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Human gut Micro biota of Obese and lean individual

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Abstract

Recent studies of 16s rRNA genes in the mammalian gut microbiota distinguished a higher Firmicutes/Bacteriodetes ratio in obese individuals compared to lean individuals. This ratio was estimated using a Clonal Sanger Sequencing approach which is time-consuming and requires laborious data analysis. In contrast new high throughput Pyrosequencing Technology offer an inexpensive alternative to Clonal Sanger Sequencing and would significantly advance our understanding of obesity by the development of clinical diagnostic method. Here we present a cost effective method that combines 16s RNA Pyrosequencing and DNA barcodes of the Firmicutes/Bacteroidetes ratio in the gut microbiota of obese human. Excessive free radicals initial mitochondria dysfunction due to dysfunction mitochondria sugar and fat molecules remain unabsorbed and metabolized by dysfunction mitochondria. According to invention, the mitochondria double membrane and donate the electron to unstable molecules and balance them and also stimulate the other antioxidants enzyme production. By this activity the mitochondria is regenerated again, fat and sugar then absorbed by the mitochondria for energy production and then supplying energy to the cell.

Keywords: Human gut, Micro biota, obese, lean, Firmicutes

Contents

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1. Introduction

Adult human gut may contain up to 100 trillion microbial organisms known as the micro biota, this micro biota may serve various function including promoting development of the human immune system, modulating inflammation and effective calorie extraction. Two major groups of bacteria – Bacteriodetes and Firmicutes together make upto

90% of microbes found in intestine of mice and human, help to break indigestible food. When compared the microbiota of a obese and the lean mice the obese mice had 50% fewer Bacteriodetes and proportionally more Firmicutes. Obese mice= more Firmicutes, less Bacteriodetes

Transplanting the gut microbiota from normal mice into the germ recipient, increases their body fat without any increase in food consumption, raising the possibility that the composition of microbial community in the gut affects the amount of energy extracted from diet. The abundance of Bacteriodetes was decreased in obese people compared to lean people and this proportion increase with weight loss. Studies were carried out to determine whether body fat is related to gut microbial communities in common breeds of pigs. The real time PCR analysis was used together with a group specific primers to detect population of all bacteria, the Bacteriodetes, the Firmicutes and the Bacteroides species in the ceacum of obese and lean pigs, the lean is Landrace pig and obese is Meishan pig.

Pigs share a high similarity with human with respect to the anatomy, physiology and metabolism of the digestive system and have strong capacity for fat storage. Pork producers and consumers now pay more attention to meat quality and they expect that pig's produces leaner meat so we want to know whether body fat correlated with the gut microbial communities can control fat storage In pigs. The coexistence of Bacteriodetes and Firmicutes in the gut implies minimized competition for resources through cooperation. The obese gut has as yet uncharacterized properties that tip the balance towards the Firmicutes.

The dynamic linkage between adiposity and the gut microbial ecology indicates the manipulation of gut microbial communities could be another approach in the treatment of obesity. The patented probiotic Theralac contains 5 bilions CFU of *Lactobacillus paracasei* F19 per capsule another 15 billion CFU from four complementary probiotics strains. All five of the probiotic strains in Theralac are protected from stomach acid by patented Sodium Alginate delivery system that assures live delivery into intestinal tract.

Thus Firmicutes isolated from feaces of obese person can be used as a source of fatty acid accumulation in the lean person and thus can be used as probiotics, by using it in the form of capsule.

Bacteria: Firmicutes

The Firmicutes are a phylum of bacteria, most of which have gram positive cell wall structure. Firmicutes are low GC content of DNA. Many firmicutes produce endospore which are resistant to dessication and can survives in extreme conditions, they are found in various environments and the group includes some notable pathogens. Firmicutes plays an important role in beer, wine and cider spoilage. The division firmicutes is further sub divided into 3 classes:

Clostridia, the Bacilli, the Mollicutes

Bacteria: Bacteriodetes

Bacteriodetes share a common route with the chlorobi in the ARB and RDP trees. Within the Bacteriodetes there are three distinct lineages that have been accorded the rank of class:

A. The Bacteriodetes
B. The Flavobacteria
C. The Sphingobacteria.

The Bacteriodetes are phenotypic ally diverse and overlap significantly with members of other phyla. Member species can be ascribed to the following broad categories:

a. Gram negative aerobic
b. Anaerobic Gram negative rods
c. Non photosynthetic, non-fruiting bacteria
d. Sheathed bacteria
e. Symbiotic bacteria
f. Non motile, curved bacteria
g. Gut microbial gene catalogue established by metagenomic sequencing

Metagenomic is the genomic analysis of the microorganism by direct extraction and cloning of DNA from an assemblage of microorganism, analyses of 16s rRNA gene sequences amplified directly from the environment, an approach that avoided the bias imposed by culturing and led to the discovery of vast lineages of microbial life.Analysis of 16srRNA gene, such studies yielded only a phylogenetic description of community membership, providing little insight into the genetics, physiology, and biochemistry of the members. Novel genes and gene products discovered through metagenomics include the first bacteriorhodopsin of bacterial origin, novel small molecules with antimicrobial activity, and new members of families of known proteins such as Na^+ (Li^+)/H^+ antiporter, Rec A, DNA polymerase and antibiotic determinants.

