



Interplay between research question and
in vitro/ex vivo model selection in prebiotic R&D

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Company Overview

Expert in *in vitro* simulation of the gastrointestinal tract, offering various **cutting-edge technology platforms & profound expertise** for these simulations.



Company Background

Spin-off company from Ghent University.
Founded in 2008.
Located in Gent, Belgium - active worldwide.
42 employees (10 PhDs with on avg. 8 years of experience)



Recognition

Highly validated – IVIV correlations
Over 300 publications
Recognized by regulatory agencies
Official biobank since 2023



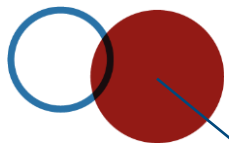
Company Mission

We make our clients successful by **de-risking their product development**, providing **mechanistic insights** using cutting edge technologies with proven **in vitro - in vivo validation** in the area of gastrointestinal and (host) microbiome research.



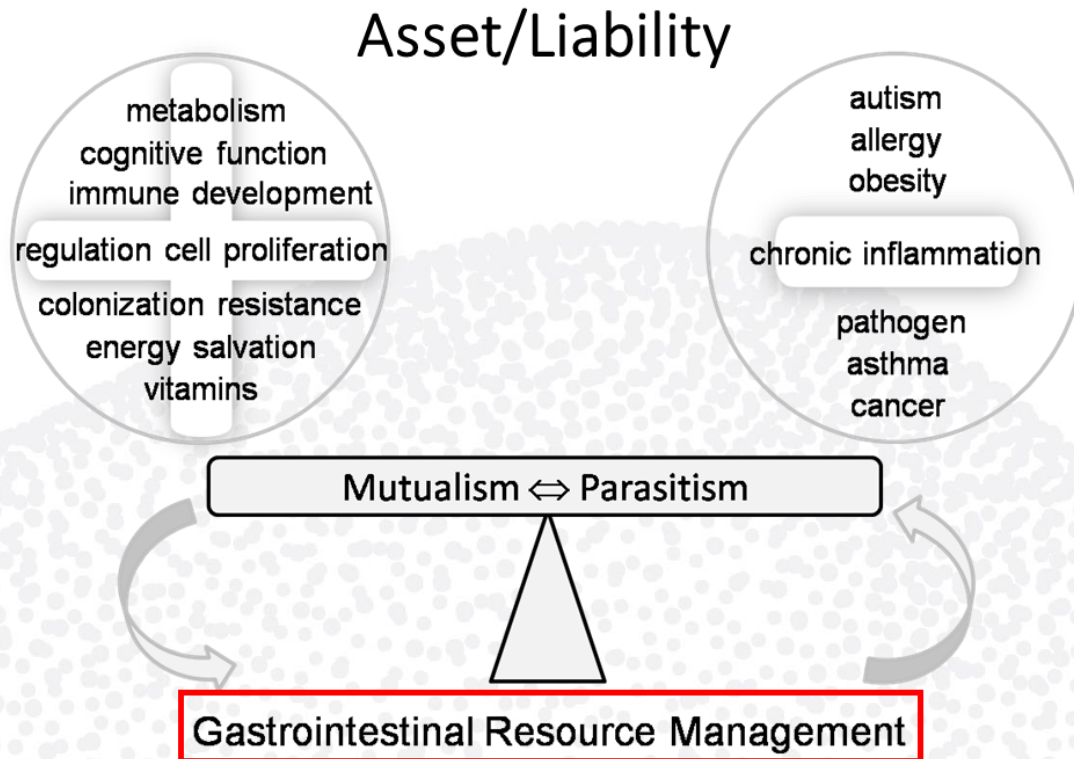
Company Vision

Global reference in gastrointestinal and host-microbiome research, offering a comprehensive "**one-stop shop**" for the development of new ingredients and drugs.



Three core pillars and our approach

PREBIOTIC: a substrate that is selectively utilized by host microorganisms conferring a health benefit to the host. Thus, the concept includes a microbiota-mediated mechanism.



- ***In vivo* human studies**
 - **Cannot “look inside” the gut**
 - Black box effect or invasive methods
 - **Only administer products recognized as safe**
 - **Environmental factors cause high variability**
- ***In vivo* animal studies**
 - **Ethical approval**
 - **Different physiology & microbiome**
- ***In vitro/Ex vivo* studies**



Three core pillars and our approach

3 Rs

Market trend aimed at the Replacement, Reduction, and Refinement of animals used in research.



