

Co-metabolism of amino acids and polyfructans by *Bacteroides thetaiotaomicron* in defined media. By *S. Adamberg*¹, *K. Tomson*², *H. Vija*³, *T. Visnapuu*⁴ & *K. Adamberg*^{1,2}. ¹Department of Food Processing, Tallinn University of Technology, 19086 Tallinn, Estonia; ²Competence Center of Food and Fermentation Technologies, 12618 Tallinn, Estonia; ³National Institute of Chemical Physics and Biophysics, 12618 Tallinn, Estonia; ⁴Department of Genetics, Institute of Molecular and Cell Biology, University of Tartu, 51010 Tartu, Estonia

Introduction. *Bacteroides thetaiotaomicron* is considered to be human symbiont that stabilizes the colon ecosystem by multiple functions including catabolism of wide variety of polysaccharides (1). Levan (β 2-6 fructan) and levan-type oligosaccharides are produced mostly by extracellular enzymes of several bacteria (2, 3) but can also be synthesized within plants. Compared to inulin, the functions and applications of levan and levan-type FOS are only moderately investigated. Moreover, cross-talk between fructan and amino acid metabolism in *B. thetaiotaomicron* has not been shown earlier.

Materials and Methods. The growth of *Bacteroides thetaiotaomicron* DSMZ 2079 in defined media (mineral salts, buffers, vitamins, pH 7.1) with two different amino acid compositions – 20 and 2 amino acids (Cys, His), was comparatively studied in isothermal microcalorimeter (IMC) TAM III (TA Instruments, USA) at 37°C for 168 hours. Polyfructans inulin (Beneo Orafti[®] HP, Belgium) and levan (synthesized using levansucrase from *Pseudomonas syringae*, University of Tartu, Estonia), fructooligosaccharides from the levan synthesis (FOS_{mix}), sucrose and fructose were tested as substrates. The analyses comprised substrate concentrations, organic acids, amino acids and pH.

Results and Discussion. The power-time curves were substrate specific and reflected multiple growth phases on all substrates (Fig 1), whilst growth was not detected in medium without carbohydrate source (only free amino acids) nor with inulin as a sole saccharide. Consumption of fructans and production of organic acids were in accordance with biomass formation and acidity change with the final pH around 5.0. From the FOS_{mix} oligosaccharides with DP 3 – 9 were metabolised with simultaneous accumulation of monosaccharide (Fig 2). More oligosaccharides were consumed in conditions of amino acids deficiency, obviously because of protein synthesis from these saccharides. The highest specific growth rates ($\mu_{max} = 0.55$ and 0.51 h^{-1}) were obtained with FOS_{mix} in media M1 and M2, respectively, followed by levan, fructose and sucrose ($\sim 0.4 \text{ h}^{-1}$).

The total amount of acids produced were similar in both media, however, with different proportions (Fig 3). In the beginning of growth (up to 24 h) acetic acid fermentation dominated followed by increased lactate production and ended with production of mainly propionic (and succinic) acids in the medium containing 20 amino acids (M1). In medium M1, all acids were produced throughout incubation, in medium M2 lactate was not produced during the first 24 h, and succinate was not produced after 48 h. Lack of amino acids enhanced production of propionic acid (Fig 3). The highest production of D-lactate was observed in the medium M1 in parallel with extensive consumption of amino acids (up to 17 mmol/gDW), especially energetic

amino acids Ser, Thr, and Asp (Table 1). Production of Ala, especially in amino acid deficient medium, was observed on all substrates and (Tab 1). Glu was produced up to 48 h and consumed thereafter (Tab 1).

