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An Introduction to the Microbiome: Challenges and Resolutions for Research

Acknowledgements

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Front Cover: The wild garden of gut bacteria.
Source: Dr Chris Wood and Nicola Fawcett, Modernising Medical Microbiology (Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0))

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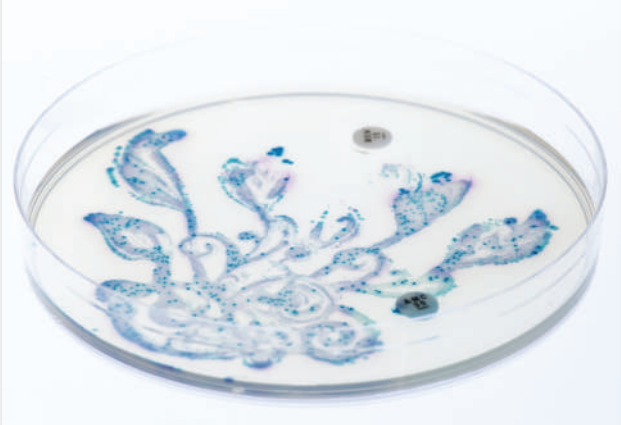
1. What is the microbiome?

The microbiome is made up of a community of microorganisms which include bacteria, viruses, fungi, and protozoa[1]. The microbes that are resident in each microbiome do not function in isolation and are found in diverse communities within specific niches; humans, plants, and other animals all have multiple microbiomes per organism. There is considerable inter-individual microbiome diversity, and within an individual there can be extensive variation in the makeup of each microbiome. In humans, each microbiome is distinct, depending on its location in the body and the microorganisms which inhabit it. Gut microbiota can be radically different compared to skin microbiota – this needs to be taken into consideration when referring to the microbiome under investigation. Currently, microbiome research is experiencing phenomenal growth with particular foci being gut and skin microbiomes, and the microbiota which inhabit these niches.

Genetic diversity of microbiota within a microbiome is huge, far more diverse that of the host, even in the case of humans. It is therefore not surprising that evidence is accumulating which suggests the microbiome plays a critical role in host health. A cohort study as part of the Human Microbiome Project found that there may be more than eight million unique genes associated with various microbiomes in the human body[2]. It is possible that the total microbiome genome may give a genetic contribution to each person several hundred times greater than their own genome.

Microbiomes have been found to broadly influence four different areas for humans: nutrition, immunity, behaviour and disease. There is growing interest in the role of the microbiome in human health and disease, with microbiome influence reported in an increasing number of diseases. Furthermore, host-microbiome interplay provides an additional level of complexity.



Gut bacteria. Source: Wikimedia Commons

An associative role has been identified between the microbiome and chronic diseases such as rheumatoid arthritis, allergies and asthma. Additionally, for chronic inflammatory disease, evidence suggests that the infant microbiome plays a role in asthma and allergies in later life. Research into neuropsychiatric disorders has also found associations of certain compositions of the microbiome with people with schizophrenia and other neuropsychiatric disorders. For diseases such as colon cancer, the microbiome has been identified not just as causative, but also under investigation as a preventative and therapeutic agent[3] [4].

2. Importance in human health

Microbiomes are thought to play key roles in human health, from nutrition to chronic conditions. Their role in chronic inflammatory conditions such as asthma, allergies, and rheumatoid arthritis is an area of active research, however the exact mechanistic link is unclear. There are three possibilities; firstly, that the microbiome changes in response to a disease; secondly, that the disease drives changes in the constitution of the microbiome; or finally, pathological alterations to the microbiome lead to, or accelerate, disease progression. What is clear, however, is that the microbiome plays a critical role in a diverse range of diseases.

Gut microbes play an important role in nutritional health. The fetal digestive tract is believed to be sterile until childbirth, with the first exposure of the immune system to commensals occurring during passage through the birth canal. This early contact with the maternal microbiome is thought to result in the long-term shaping of the immune system. Studies performed in germ-free animals demonstrate the critical role of the microbiome in the development of the lymphoid system[5] [6] [7].

Bacteria in the gut perform the function of priming immune response and maintaining immune cell homeostasis. Essential metabolites are produced or regulated by microbes in the human gut, such as *Clostridia* bacteria which modulate retinoic acid signalling in immune cells[8]. Dysbiosis of the intestinal microbiota has been shown to be sufficient to induce exacerbated intestinal TH17 cellular responses in

mouse models[9]. Gut microbiota can also act as biomarkers for chronic inflammatory diseases including inflammatory bowel disease (IBD), type 2 diabetes and asthma[10 11].

Gut bacteria have a demonstrable ability to influence patient response to treatment. For example, bacteria were shown to metabolise the Parkinson's treatment Levodopa, resulting in both reduced bioavailability of the drug and potentially unwanted side effects. Gut bacteria are also able to inactivate metabolic pathways, highlighting their potential utility as modulators of the microbiome to optimise treatment efficacy[12].

The microbiota that make up a microbiome can influence immunotherapy responses. Tumour cells can evolve to evade the host immune system, facilitating growth and metastases. Immunotherapy aims to boost the host immune system in order to treat cancer. As the microbiome plays a key role in immune modulation, it is plausible that microbiome composition can impact upon response to immunotherapy. Multiple studies have shown an association between microbiome composition and response to anti-PD1 therapy[12 13 14].

Given the above, there is growing interest into human microbiome research. Since 2000, there have been over 1,500 clinical trials listed on the ClinicalTrials.gov database that involve the microbiome as a biomarker or therapeutic intervention[15]. Nearly 600 of these were registered since the start of 2018.

3. Challenges

There are a number of challenges for microbiome research. Some of the main challenges include making credible sense of data generated, the handling, processing and storage of that data, deciding between different sequencing methods available, and other longstanding issues that arise when working with microorganisms.