There is no such a thing as “A” gut

Simulation of micro-niches (lumen vs mucus; upper GIT vs colon) is key to provide sounding results

Personalization

Precision nutrition and precision medicine are the base for the development of new innovative products



There is no such a thing as “A” person

Need of taking into account interindividual variability (healthy vs disease; variability in the target population)

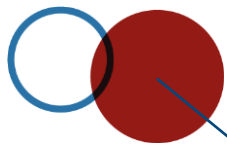
Throughput vs MoA

Market trend directed to the test of multiple lead products under different physiological conditions to generate mechanistic data



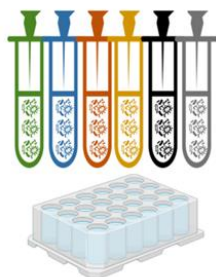
There is no such a thing as “A” one-model-fits-all

The selection of the test model depends on the research question and the physiology of the target population



The choice of the technology depends on the research question

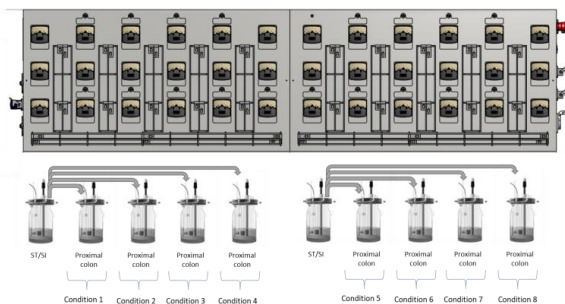
Colon-on-a-plate®



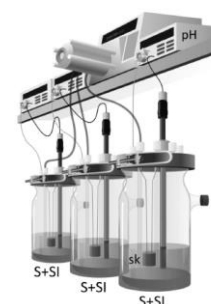
Short-term colonic simulation



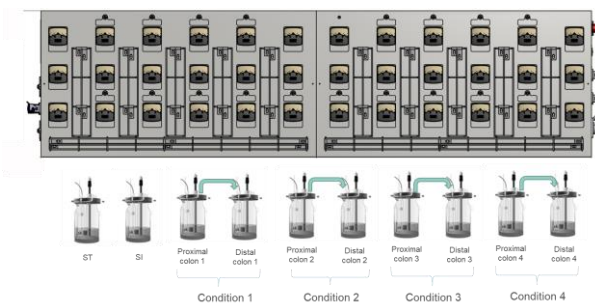
Screening SHIME®



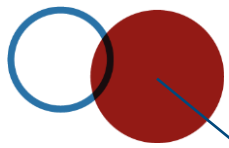
Upper GIT



Short- and long-term SHIME®



Arms	10s to 100s	10s	8 arms per instrument	2 in triplicate	4 arms per instrument
Volume	7 mL	50 mL	500 mL	250 mL	500-800 mL
Dose	Single	Single	Repeated	Single	Repeated
Time	24-48h	24-48h	7 days	6h	10 days to 9 weeks
Aim	Screening of multiple conditions, interindividual variability, high throughput	Screening of multiple actives, measurement of multiple endpoints to establish a kinetic	Screening of actives for which a daily repeated doses is needed to appreciate the effect/differential effect from other test products	Digestibility, targeted delivery, survival probiotic, bioaccessibility, bioavailability	Execution of a clinical trial <i>in vitro</i> , focus on the effect of actives in terms of temporal onset of the effect and its localization.



ProDigest technology platform

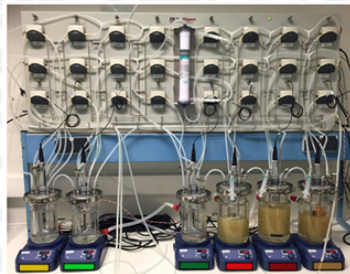
Colon-on-a-plate®



Short-term colonic simulation



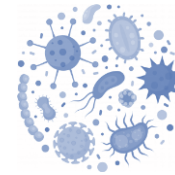
SHIME®



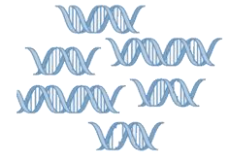
Throughput

Complexity of the simulation

Microbial fermentation activity



Metagenomics at luminal and mucosal level



Multi-omics data integration and data correlation

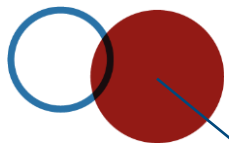


Metabolic fingerprinting & metabolomics



Microbiome-host interaction



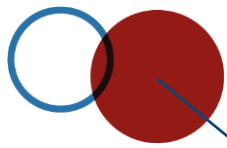


Possible research questions to answer

- I have a **high number** of lead compounds and I want to see the impact in terms of interindividual variability
- I want to test the same active with different formulations in **multiple individuals** of a specific target population
- I have a selected numbers of potential prebiotics and I want to test them with **multiple strains** to develop a possible synbiotic