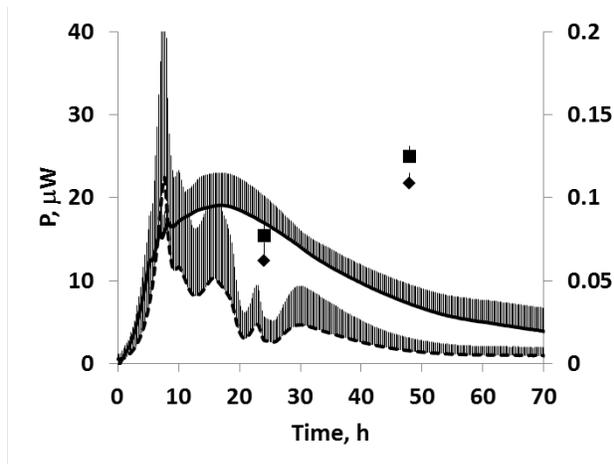


Figure 1. Power-time curves and biomass accumulation during the growth of *B. thetaiotaomicron* DSMZ 2079 in defined media with two different amino acid compositions: medium M1 (20 amino acids, solid line and squares) and medium M2 (amino acid restricted medium, dashed line and diamonds); average of duplicate experiments with each substrate: levan, FOS_{mix}, fructose and sucrose with standard deviations.

Figure 2. Concentrations oligosaccharides of FOS_{mix} (expressed as hexose equivalents) at 0, 24, 48 and 168 hours of growth of *B. thetaiotaomicron* in amino acid restricted medium (M2).

Method: Acquity UPLC (Waters, Milford, MA, USA), evaporative light scattering (ELS) detector. Alltech Prevail Carbohydrate ES column (250 mm × 4.6 mm; particle size 5 mm) (Grace, Deerfield, USA).

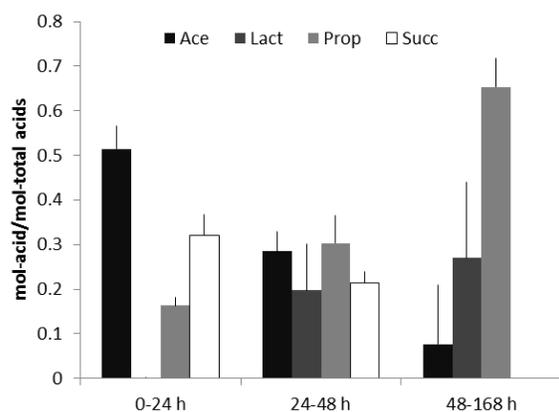
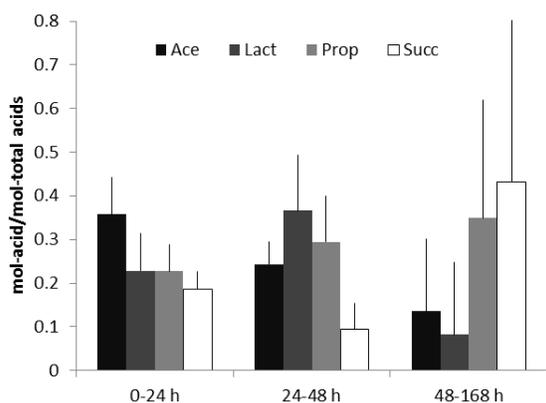
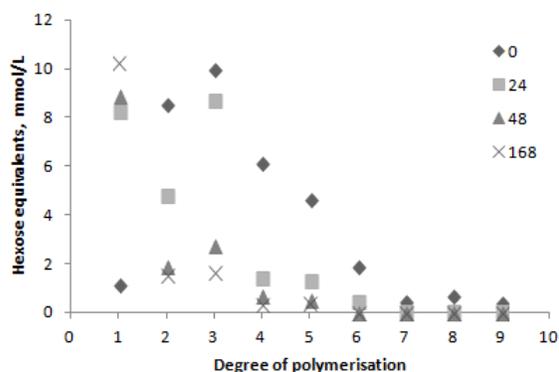


Figure 3. Proportions of organic acids produced by *B. thetaiotaomicron* in defined media M1 (20 amino acids, left figure) and M2 (2 amino acids, right figure). For each time interval (0 - 24, 24 - 48 h and 48 - 168 h) the sum of acids is normalized to 1 and ratios of acetate/total acids (Ace), lactate/total acids (Lact), propanoate/total acids (Prop) or succinate/total acids (Succ) are presented.

