



# In vitro consumption and utilization of different Microbiota Accessible Carbohydrates (MACs) by key microbial components of the infant microbiome

Verena Ducret<sup>1</sup>, Karl Perron<sup>1</sup>, Jean-Philippe Kunz<sup>2</sup>, Philippe Steenhout<sup>2</sup>, Yemi Adesokan PhD<sup>2</sup>

<sup>1</sup>University of Geneva, Department of Plant Biology, Geneva, Switzerland <sup>2</sup>Gnubiotics Sciences, Microbiome Research, Monthey, Switzerland Contact email: jpkunz@gnubiotics.com

## introduction

### GnuBiotics MAC formulations are highly diverse, structural and functional HMO-mimics

Several essential groups of commensal bacteria found in the infant gut, such as *Bacteroides* and *Akkermansia*, have been described as effective utilizers of Human Milk Oligosaccharides (HMOs). HMOs are a class of Microbiota Accessible Carbohydrates (MACs) and are naturally found in human breast milk. There are up to 200 different HMO structures and they are known to play a key role in infant development, as well as modulate infant's immune system. We have generated different formulations containing up to **130 uniquely diverse MAC structures**, that mimic HMOs (Figure 1a and 1b). The aim of this study was to investigate the ability of *Bacteroides thetaiotaomicron* (Bt) and *Akkermansia muciniphila* (Am) to utilize different formulations of structurally diverse MAC formulations.

EPITOPE	HMO (%)	GNU MAC (%)
Fucose blood group H (Fuca1-2Gal)	33%	36%
3 linked sialic acid (NeuAca2-3Gal)	8%	10%
6 linked sialic acid (NeuAca2-3Gal/GalNAc)	13%	6%

Figure 1b. Structural Mimics of Natural HMOs: Examples of terminal epitopes in HMO mimicry in GnuBiotics MAC formulations.

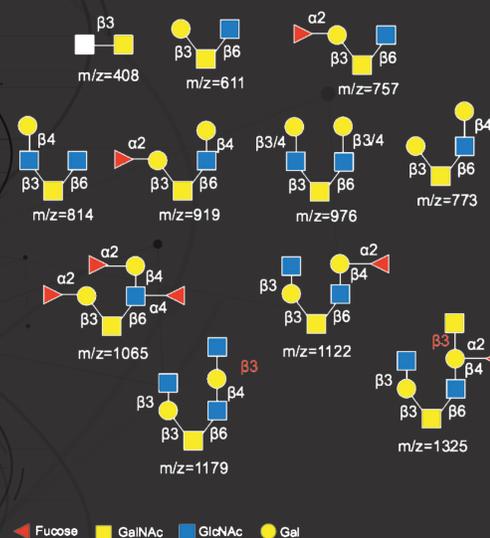


Figure 1 a. Superior Diversity of MAC Formulations: Examples of MAC structures present in the GnuBiotics MAC formulations, that structurally mimic HMOs.

## methods

*B. thetaiotaomicron* ATCC 29148 (Bt) and *A. muciniphila* ATCC BAA835 (Ak) were cultivated in minimal medium (MM) containing one of two different formulations of MACs (Mac-P1 and Mac-P2) at a final concentration of 1.5%. *B. thetaiotaomicron* ATCC 29148 was also grown in 0.5% of glucose (Glc), as a positive control. Cultures were performed in 96-well plates, in triplicate, under anaerobic conditions. The bacterial growth was determined by measuring the optical densities (OD) at 600nm at the start, after 24h, 48h and 72h of growth.

## results

### Gnubiotics MAC formulations support selective growth of key components of the infant microbiome

In the case of Bt, both formulations of MACs lead to growth, although MAC-P1 appeared to instigate more growth than MAC-P2 (Figure 2). On the other hand, in the case of Ak only MAC-P2, and not MAC-P1, was able to stimulate grow (Figure 3). Based on this observation, different formulations are selectively utilized by different bacteria.