Making sense of the data produced in microbiome research can be difficult due to their size.

Server room. Source: Getty Images

Generating huge amounts of data is relatively easy and affordable due to recent sequencing breakthroughs, however the analysis of the data requires a high level of skill and computational resources. Data mining from public datasets also returns a wealth of microbiome data; there are over 25,000 metagenome samples available from the EBI metagenomics repository alone[16].

One key caveat to both in-house generated data and data mining from external sources is the potential lack of standardisation for data outputs. Standardisation of storage databases and analysis pipelines is key in order to be able to effectively compare results across studies. Without this, the large volumes of data generated need to be translated into the required format and stored, taking time and computational resources which could otherwise be better utilised analysing the data.

Research into the microbiome has previously been hindered by the cost of sequencing. The cost is now within reach for in-house research, whereas before costs for sequencing metagenomic samples may have been prohibitive. Previously only the 16S region of bacterial strains could realistically be sequenced, however incoming techniques such as shotgun metagenomic sequencing allow for a picture of the whole bacterial community in a microbiome to be built. This allows for better resolution to be found regarding the function of species and what genes are being expressed within a microbiome, rather than just bacterial composition. While a huge benefit, this has also created challenges as datasets generated are larger to accommodate the whole genomes of entire bacterial communities, rather than just the 16S regions.

Working with microorganisms themselves pose a unique issue. Many microorganisms are not culturable in a laboratory, with traditional methods of identification for microorganisms inadequate for in-depth study. Previously, microbiomes were not able to be studied in detail due to this issue. With advances in technology, and new sequencing methods, more detailed studies can now be undertaken. Even still, nearly 44% of genes derived from the Integrated Gene Catalogue (IGC) of the human gut microbiome do not have a match in functional databases, while around 20% do not match currently characterised microbial genomes and are unable to be assigned a taxonomic identifier. Metagenomics has uncovered many new taxa and genes, yet many unknowns for research still remain.

Other challenges for the area are not new; taking samples of the gut microbiome can be extremely invasive, while other microbiomes (such as the fetal digestive tract) are both time-sensitive and delicate for sample collection. Both feed into the issue of sample size for research studies. Samples that are difficult to acquire can affect the ability to recruit a large sample size for microbiome studies.

4. Resolutions

As with any challenges, there are resolutions which can overcome them. Whether 16S or shotgun sequencing was used, there will be a large dataset produced that needs to be handled and processed in the correct manner. Bioinformatics is able to resolve this central challenge. Typically, bioinformatic approaches follow a similar pipeline[17]. With the right technical expertise, practical and theoretical knowledge, equipment and skills, bioinformaticians will perform a quality control (QC) evaluation, filter data, and identify the microbes present in the samples and their relative abundances. Once this has been completed, this information can be used to perform functional profiling and both differential abundance and functional enrichment analyses to contextualise the information gained.

The usage of 16S sequencing versus shotgun metagenomics may solve some common challenges. Depending on what research is focused on, a more dynamic

approach (whole-genome shotgun metagenomics) may be more appropriate than a 'snapshot' approach (16S sequencing), especially given the dynamic nature of microbiomes and their associated microbiota. With shotgun metagenomics, bacteria in each microbiome can be identified at the strain level. This is not possible using the 'gold-standard' approach of community profiling, 16S sequencing.
With 16S sequencing, a fraction of a single ribosomal gene is profiled. Identification of the bacterium may be to the level of genus, however species or strain level may not necessarily be possible. While 16S sequencing is a relatively cost-effective and quick approach, a large number of assumptions need to be made from the results. It cannot give information on the variation in the bacterial genome, which are highly dynamic and variable. 16S sequencing could identify that *E. coli* is present in a sample, however it would not be able to give further information on whether it was a harmless commensal or pathogenic variant. Bias could also be introduced when using 16S sequencing as PCR is used to amplify the sample. 16S sequencing is also limited to bacterial species - viruses and fungi present in the sample will not be sequenced.

As shotgun metagenomics samples the entire genome of any organism present in a sample, it is less susceptible to the biases of targeted gene amplification. It is much more expensive and computationally more intensive than 16S sequencing, as it returns a much higher level of resolution of what genes are present.

Shotgun metagenomics provides direct evidence about the presence or absence of specific functional pathways in a sample, rather than having to extrapolate or make assumptions. Shotgun metagenomics provides the full picture of what species present in the microbiome, with the ability to identify to strain level, as well as determine what virulence or resistance genes may be present in that microbiome.

5. Future of microbiome research

Microbiome research is a high-growth area and looks set to continue as one. Between 2005 and 2015, the number of published articles on the topic of cancer-microbiome interactions increased by nearly 2,000%[18].

With new sequencing technologies, microbiome research is not limited by data collection methods. Challenges for research may well relate to whether the microbiome can be used as a predictive tool. Currently, there are known associations of the microbiome with diseases such as asthma and type two diabetes; whether these associations are caused by the microbiome or whether the microbiome changes composition as a side effect of the conditions is still unknown. However, there are exciting examples of the microbiome being manipulated or utilised to therapeutic advantage.

In the future, research efforts may well focus on whether the role of the microbiome can be used to predict disease incidence or to highlight microbiome risk-factors for various chronic diseases. Finally, therapeutic interventions will also be a major focus.

6. Fios Genomics' expertise

Fios Genomics has experience with a number of therapeutic areas related to microbiome analysis, including the gastrointestinal and respiratory systems. We have worked with clients on microbiome analysis, projects specifically related to the interplay of the microbiome and oncology, as well as on a European project for MAARS.

Fios Genomics' offices in Edinburgh, UK. Source: Fios Genomics Ltd

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