Reassembly of multiple genomes has provided insight into the energy and nutrients cycling within the community, genome structure, gene function, population and microheterogenecity and lateral gene transfer among members of an uncultured community. The application of metagenomic sequence information will facilitate the design of better culturing strategies to link genomic analysis with pure culture studies.

2. Materials and Methods

Sample Collection:

a. Faecal sample from human was collected none of the human has been exposed to antibiotic
b. Adult and elderly subjects consumed an unrestricted western type diet
c. All subjects were not under antibiotic treatment or any other drugs known to influence the faecal microbiota composition for atleast three months prior to sampling.
d. All subjects were free of known metabolic and gastrointestinal disease. Whole stool were collected in sterile boxes and immediately stored at 4°C under anaerobic conditions using an Anaerocult A
e. Samples were frozen within 4h at -20°C as 200mg aliquots and stored for further analysis.

Exraction of Dna From Stool Sample

DNA from stool samples (wet weight 0.2g) will be isolated through Q1A amp DNA stool kit (qiagen).

PCR Analysis:

Real time PCR analysis of Firmicutes and Bacteriodetes bacterial division will be done with these groups specific primers. Primer and probes used in this study were designed based on 16s RNA sequence.

Sequence of Oligonucleotides Primer and Probes:

Bacteriodetes: Bact934F, GGARCATGTGGTTTAATTCGATGAT
Bact1060R, AGCTGACGACAACCATGCAG

Firmicutes: Firm934F, GGAGYATGTGGTTTAATTCGAAGCA
Firm1060R, AGCTGACGACAACCATGCAC

PCR Conditions

a. Primers and probes used in this PCR were synthesized commercially by invitrogen.
b. Real time quantitative PCR (qPCR) was carried out with an ABI-PRISM 7900 sequence detection system.
c. Analysis was performed in triplicate and the mean value was calculated.
d. Each PCR mixture was composed of 5microlitres of power SYBR PCR Master Kit, 100nm of each group specific primer and 1microliter DNA in each reaction for detecting Bacteriodetes, Firmicutes and all Bacteria.

The amplication program consisted of

a. 1 cycle of 50°C for 2min;
b. 1 cycle of 95% for 10min;
c. 40 cycles of 95°C for 15s and 60°C for 1min.
d. A melting curve analysis was alone after amplification

For Bacteriodes a primer script PCR kit was used with 100nm of Bacteroides specific primer and specific primer.
The reaction protocol comprised of

a. 1 cycle of 50°C for 2min;
b. 1 cycle of 95°C for 10s;
c. 40 cycles of 95°C for 5s and 60°c for 30s
d. The threshold cycles values and baseline settings were determined by automatic analysis settings.

3. Results and Discussion

The gut microbial communities are affected by many factors including kinship relationship, food composition, environment and age. Recent studies showed body fat storage affects the gut microbial ecology in mice and human. The obese person had a 50% reduction in the abundance of Bacteriodetes and a significantly greater proportion of Firmicutes compared to lean person. The obese person had fewer Bacteriodetes and more Firmicutes in their faeces than did lean controls. More researches are needed to investigate whether the percentage of Firmicutes is effected by a body fat and whether one or more groups among the Firmicutes may be biomarkers of obesity.

4. Conclusion

The composition of microbe's biomass in our gut may influence our weight in a significant way. A new science called nutrigenomics deals with the effects of more than 600 genes that are associated with the obesity. These genes interrupt with our diet and with microbes in our intestinal tract in ways that impact our chances of becoming obese.

Previous studies have shown that obese person have a higher ratio of Firmicutes, bacteria in their colons, this ratio is lower in lean person. It turns out that Firmicutes are more efficient at extracting energy and nutrition from dietry carbohydrates than Bacteriodes. So individuals with high Firmicutes number appear more prone to be overweight. There is emerging evidence that certain strains of Bifidiobacterium and Lactobacillus probiotics improves the numbers of Bacteriodes versus Firmicutes and therefore reduce weight gains and obesity in individuals consuming such probiotics.

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