### *B. Thetaiotaomicron* growth

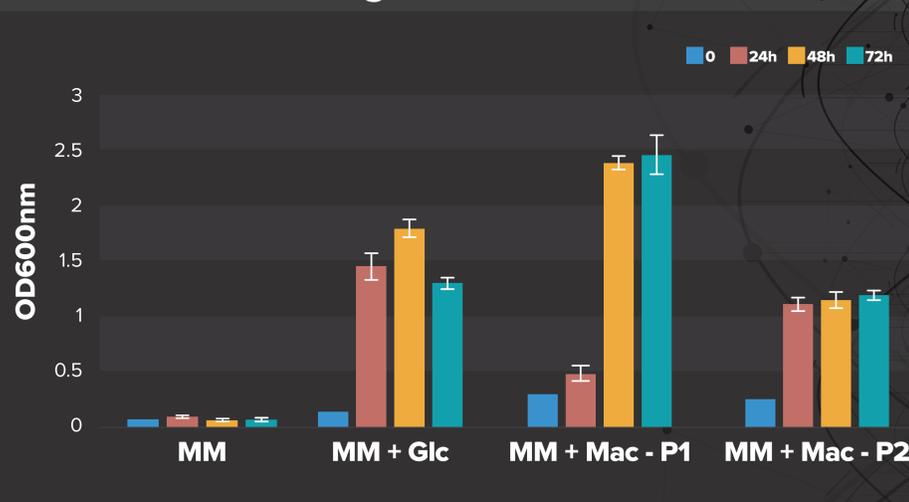


Figure 2. In vitro growth of *B. Thetaiotaomicron* in the presence of two different formulations of microbiota accessible carbohydrates (MACs). Bacteria were cultured under 4 different conditions: minimal media (MM) alone, MM plus glucose as positive control (MM+Glc), MM plus MAC formulation 1 (MM+Mac-P1), or MM plus MAC formulation 2 (MM+Mac-P2). Bacterial growth was measured by determining optical density at OD 600nm, at 0h, 24h, 48h, and 72h.

### *A. muciniphila* growth

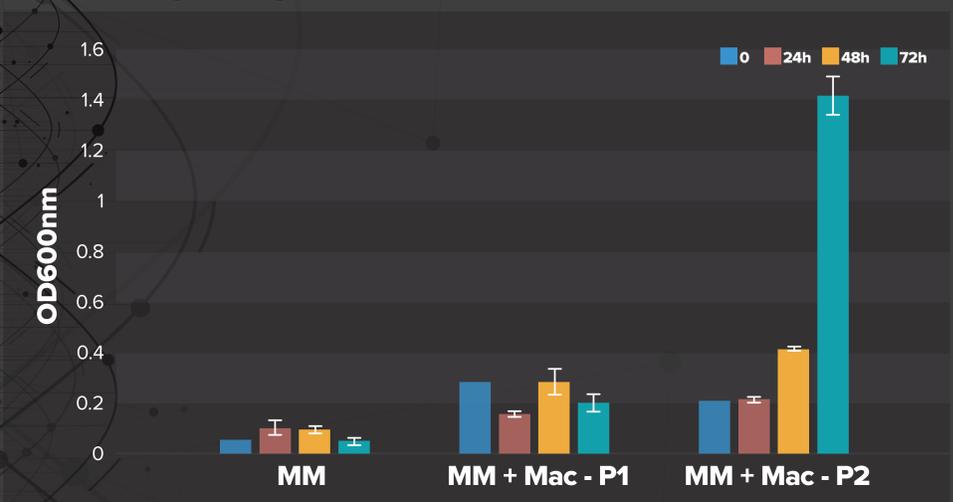


Figure 3. In vitro growth of *A. muciniphila* in the presence of two different formulations of microbiota accessible carbohydrates (MACs). Bacteria were cultured under 3 different conditions: minimal media (MM) alone, MM plus MAC formulation 1 (MM+Mac-P1), or MM plus MAC formulation 2 (MM+Mac-P2). Bacterial growth was measured by determining optical density at OD 600nm, at 0h, 24h, 48h, and 72h.

## conclusion

Gnubiotics MAC formulations are closer to mother's milk in diversity than any other HMO product and may potentially have an effect in restoring infant gut dysbiosis

This study shows that MAC formulations with a structural diversity similar to HMOs present in breast milk may have similar function to naturally occurring HMOs, by stimulating key components of infant microbiota *in vitro*. Moreover, different formulations may have different effects on the microbiome, suggesting a possibility of a more personalized approach to microbiome modulation. Our diverse HMO-mimicking MAC formulations could be helpful in establishing the correct gut microbiome in C- section infant populations that are known to have dysbiosis.