (2021) The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *National Reviews in Gastroenterology & Hepatology, 18*(9), 649–667.

Sampson, T. R., Debelius, J. W., Thron, T., Janssen, S., Shastri, G. G., Ilhan, Z. E., Challis, C., Schretter, C. E., Rocha, S., Gradinaru, V., Chesselet, M. F., Keshavarzian, A., Shannon, K. M., Krajmalnik-Brown, R., Wittung-Stafshede, P., Knight, R., & Mazmanian, S. K. (2016) Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell, 167*(6), 1469-1480.

Scheperjans, F., Derkinderen, P., & Borghammer, P. (2018). The gut and Parkinson's disease: Hype or hope? *Journal of Parkinson's Disease, 8*(s1), S31-S39.

Schwendner, P., Mahnert, A., Koskinen, K., Moissl-Eichinger, C., Barczyk, S., Wirth R, Berg G, & Rettberg P. (2017). Preparing for the crewed Mars journey: microbiota dynamics in the confined Mars500 habitat during simulated Mars flight and landing. *Microbiome, 5*(1), 1-23.

Scientific Committee on Emerging and Newly Identified Health Risks [SCENIHR], Scientific Committee on Consumer Safety (SCCS) & Scientific Committee on Health and Environmental Risks (SCHER) (2014). Synthetic Biology I Definition. https://health.ec.europa.eu/publications/synthetic-biology-i-definition_en

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Segal, A., Zlotnik, Y., Moyal-Atias, K., Abuhasira, R., & Ifergane, G. (2021). Fecal microbiota transplant as a potential treatment for Parkinson's disease - A case series. *Clinical Neurology and Neurosurgery, 207*, 106791.

Selma-Royo, M., Calvo-Lerma, J., Bäuerl, C., Esteban-Torres, M., Cabrera-Rubio, R., & Collado, M. C. (2022). Human milk microbiota: what did we learn in the last 20 years? *Microbiome Research Reports, 1*, 19.

Selma-Royo, M., García-Mantrana, I., Collado, M. C., & Perez-Martínez, G. (2022). Intake of Natural, Unprocessed tiger nuts (Cyperus esculentus L.) drink significantly favors intestinal beneficial bacteria in a short period of time. *Nutrients, 14*(9), 1709.

Sharif, S., Greer, A., Skorupski, C., Hao, Q., Johnstone, J., Dionne, J. C., Lau, V., Manzanares, W., Eltorki, M., Duan, E., Lauzier, F., Marshall, J. C., Heels-Ansdell, D., Thabane, L., Cook, D. J., & Rochwerg, B. (2022). Probiotics in critical illness: A systematic review and meta-analysis of randomized controlled trials. *Critical Care Medicine, 50*(8), 1175-1186.

Shi, C., & Maktabdar, M. (2022) Lactic acid bacteria as biopreservation against spoilage molds in dairy products – A review. *Frontiers in Microbiology, 12*, 819684.

Shida, K., Sato, T., Iizuka, R., Hoshi, R., Watanabe, O., Igarashi, T., Miyazaki, K., Nanno, M., & Ishikawa, F. (2017). Daily intake of fermented milk with *Lactobacillus casei* strain Shirota reduces the incidence and duration of upper respiratory tract infections in healthy middle-aged office workers. *European Journal of Nutrition, 56*(1), 45-53.

Siedler, S., Balti, R., & Neves, A. R. (2019). Bioprotective mechanisms of lactic acid bacteria against fungal spoilage of food. *Current Opinion in Biotechnology, 56*, 138-146.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Staudacher, H. M., & Whelan, K. (2017). The low FODMAP diet: recent advances in understanding its mechanisms and efficacy in IBS. *Gut, 66*(8),1517-1527.

Suda, K., & Matsuda, K. (2022). How microbes affect depression: Underlying mechanisms via the gut-brain axis and the modulating role of probiotics. *International Journal of Molecular Science, 23*(3), 1172.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X. N., Kubo, C., & Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *Journal of Physiology, 558*(Pt 1), 263-275.

Szajewska, H., & Kołodziej, M. (2015a). Systematic review with meta-analysis: *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea. *Alimentary Pharmacology & Therapeutics, 42*(7), 793-801.

Szajewska, H., & Kołodziej. M. (2015b). Systematic review with meta-analysis: *Lactobacillus rhamnosus* GG in the prevention of antibiotic-associated diarrhoea in children and adults. *Alimentary Pharmacology & Therapeutics, 42*(10), 1149-1157.

Tae Yu, H., Youn, J. C., Lee, J., Park, S., Chi, H. S., Lee, J., Choi, C., Park, S., Choi, D., Ha, J. W., & Shin, E. C. (2015). Characterization of CD8(+)CD57(+) T cells in patients with acute myocardial infarction. *Cellular & Molecular Immunology, 12*(4), 466-473.

