The Intestinal Microbiota and Gut Health:

Contribution of the Diet, Bacterial Metabolites, Host Interactions and Impact on Health and Disease

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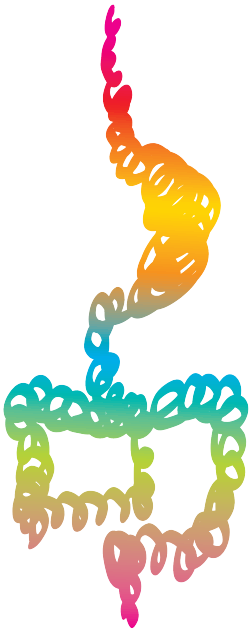

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Metagenomics view of gut microbiota structure in old and young age by 454-pyrosequencing. By E. Franciosi*[1], I. Carafa[1], C. Maidens[2], A. Przemska[2], H. Dong[2], I. Bin Dayel[2], C. Childs[2], R. Viola[1], P. Yaqoob[2] and K.M. Tuohy[1], *[1]Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Italy and [2]Department of food Science and Nutrition, University of Reading, Reading, UK*

Introduction

The human gut microbiota has been the subject of intense research in recent years. This has been due in large part to the development of culture-independent techniques and in particular high-throughput sequencing. The general availability of these powerful methodologies has resulted in an explosion of reports describing variations in the gut microbiota as a function of age, health status, diet and location.

We focused our study on the gut microbiota in old and young age. The proportion of elderly citizens is increasing. Analysis of the gut microbiota, could give elements in the overall process of improving the health of the elderly. Physiological processes in the elderly are particularly likely to have connections with the intestinal microbiota considering the concomitant changes in immune function and diet which occur along side microbiota senescence in old age. The motility of the intestine decreases with age, leading to longer transit times and thus altered dynamics of nutrient turn-over. Combined with other physiological aspects of altered bowel function (3), loss of dentations, the physical environment of the elderly gut is significantly different from that of younger adults. Similarly, elderly people are often on long term medication and radically change their dietary intake and energy expenditure via physical exercise. All these factors could play a role in altering the gut microbiota in old age.

Materials and Methods

Here, we present comparative metagenomic data from the fecal microbiota of 29 healthy young subjects (20-35 y) and 32 healthy older subjects (60-75 y). The analysis was carried out using bacterial tag encoded FLX amplicon pyrosequencing generating a 520bp sequence targeting V1, V2 and V3 regions located on the 16S rRNA gene. We generated 736,000 sequence reads from 16S rRNA gene V1-V3 amplicons, with an average of 12,000 reads per subject. The phylogenetic analysis was performed using Qiime (Quantitative Insights Into Microbial Ecology) and RDP (Ribosomal Database Project) (1, 2). The 454 sequences were processed and analyzed in an Unix-environment.

After a first filtering for small and low quality reads, the sequences were assigned to samples according to sample-specific barcodes. Sequences were then checked for the following criteria: (i) no more than 1 mismatch in the forward primer; (ii) length of at least 200 and no more than 700 nucleotides (barcodes and primers excluded); (iii) no more than two undetermined bases (denoted by N). Sequences were binned into phylotypes at 97% sequence similarity. For each phylotype group only one copy was conserved called OTU (Operational Taxonomic Unit). Each originated OTU was assigned to a family by the RDP classifier.

Results and Discussion

When we examined OTU abundance (Fig.1), phylum-level microbiota assignments showed that older subjects microbiota had a significantly ($p = 0.0046$) higher proportion of *Bacteroidetes* (25.4 and 16.7 % in old and young respectively), while a significantly ($p = 0.0012$) higher proportion of *Firmicutes* was found in young subjects (80.1 and 69.4 % in young and old respectively). Smaller, but significant, differences were also apparent in the *Proteobacterium* and *Tenericutes* phyla, which were both higher in the older subjects. The Families associated with the differences observed in *Bacteroidetes* were *Bacteroidaceae* and *Rikenellaceae* which were present in significantly higher numbers in stools of older compared with younger subjects. The Families associated with differences in *Firmicutes* were *Lachnospiraceae* and *Ruminococcaceae,* which were present in higher numbers in stools of younger compared with older subjects.

UniFrac PCoA (principal co-ordinate) analysis of 21,679 OTUs indicated a separation of the old subjects from the young. In particular old subjects clustered in the right side of the graph with the exception of six subjects clustering in the left side with the young subjects (Fig. 2). To a deeper analysis, the six old subjects clustering with the younger in the left side and indicated by an arrow in Fig.2, were free from any medication.

Our observations confirm that there are substantial changes in the gut microbiota of young and older adults, there is a significant lowering of *Firmicutes* that are appear to be replaced by *Bacteroidetes* with aging. In addition there are indications about the influence that medication could have on the gut microbiota in the elderly.

