The study of fructan metabolism by fecal culture in isothermal microcalorimeter. By K. Adamberg[1,2], K. Tomson[2], T. Visnapuu[3] & S. Adamberg[1], *[1]Department of Food Processing, Tallinn University of Technology, 19086 Tallinn, Estonia; [2]Competence Center of Food and Fermentation Technologies, 12618 Tallinn, Estonia; [3]Department of Genetics, Institute of Molecular and Cell Biology, University of Tartu, 51010 Tartu , Estonia*

Introduction. The impact of dietary carbohydrates, including prebiotics, on human health requires understanding of the complex relationship between diet composition, the gut microbiota and metabolic outputs (1). The aim of this study was to elucidate the effect of amino acids on metabolism of polyfructans by fecal microbiota by using isothermal microcalorimetry - a method for continuous monitoring of heat flow that is proportional to the rate of bacterial growth (2).

Materials and methods. Fecal inoculum from a single donor was grown in a defined medium containing 20 amino acids, vitamins and mineral salts, in a multichannel isothermal microcalorimeter TAM III ((TA Instruments, USA). Polyfructans inulin (from chicory, Orafti® HP, Belgium) and levan (synthesized using levansucrase from *Pseudomonas syringae*, University of Tartu, Estonia), and the corresponding fructooligosaccharides (FOS_{inu} and FOS_{mix}) were used as substrates. Consumption of fructans and amino acids, production of organic acids and changes in microbial population were analyzed.

Results and Discussion. The power-time curves generated during sequential degradation of oligo- and polysaccharides were substrate-specific and multiauxic (Fig 1). Compared to inulin levan was metabolized with higher growth rates ($\mu = 0.8\pm0.1$ h^{-1} vs 1.2 ± 0.2 h^{-1}, respectively) that might be caused by different glycoside linkage types (β2-1in inulin and β2-6 in levan) or/and degree of polymerisation of the substrate. Gradual switch from short chain to long chain oligosaccharides in parallel to increased consumption of Asx, Gln and Ser was observed during the growth on inulin.

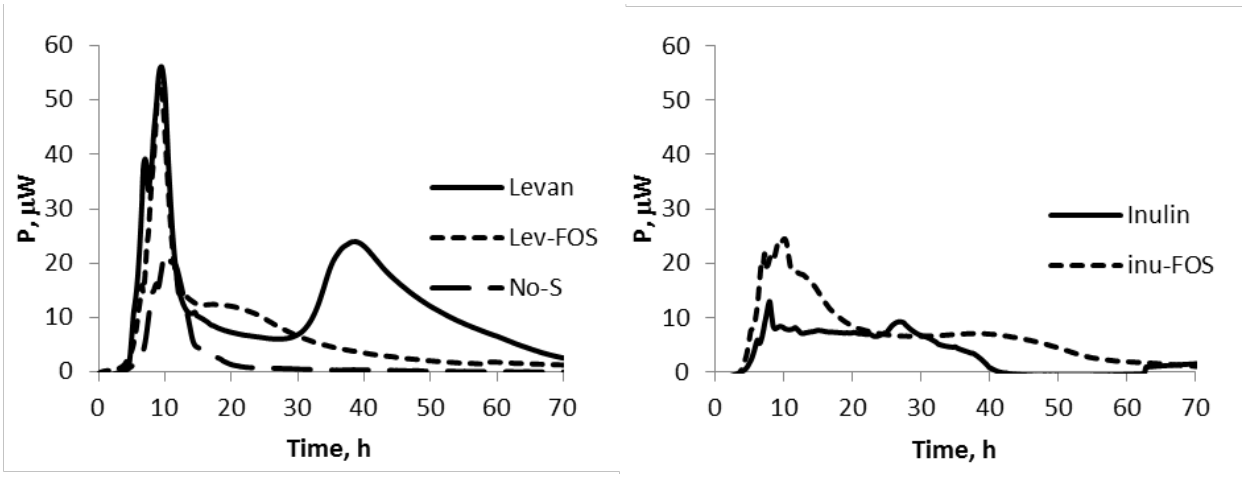

Figure 1. Microcalorimeter power-time curves of fecal microbial consortium on different substrates. No-S indicates medium without added carbohydrate; . Error bars are not shown but in all cases absolute deviations of two parallel experiments remained within 20 % difference.