Takada, M., Nishida, K., Kataoka-Kato, A., Gondo, Y., Ishikawa, H., Suda, K., Kawai, M., Hoshi, R., Watanabe, O., Igarashi, T., Kuwano, Y., Miyazaki, K., & Rokutan, K. (2016). Probiotic *Lactobacillus casei* strain Shirota relieves stress-associated symptoms by modulating the gut-brain interaction in human and animal models. *Neurogastroenterology and Motility, 28*(7), 1027-1036.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Tenenbaum, M., Deracinois, B., Dugardin, C., Matéos, A., Romelard, A., Auger, J., Boulier, A., Ravallec, R., Flahaut, C., & Cudennec, B. (2022). Identification, production and bioactivity of casein phosphopeptides - A review. *Food Research International, 157*, 111360.

Treadway, M. T., Waskom, M. L., Dillon, D. G., Holmes, A. J., Park, M. T. M., Chakravarty, M. M., Dutra, S. J., Polli, F. E., Iosifescu, D. V., Fava, M., Gabrieli, J. D. E., & Pizzagalli, D. A. (2015). Illness progression, recent stress, and morphometry of hippocampal subfields and medial prefrontal cortex in major depression. *Biological Psychiatry, 77*(3), 285-294.

Tsilika, M., Thoma, G., Aidoni, Z., Tsaousi, G., Fotiadis, K., Stavrou, G., Malliou, P., Chorti, A., Massa, H., Antypa, E., Vasiliadou, G., Pagdatoglou, K., Voudouris, A., Vasiliagou, S., Mitos, G., Kontopoulou, N., Paraforou, N., Antoniadou, E., Mouloudi, H. … Kotzampassi, K. (2022). A four-probiotic preparation for ventilator-associated pneumonia in multi-trauma patients: results of a randomized clinical trial. *International Journal of Antimicrobial Agents, 59*(1), 106471.

Tuikhar, N., Keisam, S., Labala, R. K., Imrat, Ramakrishnan, P., Arunkumar, M. C., Ahmed, G., Biagi, E., & Jeyaram, K. (2019). Comparative analysis of the gut microbiota in centenarians and young adults shows a common signature across genotypically non-related populations. *Mechanisms of Ageing and Development, 179*, 23-35.

van Kessel, S. P., Frye, A. K., El-Gendy, A. O., Castejon, M., Keshavarzian, A., van Dijk, G., & El Aidy, S. (2019). Gut bacterial tyrosine decarboxylases restrict levels of levodopa in the treatment of Parkinson's disease. *Nature Communications, 10*(1), 310.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

Vandenbroucke, R. E. (2016). A hidden epithelial barrier in the brain with a central role in regulating brain homeostasis. Implications for aging. *Annals of the American Thoracic Society, 13 Suppl 5*, S407-S410.

von Mutius, E., Braun-Fahrländer, C., Schierl, R., Riedler, J., Ehlermann, S., Maisch, S., Waser, M., & Nowak, D. (2000). Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clinical & Experimental Allergy, 30*, 1230–1234.

Voorhies, A. A., Mark Ott, C., Mehta, S., Pierson, D. L., Crucian, B. E., Feiveson, A., Oubre, C. M., Torralba, M., Moncera, K., Zhang, Y., Zurek, E., & Lorenzi, H. A. (2019). Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome. *Scientific Reports, 9*(1), 9911.

Wacklin, P., Mäkivuokko, H., Alakulppi, N., Nikkilä, J., Tenkanen, H., Räbinä, J., Partanen, J., Aranko, K., & Mättö, J. (2011). Secretor genotype (FUT2 gene) is strongly associated with the composition of Bifidobacteria in the human intestine. *PLoS One, 6*(5), e20113.

Walsh, A. M., Crispie, F., Kilcawley, K., O'Sullivan, O., O'Sullivan, M. G., Claesson, M. J., & Cotter, P. D. (2016). Microbial succession and flavor production in the fermented dairy beverage kefir. *mSystems, 1*(5), e00052-16.

Waters, J. L., & Ley, R. E. (2019). The human gut bacteria Christensenellaceae are widespread, heritable, and associated with health. *BMC Biology, 17*(1), 83.

Wu, J., Masuy, I., Biesiekierski, J. R., Fitzke, H. E., Parikh, C., Schofield, L., Shaikh, H., Bhagwanani, A., Aziz, Q., Taylor, S. A., Tack, J., & Van Oudenhove, L. (2022). Gut-brain axis dysfunction underlies FODMAP-induced symptom generation in irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics, 55*(6), 670-682.

1949 Xie, J., Li, Q., Haesebrouck, F., Van Hoecke, L., & Vandenbroucke, R. E. (2022).
1950 The tremendous biomedical potential of bacterial extracellular vesicles. *Trends in*
1951 *Biotechnology, 40(*10), 1173-1194.

