GENOME WATCH

Keeping tally in the microbiome

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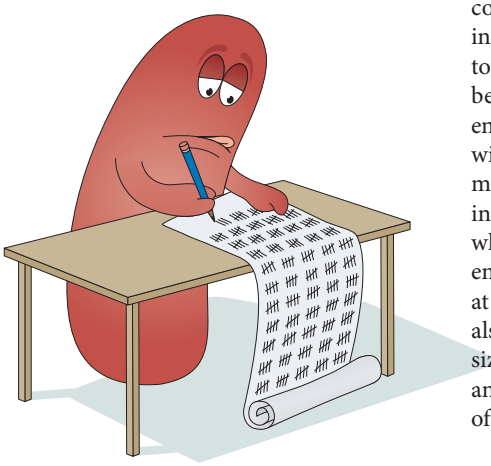
This month's Genome Watch highlights how the development of new approaches for quantifying the human microbiome may pave the way for a better understanding of microbial shifts in the context of human health and disease.

The use of metagenomics to analyse the human microbiota is a relatively new field of study, having risen to the forefront of microbiology research over the past decade. As scientific and methodological breakthroughs continue at an ever-increasing pace, we are observing vast increases in the availability of high-dimensional sequencing data and a myriad of microbiome studies, raising awareness and concerns of potential technical issues and biases that affect different stages of the experimental design, from sampling to analysis[1].

One major limitation of culture-independent microbiome profiling approaches is that current sequencing technologies have a finite sequencing yield (that is, it is not possible to sequence the entirety of the sample), so the resulting read count only represents a fixed, random portion of the diversity within the sample. Hence, the prevalence of specific species within the microbiome has, until now, been mostly evaluated as relative abundances that are determined from the sequencing output. This technical limitation requires a unique statistical approach, making use of methods that are appropriate for compositional-type data[2] (that is, that represent relative proportions instead of absolute values). To address this issue, new techniques to approximate absolute numbers of bacterial counts within samples have been applied to different input materials, such as the spiking of a controlled amount of exogenous bacteria into mice stool samples[3] or combining 16S ribosomal RNA (rRNA) sequencing with flow cytometry to quantify taxon abundances in environmental samples[4]. Yet, determining absolute abundances has remained a challenge in human microbiome research.



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Now, Vandeputte et al.[5] have developed a new flow cytometry-based approach to quantify microbial abundances in faecal material, which they termed 'Quantitative Microbiome Profiling' (QMP), and applied it to investigate a cohort of individuals with Crohn's disease (a type of inflammatory bowel disease) and healthy individuals. Their workflow consisted of combining flow cytometric bacterial cell counts with the amplification and sequencing of the 16S rRNA gene. Generated data was then normalized for sequencing depth and for differences in the number of 16S rRNA gene copies between bacterial species. Using this innovative approach, the authors showed that total microbial abundances vary substantially between individuals (up to tenfold differences) and that this variation is also associated with host status. They found that absolute microbial counts were three times lower in individuals with Crohn's disease, most of whom were colonized by a newly discovered enterotype of *Bacteroides* sp. that was present at a low cell count. Surprisingly, the authors also observed that total microbial population sizes fluctuated substantially from day to day and differed by up to fivefold over the course of a week, even within healthy individuals.

These results represent an important proof of concept with key implications for future 'omics'-based research. The next crucial steps towards widespread adoption involve extending these approaches from amplicon-based studies (for example, on the 16S rRNA gene) to shotgun metagenomics methods and scaling up to analyse hundreds and thousands of samples. However, introducing additional stages during sample processing and storage might pose new technical challenges that must be overcome. The design of future microbiome studies will likely be adapted according to the sample size and the specific biological question that is being addressed, with large-scale exploratory analysis starting with approaches that measure relative abundances of bacteria within a sample and then progressing to quantitative assessment of sub-cohorts of interest by employing innovative microbiome-quantification techniques.

In summary, these novel methodological approaches have the potential to reveal associations between bacterial abundance and other host phenotypes of interest. Ultimately, this may open the way for uncovering important biological mechanisms about the role of the microbiota in both human health and disease.

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Competing interests
The authors declare no competing interests.