Let's Get Meta- With It:
Current Methods in Polar Microbiome Research

Julianne Peralta[1]
[1]Department of Molecular Genetics, University of Toronto, Toronto, Canada

What is a microbiome?

Microorganisms such as bacteria, archaea, protists, and fungi form associations with every animal on Earth. They colonise mucosal surfaces of the body such as the respiratory tract and gut, conferring benefits to their hosts, and underpin the planet's biogeochemical cycles and food chains [1]. The **collective genetic material of a community of microbes** in an ecological niche—a microbiota—is often referred to as a **"microbiome".**

The study of microbiomes originally emerged from the field of microbial ecology. In the nineteenth century, microbial ecology shifted the view of microorganisms existing as single cells to existing as assemblages where species interactions were integral to community dynamics and function [2].

"Dysbiosis" of the microbiome is usually defined as a loss or reduction in species diversity or a change in community composition [3] but as the scope of microbiome research is broadened, the concept of a dysbiotic microbiome can also include alterations in its metabolic activities.

How do we study microbiomes?

Over the past two decades, biological research has been transformed by the decreasing costs of next-generation sequencing (NGS) technologies and increases in computational power. These high-throughput sequencing methods allow for the study of not just individual species but the entire community sampled from its niche.

There are three main methods used to profile microbiomes:

Who is present?

- The 16S (in prokaryotes) and 18S rRNA genes (in eukaryotes) code for a component of the ribosome, the cellular machinery that synthesises proteins.
- Polymerase chain reaction (PCR) amplification and sequencing of the hypervariable regions for **gene-marker surveys** can be used to identify which taxa are present in a community, like barcodes.
- However, the ability to tell apart closely related species may depend on downstream computational methods such as: *de novo* vs. reference-based clustering, and use of Operational Taxonomic Units (OTUs) vs. Amplicon Sequence Variants (ASVs) [4,5].

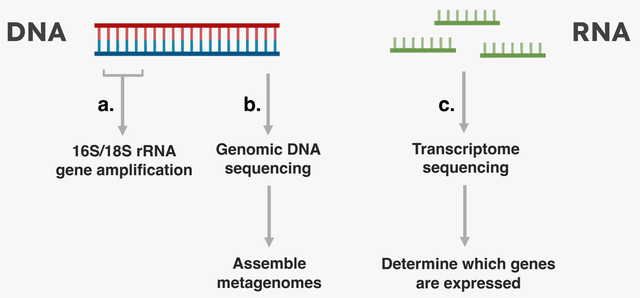

Figure 1. Methods of microbiome profiling. In cells, deoxyribonucleic acid (DNA) is transcribed into single-stranded ribonucleic acid (RNA) by ribosomes. Both molecules are essential for biological function and can be leveraged to study microbiomes through **(a)** Gene marker-surveys, **(b)** Metagenomics, and **(c)** Metatranscriptomics (*i.e.* whole microbiome RNA-sequencing).

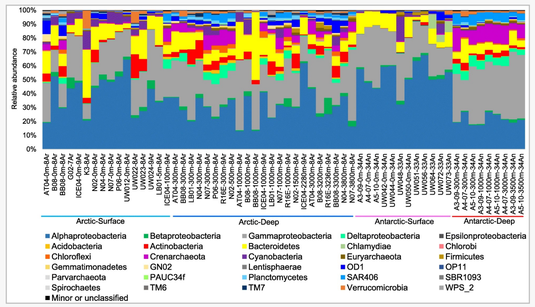

Figure 2. Phylum-level (and Class-level Proteobacteria) taxonomic composition of 60 polar seawater metagenomic samples, based on recovery of 16S miTag sequences from assembled metagenomes. The top 30 phyla based on maximum relative abundance are shown. **Cao et al.** [6].

What are they capable of?

- Metagenomic studies can shed light on not only the composition of a microbiome (**Fig. 2**) but also its functional potential.
- After DNA-sequencing of a bulk sample, computational tools are used to assemble short 'reads' of DNA into longer contiguous sequences (contigs) that are then binned into **Metagenome Assembled Genomes (MAGs).**
- A MAG is a reconstruction of a genome that is meant to represent a single species.
- Identifying genes involves comparing the MAGs to databases of high-quality microbial sequences and looking for similar sequences that are already annotated.

What are they doing?

- Just because a gene or a species is present, it doesn't mean that it's active!
- RNA-sequencing of bulk samples provides information about which genes are actually transcribed into coding or non-coding RNA.
- **Metatranscriptomics** is a more sensitive method that can detect functional differences between microbiomes (*E.g.* which enzymes are highly expressed) and may be a better predictor of microbiome dynamics than taxonomic composition alone [7,8].

Polar and Subpolar regions

Cold-adapted microbiomes of the Arctic and Antarctic, both soil- and marine-based, have captured the attention of researchers due to their particular vulnerability to anthropogenic climate change. Microbial niches in these environments are typically found between frozen substrates, such as in **permafrost** or **weathering crusts** (a porous layer near the surface of a glacier) which can be rapidly altered by changes in temperature [9].

Figure 3. Microbial mat collected from a meltwater pond on McMurdo Ice Shelf, Antarctica. **Jackson et al.** [10].

In spite of the challenges associated with remote sampling sites and the availability of high-quality genomic sequences, these studies provide essential insight into not only fundamental microbiology but also the interdisciplinary fields of ecology, biogeography, and biomedicine.

Permafrost as a potential pathogen reservoir

- Permafrost is the part of the cryosphere composed of soil that remains below 0°C for at least two years, although most permafrost is much older.
- It underlies 15% of exposed land in the Northern Hemisphere, with 5 million people living in these regions [12,13].