1952 Xie, J., Cools, L., Van Imschoot, G., Van Wonterghem, E., Pauwels, M. J.,
1953 Vlaeminck, I., De Witte, C., Andaloussi, S. E. L.,Wierda, K., De Groef, L.,
1954 Haesebrouck, F., Van Hoecke, L., & Vandenbroucke, R. E. (2023). Helicobacter
1955 pylori-derived outer membrane vesicles contribute to Alzheimer's disease
1956 pathogenesis via C3-C3aR signalling. Journal of Extracellular Vesicles, e12306.
1957 https://doi.org/10.1002/jev2.12306.

1958 Yeoh, Y. K., Zuo, T., Lui, G. C., Zhang, F., Liu, Q., Li, A. Y., Chung, A. C., Cheung,
1959 C. P., Tso, E. Y., Fung, K. S., Chan, V., Ling, L., Joynt, G., Hui, D. S., Chow, K. M.,
1960 Ng, S. S. S., Li, T.C., Ng, R. W., Yip, T.C., … Ng, S. C. (2021). Gut microbiota
1961 composition reflects disease severity and dysfunctional immune responses in
1962 patients with COVID-19. *Gut, 70*, 698–706.

1963 You, J., & Yaqoob, P. (2012). Evidence of immunomodulatory effects of a novel
1964 probiotic, *Bifidobacterium longum* bv. *infantis* CCUG 52486. *FEMS Immunology and*
1965 *Medical Microbiology, 66*(3), 353-362.

1966 Sato, Y., Atarashi, K., Plichta, D. R., Arai, Y., Sasajima, S., Kearney, S. M., Suda,
1967 W., Takeshita, K., Sasaki, T., Okamoto, S., Skelly, A. N., Okamura, Y., Vlamakis, H.,
1968 Li, Y., Tanoue, T., Takei, H., Nittono, H., Narushima, S., Irie, J. … Honda, K. (2021).
1969 Novel bile acid biosynthetic pathways are enriched in the microbiome of
1970 centenarians. *Nature, 599*(7885), 458-464.

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

1971 Zeilstra, D., Younes, J. A., Brummer, R. J., & Kleerebezem, M. (2018). Perspective:
1972 fundamental limitations of the randomized controlled trial method in nutritional
1973 research: The example of probiotics. *Advances in Nutrition, 9*(5), 561-571.

1974 Zhao, L., Zhang, F., Ding, X., Wu, G., Lam, Y. Y., Wang, X., Fu, H., Xue, X., Lu, C.,
1975 Ma, J., Yu, L., Xu, C., Ren, Z., Xu, Y., Xu, S., Shen, H., Zhu, X., Shi, Y., Shen, Q. …
1976 Zhang, C. (2018). Gut bacteria selectively promoted by dietary fibers alleviate type 2
1977 diabetes. *Science, 359*(6380), 1151-1156.

1978 Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., Zeng, L., Chen, J., Fan, S.,
1979 Du, X., Zhang, X., Yang, D., Yang, Y., Meng, H., Li, W., Melgiri, N. D., Licinio, J.,
1980 Wei, H., & Xie, P. (2016). Gut microbiome remodelling induces depressive-like
1981 behaviors through a pathway mediated by the host's metabolism. *Molecular*
1982 *Psychiatry, 21*(6), 786-796.

1983 Zhong, C., Qu, C., Wang, B., Liang, S., Zeng, B. (2017). Probiotics for preventing
1984 and treating small intestinal bacterial overgrowth: A meta-analysis and systematic
1985 review of current evidence. *Journal of Clinical Gastroenterology, 51*(4), 300-311.

1986

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press

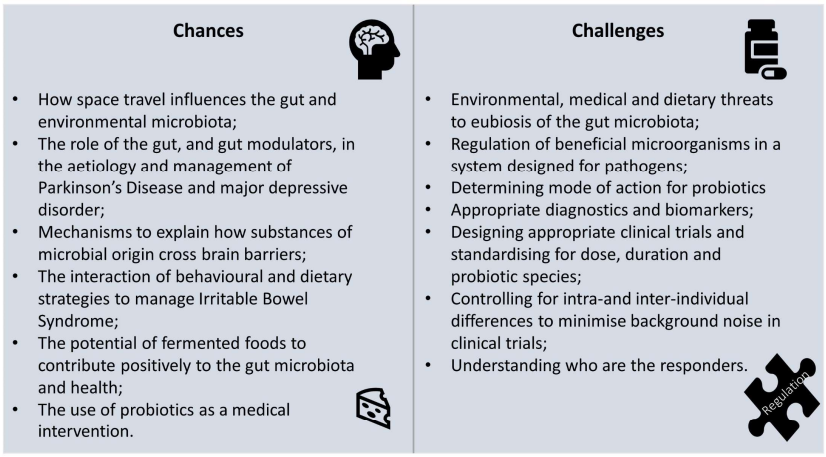

Figure 1: Summary of the overarching themes of the Symposium

1987

https://doi.org/10.1017/gmb.2023.4 Published online by Cambridge University Press