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Short Communication

Profiling of Microbes: Tools, Techniques, and Challenges

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ABSTRACT

High-throughput sequencing studies and new software tools are revolutionizing microbial community analyses, yet the variety of experimental and computational methods can be daunting. In this review, we discuss some of the different approaches to community profiling, highlighting strengths and weaknesses of various experimental approaches, sequencing methodologies, and analytical methods. We also address one key question emerging from various Human Microbiome Projects: Is there a substantial core of abundant organisms or lineages that we all share? It appears that in some human body habitats, such as the hand and the gut, the diversity among individuals is so great that we can rule out the possibility that any species is at high abundance in all individuals: It is possible that the focus should instead be on higher-level taxa or on functional genes instead.

Keywords: - Microbes; Profiling; Human microbiota

INTRODUCTION

The human microbiota (the collection of microbes that live on and inside us) consists of about 100 trillion microbial cells that outnumber our "human" cells 10 to 1, and that provide a wide range of metabolic functions that we lack. If we consider ourselves as supraorganisms encompassing these microbial symbionts, by far the majority of genes in the system are microbial [1]. In this sense, completing the human genome requires us to characterize the microbiome (the collection of genes in the microbiota). Currently, there are two main methods for performing this characterization that do not rely on growing organisms in pure culture: small-subunit ribosomal RNA (rRNA) studies, in which the 16S rRNA gene sequences (for archaea and bacteria) or the 18S rRNA gene sequences (for eukaryotes) are used as stable phylogenetic markers to define which lineages are present in a sample, and metagenomic studies, in which community DNA is subject to shotgun sequencing. Small subunit rRNA-based studies are sometimes also considered to be "metagenomic" in that they analyze a heterogeneous sample of community DNA [2]. Community profiling, or determining the abundance of each kind of microbe, is much cheaper using rRNA because only one gene out of each genome is examined, but metagenomic profiles are essential for understanding the functions encoded in those genomes. Techniques that probe gene expression directly such as metatranscriptomics and metaproteomics (analysis of the transcripts or proteins in a community, respectively), although useful in simpler microbial communities such as acid mine drainage, are just beginning to be applied to human-associated microbial communities.

Through the use of metagenomic and rRNA-based techniques, much progress has been made in characterizing the human microbiome and its role in health and disease in the past few years, especially with the advent of high-throughput sequencing. These studies are challenging because of the scale and complexity of the microbiome and because of the unexpected variability between individuals [3]. In this review, we cover the combination of experimental and analytical techniques used to characterize the microbiomes of humans and of other mammals. In particular, we describe how recent advances in technology and experimental techniques, together with computational methods that draw on the long tradition of community analysis in large-scale ecological studies, are essential for uncovering large-scale trends that relate the microbiomes of many individuals.

Key questions facing investigators

The wide array of sequencing technologies and analytical tools can be daunting. The path to a successful study is first to define what hypothesis is being tested and then to select the appropriate technology. For example, it would be unfortunate to spend months and millions of dollars performing a metagenomic study solely to find changes such as the shift in the Bacteroidetes:Firmicutes–Actinobacteria ratio in the gut of obese individuals when a much faster and cheaper assay could have provided the same result at a much lower cost. Such studies must be justified by additional analyses that can only be performed with metagenomic sequences [4]. Here we cover some of the key questions facing investigators: whether to use sequencing or to use lower resolution but cheaper methods that allow more samples to be processed for the same cost,

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which type of sequencing to perform, and how the data should be analyzed. These decisions, especially with respect to data analysis, often differ between rRNA and metagenomic surveys. For example, phylogenetic methods are increasingly useful for rRNA surveys because this gene allows accurate reconstruction of phylogeny, whereas functional or taxon-based methods are typically more useful for metagenomic surveys because of the range of functions represented and because of the difficulty of reconstructing the phylogenies of small fragments of many gene families.

Although the cost of sequencing is dropping, fingerprinting techniques (techniques that provide limited information about the microbial community) are still orders of magnitude cheaper and faster to perform [5]. Fingerprinting techniques include T-RFLP, DGGE, and TGGE: These methods have been reviewed comprehensively. Briefly, they rely on amplification of a specific gene, typically but not always 16S rRNA, then separating different variants of the gene in the community sample by electrophoresis. These methods can be used to analyze large numbers of samples, including clustering of the banding patterns with statistical techniques such as Principal Coordinates Analysis (PCoA), but typically the dynamic range is limited (so only the few most abundant members of the community can be observed), and it is difficult to relate banding patterns to changes in particular species or lineages. It is also generally impossible to combine data from different studies into a single analysis [6]. However, these techniques can be useful for checking for stability in the dominant members of a community and for clustering communities according to changes in the dominant members across large numbers of samples.

The main advantages of sequencing studies over fingerprinting are that sequences can be classified according to taxonomy and function, that sequencing provides much greater dynamic range and ability to compare complex samples, and that sequences from different studies can be compared to one another and placed in the same phylogenetic tree. Sequencing is especially useful when asking which specific genes or species contribute to differences among communities.

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