- In 2016, *Bacillus anthracis*-infected animal carcasses in Siberia that were previously frozen in permafrost were exposed by thaw, causing an outbreak in anthrax disease that resulted in the deaths of 2000 reindeer and one person due to ingestion of the highly resistant spores [14].
- A modeling study investigating permafrost and disease transmission dynamics in the Arctic suggested that annual risk of anthrax infection is correlated with the depth of the active soil (**Fig. 4**) [15].

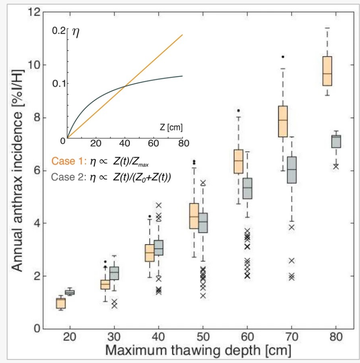

Figure 4. Results from stochastic realisations of anthrax transmission processes, with annual cumulative incidence versus maximum thawing depth of the active soil layer. Case 1 (orange) is linear while Case 2 (gray) includes a saturating function (see inset). **Stella et al.** [15].

Mining microbiomes to combat superbugs

- Many current antibiotics, such as Amoxicillin and Azithromycin, are semisynthetic versions of compounds naturally produced by microbes that were originally discovered during the 'Golden Age' of antibiotic discovery (1940-1962) [16].
- In 2019, the World Health Organization named **antimicrobial resistance** as one of the top ten threats to global health.
- Rising rates of infections by antimicrobial-resistant 'superbugs' such as multidrug-resistant tuberculosis underscores the need to study how resistance evolves or is acquired by horizontal gene transfer (HGT), as well as their mechanisms [17].

- A metagenomic study of ancient soil permafrost samples found that **antimicrobial resistance genes (ARGs)** against some classes of antibiotics were present in bacteria even before antibiotics were used in clinical settings [18].
- Any diverse microbiome—including polar and other environmental microbiomes—has potential to be a reservoir for ARGs. These "resistomes" represent opportunities to identify novel targets for new antimicrobials and antimicrobial adjuvants to treat resistant infections [19].

References

1. Sender, R., Fuchs, S. & Milo, R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* 164, 337–340 (2016).
2. Berg, G. *et al.* Microbiome definition re-visited: old concepts and new challenges. *Microbiome* 8, 1–22 (2020).
3. Perez, N. B., Dorsen, C. & Squires, A. Dysbiosis of the gut microbiome: a concept analysis. *Journal of Holistic Nursing* 38, 223–232 (2020).
4. García-López, M.-L., Santos, J. A., Otero, A. & Rodríguez-Calleja, J. M. in *Encyclopedia of Food Microbiology (Second Edition)* (eds. Batt, C. A. & Tortorello, M. L.) 261–268 (Academic Press, 2014).
5. Callahan, B. J., McMurdie, P. J. & Holmes, S. P. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal* 11, 2639–2643 (2017).
6. Cao, S. *et al.* Structure and function of the Arctic and Antarctic marine microbiota as revealed by metagenomics. *Microbiome* **8,** 1–12 (2020).
7. Jiang, Y., Xiong, X., Danska, J. & Parkinson, J. Metatranscriptomic analysis of diverse microbial communities reveals core metabolic pathways and microbiome-specific functionality. *Microbiome* 4, 1–18 (2016).
8. France, M. T. *et al.* Insight into the ecology of vaginal bacteria through integrative analyses of metagenomic and metatranscriptomic data. *Genome Biology* 23, 1–26 (2022).
9. Edwards, A. *et al.* Microbial genomics amidst the Arctic crisis. *Microbial Genomics* **6,** (2020).
10. Jackson, E. E., Hawes, I. & Jungblut, A. D. 16S rRNA gene and 18S rRNA gene diversity in microbial mat communities in meltwater ponds on the McMurdo Ice Shelf, Antarctica. *Polar Biology* **44,** 823–836 (2021).
11. Wu, R., Trubl, G., Taş, N. & Jansson, J. K. Permafrost as a potential pathogen reservoir. *One Earth* **5,** 351–360 (2022).
12. Obu, J. How much of the earth's surface is underlain by permafrost? *Journal of Geophysical Research: Earth Surface* **126,** e2021JF006123 (2021).
13. Ramage, J. *et al.* Population living on permafrost in the Arctic. *Population and Environment* 1–17 (2021).1.
14. Popova, A. Y. *et al.* Outbreak of anthrax in the Yamalo-Nenets autonomous district in 2016, epidemiological peculiarities. *Problems of Particularly Dangerous Infections* 42–46 (2016).
15. Stella, E., Mari, L., Gabrieli, J., Barbante, C. & Bertuzzo, E. Permafrost dynamics and the risk of anthrax transmission: a modelling study. *Scientific reports* **10,** 16460 (2020).
16. Morehead, M. S. & Scarbrough, C. Emergence of global antibiotic resistance. *Primary care: clinics in office practice* **45,** 467–484 (2018).
17. Adu-Oppong, B., Gasparrini, A. J. & Dantas, G. Genomic and functional techniques to mine the microbiome for novel antimicrobials and antimicrobial resistance genes. *Annals of the New York Academy of Sciences* **1388,** 42–58 (2017).
18. D'Costa, V. M. *et al.* Antibiotic resistance is ancient. *Nature* **477,** 457–461 (2011).
19. Singh, S., Verma, N. & Taneja, N. The human gut resistome: Current concepts & future prospects. *The Indian journal of medical research* **150,** 345 (2019).

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