A revolutionary approach to studying the intestinal microbiota

July 7 2014

An international research team within the MetaHIT consortium coordinated by INRA and involving teams from CEA, CNRS and Université d'Evry, has developed a new method to analyse the global genome, or the metagenome of the intestinal microbiota. This method markedly simplifies microbiome analysis and renders it more powerful. The scientists have thus been able to sequence and assemble the complete genome of 238 intestinal bacteria, 75% of which were previously unknown. This work is being published on the 6th of July 2014 in *Nature Biotechnology*.

Research carried out in recent years on the intestinal microbiota has completely overturned our vision of the human gut ecosystem. Indeed, from "simple digesters" of food, these bacteria have become major factors in understanding certain diseases such as obesity, type 2 diabetes, or Crohn's disease. Important and direct links have also been demonstrated between these bacteria and the immune system, as well as with the brain. It is estimated that 100,000 billion bacteria populate the gut of each individual (or 10 to 100 times more than the number of cells in the human body), and their diversity is considerable, estimated to around a thousand different bacterial species in the intestinal human metagenome. However, because only 15% of these bacteria were previously isolated and characterized by genome sequencing, an immense number of the microbial genes previously identified still need to be assigned to a given species.

Researchers from INRA, together with teams from CEA (Genoscope),

CNRS and Université d'Evry in France, and scientists from other countries, have developed a new method that can markedly facilitate analysis of the gut metagenome, while at the same time improving the quality of the data obtained. To achieve this, they based themselves on a simple hypothesis:

Within a bacterial species harboured by the gut of an individual, the abundance of genes remains constant, since every bacterium of a same species have the same genes

However, the relative abundance of these different species can vary markedly between individuals, from 10-fold to 1000-fold, so that of course the abundance of genes harboured by an individual varies to the same extent.

By measuring the abundance of bacterial genes in different individuals, it would therefore be possible to group the genes of a specific bacterial species, because their abundance is the same in a particular individual but differs between individuals.

An analysis of 396 stool samples from Danish and Spanish individuals allowed the researchers to cluster these millions of genes into 7381 co-abundance groups of genes. Approximately 10% of these groups (741) corresponded to bacterial species referred to as metagenomic species (MGS); the others corresponded to bacterial viruses (848 bacteriophages were discovered), plasmids (circular, bacterial DNA fragments) or genes which protected bacteria from viral attack (known as CRISPR sequences). 85% of these MGS constituted unknown bacteria species (or ~630 species).

Using this new approach, the researchers succeeded in reconstituting the complete genome of 238 of these unknown species, without prior culture of these bacteria. Living without oxygen, in an environment that is

difficult to characterise and reproduce, most of these gut bacteria cannot be cultured in the laboratory. And until now, analysis of the metagenome was based on comparing the genes detected in a sample with those listed in catalogues of genes from bacteria that were known and could be cultivated in a laboratory (or 15% of gut bacteria), so that it was impossible to assign genes to non-cultivable bacteria.

The authors also demonstrated more than 800 dependent relationships within the 7381 gene co-abundance groups; this was the case, for example, of phages which require the presence of a bacterium to survive. These dependent relationships thus enable a clearer understanding of the survival mechanisms of a micro-organism in its ecosystem. It is also the first time that an analysis has clarified the relationships between different biological entities in the gut microbiota, which will facilitate their detection, isolation and culture.

This study throws unequalled and very detailed light on microbial communities in humans. The method thus developed enables considerably simpler analysis of genes in the gut microbiota; it is now possible to study just a few thousand genetic elements, or hundreds of species, rather than the millions of genes that make up the metagenome. The method also markedly improves the reliability and accuracy of statistical analyses.

More information: Henrik Bjørn Nielsen et al. "A method for identifying metagenomic species and variable genetic elements by exhaustive co-abundance binning." *Nature Biotechnology*, 6 July 2014. DOI: 10.1038/nbt.2939

Provided by INRA