Table 1. Consumption of selected amino acids and gases (negative values indicate production) and formation of organic acids (mmol/gDW) by *B. thetaiotaomicron* grown in defined media M1 (20 amino acids) and M2 (2 amino acids); average values with standard deviations (Ave±SD) in three phases: 0-24, 24-48 and 48-168 hours.

	M1 medium			M2 medium		
	0-24 h	24-48 h	48-168 h	0-24 h	24-48 h	48-168 h
	Ave±SD	Ave±SD	Ave±SD	Ave±SD	Ave±SD	Ave±SD
Ala	0 ± 0	-0.6 ± 0.9	-1.3 ± 1.5		-2.0 ± 0.5	0.2 ± 1.7
Asp	0.0 ± 1.2	2.8 ± 0.6	5.3 ± 4.2		-0.1 ± 0.2	0.9 ± 1.4
Gln	-0.1 ± 0.3	0.5 ± 0.3	2.5 ± 1.4		0.0 ± 0.1	0.2 ± 0.4
Glu	0 ± 0	-0.7 ± 0.8	3.5 ± 3.1		-1.7 ± 0.9	7.9 ± 4.2
Gly	0 ± 0	0.2 ± 0.5	0 ± 0		-0.5 ± 0.4	0.7 ± 1.5
Ser	0 ± 0	2.7 ± 1.0	1.3 ± 1.8		-0.2 ± 0.1	0.2 ± 0.3
Thr	0 ± 0	1.2 ± 0.4	0.5 ± 1.0		-0.1 ± 0.0	0.0 ± 0.2
H2	6.2 ± 5.5	-0.5 ± 1.1	0.9 ± 2.8	14.3 ± 3.9	2.7 ± 2.7	-5.4 ± 7.9
CO2	19.9 ± 13.1	15.4 ± 6.8	2.92 ± 12.3	16.6 ± 4.8	12.3 ± 8.2	-11. ± 29.0
Ace	83.3 ± 46.1	52.3 ± 4.5	30.6 ± 38.2	57.1 ± 38.3	92.3 ± 55.9	15.3 ± 30.7
Lact	56.2 ± 34.2	82.1 ± 32.9	17.5 ± 35.1	0.08 ± 0.1	64.4 ± 38.2	53.2 ± 51.3
Prop	57.8 ± 39.1	63.7 ± 22.3	66.6 ± 54.3	18.2 ± 12.8	90.9 ± 31.3	128. ± 86.3
Succ	45.9 ± 31.5	22.2 ± 14.8	88.2 ± 94.2	36.5 ± 26.9	68.7 ± 37.2	0 ± 0

Conclusions. Glycan metabolism of *B. thetaiotaomicron* DSMZ 2079 depends on the amino acid supply. Surplus of amino acids reduces consumption of oligosaccharides with higher degree of polymerisation (DP > 3) and flux through reductive TCA cycle compared to amino acid deficiency. Interestingly, metabolism of mono- and difructans results in similar power-time curves as that of polyfructan. Our data suggest that levan might serve as a specific substrate for modulation of colon microbiota as only few genera/species, including *Bacteroides thetaiotaomicron* have been shown to degrade levan. Isothermal microcalorimetry combined with other analytical methods enables to detect metabolic shifts and elucidate quantitative metabolism of microorganisms in defined growth media.

Acknowledgements. The project has received funding from the European Regional Development Fund (projects No. 3.2.0701.12-0041) managed by Archimedes Foundation and EU29994), & Institutional Research Funding (IUT 1927) of the Estonian Ministry of Education and Research.

References

1. Sonnenburg JL, Xu J, Leip D *et al.* (2005). *Science*, **307**(5717), 1955–1959.
2. Bekers M, Laukevics J, Upite D *et al.* (2002) *Process Biochem* **38**: 701-706.
3. Visnapuu T, Zamfir AD, Mosoarca C, *et al.* (2009) *Rapid Comm Mass Spectrom* **23**:1337-1346.