Proposed research project: Application of Fluorescent in *situ* Hybridization for validation of qPCR results on gut microbiota.

Host Institute: Rowett Institute for Nutrition and Health, University of Aberdeen, UK

Supervisor: Dr. Karen Scott

Date of visit: 18.04.2011

Duration: 4 weeks

1. Purpose of the visit

During my PhD studies at VTT Technical Research Centre of Finland, I have optimized DNA based real-time PCR (qPCR) methods for the study of various bacterial groups of the human gastrointestinal tract. The used qPCR methods are based on 16S rRNA gene analysis as well as functional genes. In addition I have studied the effect of different DNA extraction methods on the numbers of various bacterial groups and optimized standard curves for their quantification.

The aim of the visit to the Rowett Institute for Nutrition and Health was to learn the Fluorescent in *situ* Hybridization (FISH) technique for the study of the gut microbial ecology. Rowett Institute has long experience of using this method for the microbial community analysis. FISH technique has the advantage of enabling the researcher to visualize the bacterial cells as they are enumerated and is complementary to the qPCR technique. The objective was to analyze fresh and frozen samples, including clinical sample materials, with FISH to compare and further validate the developed qPCR methods. The validated methods will be later used to study the gut microbiota in fecal samples obtained from various human interventions.

2. Description of the work carried out during the visit

A total of 27 fecal samples previously fixed with 4% paraformaldehyde were brought from VTT to the Rowett Institute for the FISH analysis. 18 of the samples were obtained from obese volunteers that were subjected to a weight loss diet. The samples were collected at the baseline and after 4 months. Additionally, 3 other samples subjected to 3 different storage conditions (fresh, frozen at -20 °C and frozen at -70 °C without any previous treatment) were analyzed with FISH in order to enumerate the effect of the storage conditions with FISH.

FISH was used to estimate the major bacterial groups in fecal samples. Total bacterial numbers were enumerated using the Eub338 probe. A panel of ten additional probes was used to estimate the numbers of bacteria belonging to the following bacterial groups: Erec482 (clostridial clusters XIVa and b), Rrec584 *(Eubacterium rectale-Roseburia* group (a component of the clostridial cluster XIVa)), Bac303 (*Bacteroides-Prevotella* group), Bif164 (bifidobacteria group), Prop853 (clostridial cluster IX), Rum (Rbro730 and Rfla729) (cluster IV ruminococci), Fprau645 (*Faecalibacterium prauznitzii* group), Lab158 (*Lactobacillus-Enterococcus* group), Ato291 (*Atopobium* group) and Ehal1469 (*Eubacterium hallii group*).

3. Description of the main results obtained

The total number of bacteria stayed stable after 4 months of diet as compared to the baseline counts. The same result was obtained for the clostridial cluster XIVa+b, *E. hallii*, *F. prauznitzii*, *Atopobium* group and *Lactobacillus*. The percentage of *E.rectale-Roseburia* group decreased from 4,8 % to 1,2 % (p=0.05) of the total number of cells after 4 months of diet. The same trend was observed for bifidobacteria with a decrease from 13 % to 6,3 % of the total number of cells (p=0.07) and for the clostridial cluster IX with a slight decrease from 15,6 % to 12,5 % in the number of cells. In contrast, the proportion of ruminococci increased from 7,7 % to 13,1 % and the *Bacteroides* spp. had a slight increase from 6,1 % to 9 %, after the 4 months of diet. The results were analyzed by comparing the means with a statistic paired t- test. No significant differences were obtained due to the small number of samples.

The effect of the storage conditions on FISH counts was also evaluated. The same 3 samples were analyzed as fresh, frozen at -20 °C, and frozen at -70 °C for two weeks. The total number of cells was not affected by the different storage conditions. Likewise, the percentage of the clostridial cluster XIVa+b, *Bacteroides* spp., bifidobacteria, *E. hallii*, *Atopobium* and *Lactobacillus* stayed the same. A slight decrease in the proportion of *F. prauznitzii*, ruminococci and clostridial cluster IX cells was observed after freezing the samples for two weeks at -20 °C or -70 °C. Although the number of samples studied was small, it seems that in case the samples used for FISH analysis are fixed as fresh, it results in higher numbers of bacteria, whereas the freezing may lower the counts for some groups.

5. Projected publications/ articles resulting or to result from the grant

Weight loss diet study