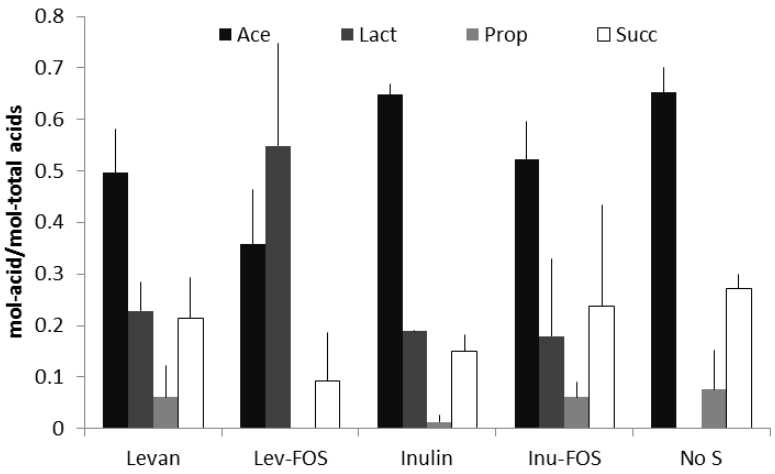

Figure 2. Proportions of acids produced by fecal consortium during growth on different carbohydrates. No-S indicates the medium without carbohydrate source, containing free amino acids. For each substrate, the ratios of acetate, lactate, propanoate or succinate to sum of all acids are shown. Error bars show standard deviation of two independent experiments (in both experiments three technical replicates were analysed).

Acetate was the major product in the medium without any carbohydrate source whilst Asx, Ser and Thr were used as the main energetic and/or carbon source for the cells (Fig. 2 and Table 1) and after exhaustion of these energetic amino acids the growth stopped. In contrast, such sharp end of the growth (heat production) was not observed in the medium supplemented with fructans. In fructan media, acetate covered 35 to 65 % of the all acids produced while propanoate was the minor product (0 to 10 %, Fig 2). FOS_{mix} was mainly converted to lactate (55±20 % of products) suggesting that acetate production was not so active by this substrate. It can be explained by the requirement of regeneration of NAD^+ which is not coupled in the case of acetate production and reductive TCA cycle should operate or hydrogen should be produced under anaerobic conditions. In general, reductive TCA partially accompanied by propanoate and succinate production recovered half of the NAD^+ excluding inulin medium where this pathway regenerated 80±2 % of NAD^+ which was caused by high consumption of amino acids (around half of total carbon consumed). Production of both D- and L-lactate was detected (except in amino acid medium without saccharides) whilst from levan mostly D-lactate was produced indicating better growth of D-lactate producers such as *Bacteroides*. This assumption was confirmed by metagenomic analyses showing detectable amount of *Bacteroides* only in the media containing levan as substrate (Fig. 2).

Incubation of fecal inoculum with different fructans changed the population composition and the initial consortium was not stable (Fig 2). The main group promoted by inulin, FOS_{inu}, FOS_{mix} and free amino acids were the genera *Escherichia/ Shigella*. Unexpectedly, the medium with no saccharides supported similar microbiota as FOS_{inu}. In medium with levan, *Bacteroides* was the dominant genus beside *Escherichia/ Shigella*, indicating high selectivity of this substrate towards *Bacteroides*.

Conclusions. It was shown that levan selectively enhances the growth of *Bacteroides* from colon microbiota. Microcalorimetry together with -omics methods is a promising approach to evaluate the selective effect of various substrates for specific bacterial groups within complex consortia. However, more reliable results in regard to colon microbiota could be achieved by using continuous culture techniques under strictly contolled conditions.

