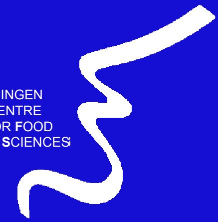
WHICH BACTERIA METABOLISE STARCH IN THE HUMAN COLON?



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Introduction

The human large intestine is a highly complex organ that plays a major role in food assimilation processes and determining the physiological effects of the diet [1].
Carbohydrates are the major component of various foods and dietetic products. So-called resistant starch (RS) escapes digestion in the small intestine and thus reaches the colon. Several studies showed the potential of RS to act as a prebiotic, when partly or totally fermented by colonic microorganisms [2]. However, it is not known which members of the gut microbiota are involved in the starch metabolism in-situ.

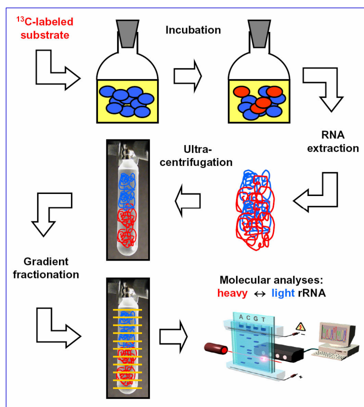

FIGURE 1: THE PRINCIPLE OF RNA-BASED STABLE ISOTOPE PROBING (RNA-SIP).

We aim to identify which gut microbes participate in the colonic starch metabolism using an in-vitro model of the human large intestine. To link the microbial diversity and metabolic function we apply the so-called RNA Stable Isotope Probing (RNA-SIP) technique (Fig. 1).
RNA-SIP is a culture-independent method, based on the incorporation of a substrate-derived isotopic label, e.g. ^{13}C, into the microbial biomass (including DNA and RNA), depending on the activity of the microorganisms present [3]. Compared to DNA-SIP, RNA-SIP is a very promising approach, because RNA is the more responsive biomarker and better reflects microbial activity [4].

Material and Methods

The TNO *in-vitro* model of the large intestine [5] was inoculated with a standardized human fecal microbiota and incubated with 1 g of 98%-labeled ^{13}C-starch, isolated from potatoes (Isolife, Wageningen, The Netherlands). RNA was extracted from lumen samples taken at different time points (0 h, 0.5 h, 1 h, 2 h, 4 h and 8 h) after starch addition.

RNA was density-resolved by means of ultra-centrifugation in cesium trifluoroacetate. Subsequently, gradients were split up into fractions of decreasing density with a fractionation device (Fig. 1).
The RNA concentration in the different gradient fractions was measured using RiboGreen®. To identify the most active starch consumers, differences in community composition of labeled vs. unlabeled (i.e. heavy vs. light) 16S rRNA was investigated using Terminal-Restriction Fragment Length Polymorphism (T-RFLP) fingerprinting.

Results

Up to 2 h after starch addition, no significant labeling of bacterial RNA was detected. However, after 4 h and 8 h of incubation, isotopically labeled RNA could be isolated (Fig. 2).

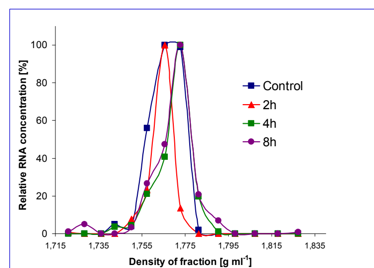

FIGURE 2: RELATIVE RNA CONCENTRATION IN GRADIENT FRACTIONS.

RT-PCR amplification of 16S rRNA (using primers 27f and 907r) [6] from gradient fractions with higher density also indicated the incorporation of the ^{13}C-label into the bacterial 16S rRNA (Fig. 3).

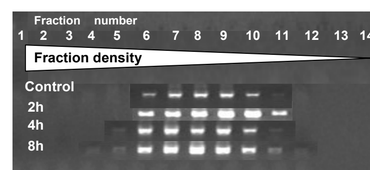

FIGURE 3: RT-PCR PRODUCTS FROM GRADIENT FRACTIONS WITH DIFFERENT DENSITIES.

T-RFLP fingerprints from the fractions with higher density obtained from the 8 h-sampling points, in comparison to the control (0 h), displayed strongly increased 69 bp and 254 bp T-RFs (Fig. 4, red arrows). Obviously, these T-RFs represent bacterial groups actively consuming starch.

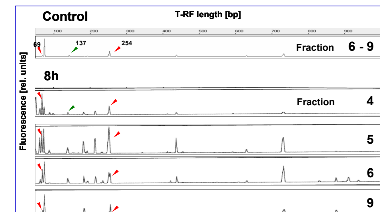

FIGURE 4: 16S rRNA T-RFLP PROFILES FROM SELECTED GRADIENT FRACTIONS. RESTRICTION ENZYME: *ALU*I.

Fingerprints from the very heavy fractions obtained from the 8 h-sampling points and generated with 35f [7] and 907rBif (CCG TCA ATT YCT TTR AGT TT) primers displayed a strongly increased 137 bp T-RF (Fig. 4, green arrow) in comparison to the fingerprints where the 27f and 907r were used for amplification (not shown). Probably, this T-RF represents *Bifidobacteria*, known to be discriminated by the latter primer pair.

Further fingerprinting analyses with different restriction enzymes and subsequent cloning of 16S rRNA from selected gradient fractions will unravel the identity of the starch consuming bacteria.

References

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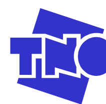
CONCLUSIONS

- Significant labeling of bacterial rRNA in an *in-vitro* colon model was detected after 4 h and 8 h of incubation with ^{13}C-labeled starch.
- T-RFLP fingerprints of isotopically labeled RNA showed the presence of bacterial groups, which degraded the starch in the *in-vitro* model of the large intestine.
- RNA-SIP has the potential to elucidate microbial structure-function relationships in the human colon, e.g. regarding the fermentation of dietary relevant carbohydrates.



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