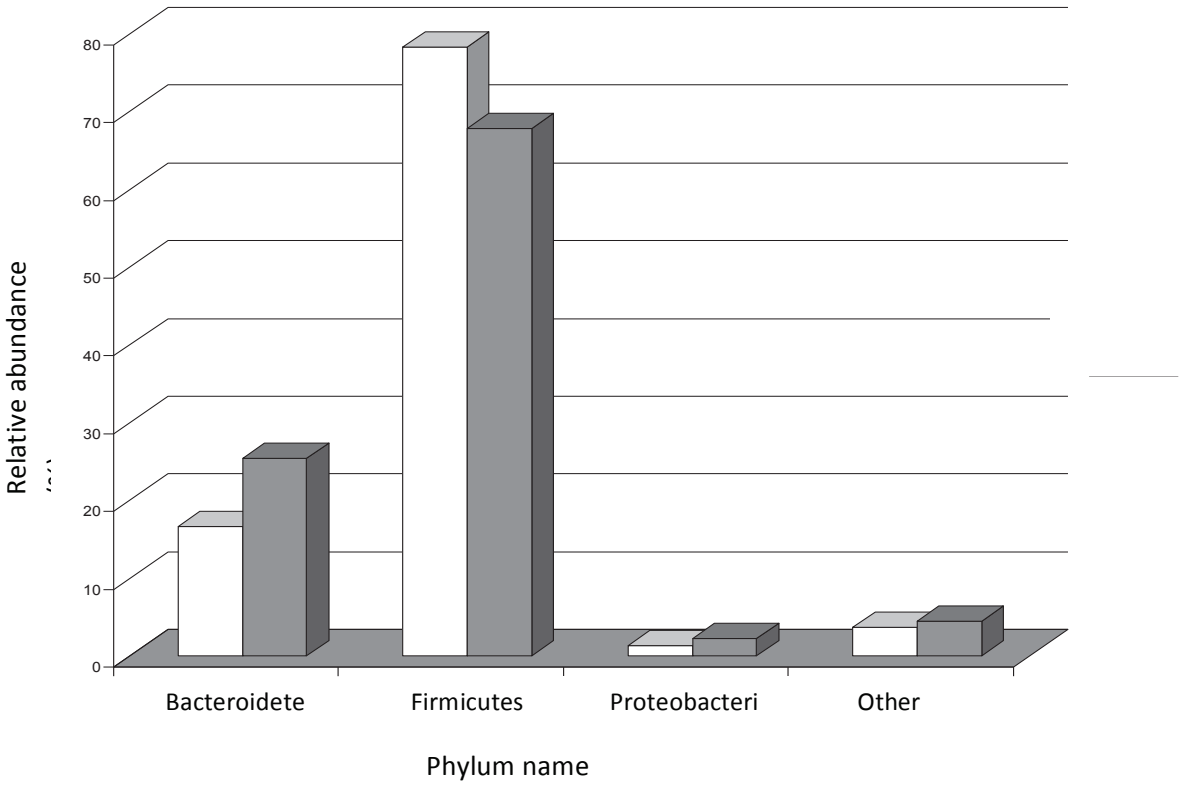

Fig. 1. Relative abundance of the bacterial community composition at Phylum-level as revealed by 454-pyrosequencing: in white bars are shown the young and in grey the old community.

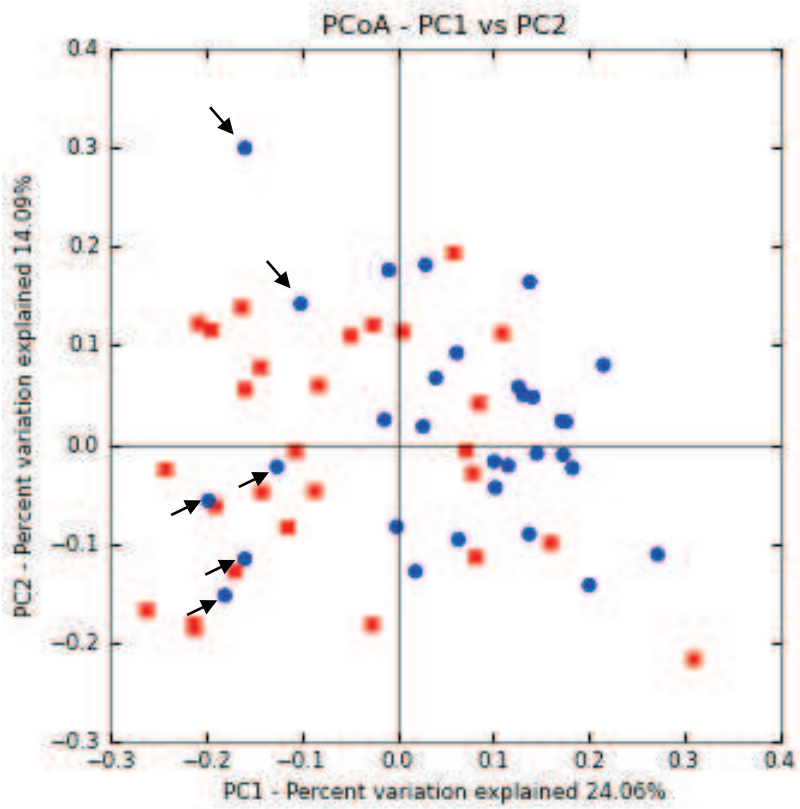

Fig. 2. 2D Scatterplot generated from the matrix of distances using Principal Coordinate Analysis. Weighted UniFrac was used to generate a matrix of pairwise distances between communities. The scatterplot is colored by subject: red square for young and blue circle for old subjects.

References

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