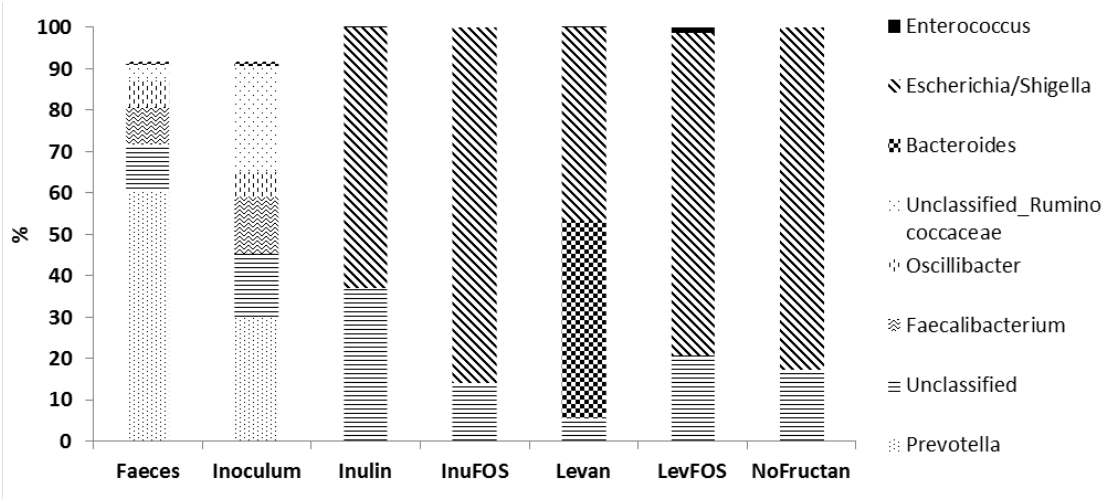

Figure 2. Metagenomic analysis of faecal inoculum and in the samples after microcalorimetry experiments on different substrates. Metagenomes has been obtained by Illumina MiSeq sequencing using region V4 primers with average reading length 290 bp and processed by MOTHUR pipeline.

Table 1. Substrate consumption and product formation (mmol/gDW) by fecal microbial consortium 72 h; averages of two experiments with standard deviations (Ave ± SD). No S indicates medium without saccharides.

	Levan	**Lev-FOS**	**Inulin**	**Inu-FOS**	**No S**
	Ave ± SD	Ave ± SD	Ave ± SD	Ave ± SD	Ave ± SD
Arg	1.7 ± 0.9	4.5 ± 3.3	2.5 ± 0.0	0.3 ± 1.1	11.5 ± 0.7
Asn	3.8 ± 2.5	0.9 ± 0.9	8.1 ± 3.7	0.6 ± 1.5	23.6 ± 3.4
Asp	4.2 ± 2.5	1.5 ± 1.5	9.5 ± 3.5	0.8 ± 2.5	25.9 ± 0.6
Gln	1.6 ± 0.9	2.5 ± 1.4	3.0 ± 0.6	0.3 ± 0.1	5.8 ± 2.4
Glu	4.1 ± 2.7	1.0 ± 1.0	3.6 ± 1.0	0.6 ± 0.6	-4.5 ± 4.57
Ser	9.8 ± 5.1	4.0 ± 4.0	22.1 ± 6.3	2.3 ± 1.2	65.1 ± 3.2
Thr	0.9 ± 0.9	1.4 ± 1.4	12.6 ± 6.1	0.9 ± 2.1	34.8 ± 6.70
H2	34.3 ± 17.2	16.0 ± 15.3	26.3 ± 5.60	22.0 ± 10.2	22.5 ± 18.9
CO2	33.5 ± 9.7	15.2 ± 16.2	31.8 ± 10.1	25.8 ± 15.4	7.9 ± 13.3
H2S	0.1 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	1.0 ± 0.9
Ace	100. ± 22.8	90.3 ± 16.6	284. ± 170.	80.2 ± 11.0	180. ± 32.1
Lact	46.5 ± 13.8	149. ± 68.4	81.0 ± 46.2	28.4 ± 34.8	0 ± 0
Prop	11.4 ± 11.4	0 ± 0	9.0 ± 9.0	11.4 ± 7.5	26.7 ± 26.7
Succ	41.8 ± 13.6	21.5 ± 21.5	56.2 ± 22.8	39.6 ± 50.6	74.8 ± 11.3

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