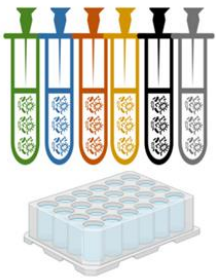



**Common feature: high number of test conditions
hence, need of a good throughput**

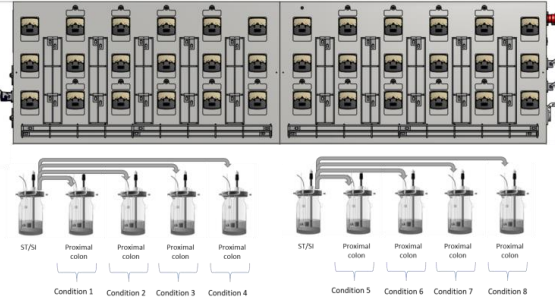


The choice of the technology depends on the research question

Colon-on-a-plate® **Short-term colonic simulation**

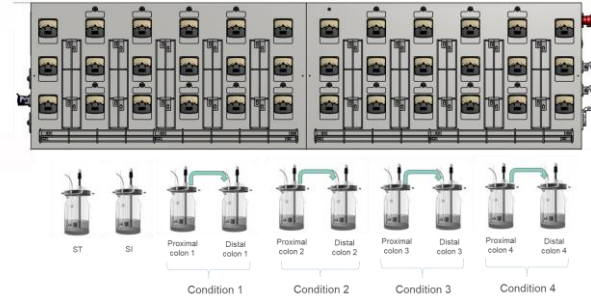
Screening SHIME®



Upper GIT

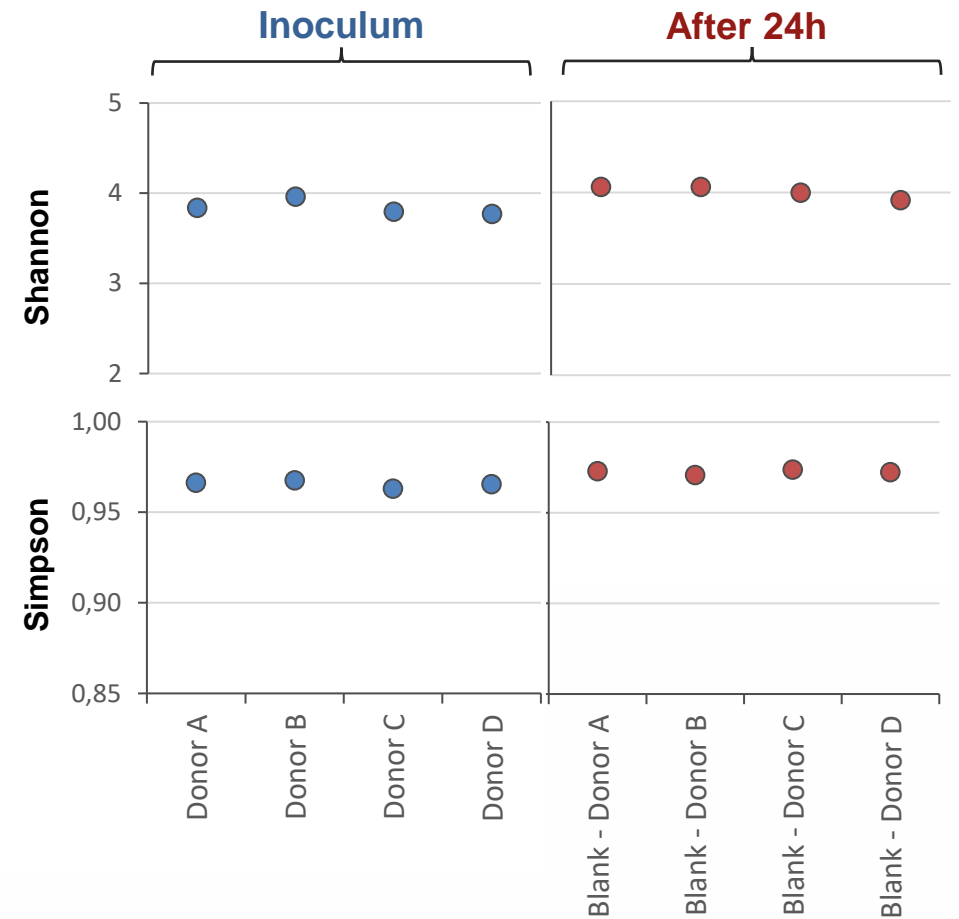
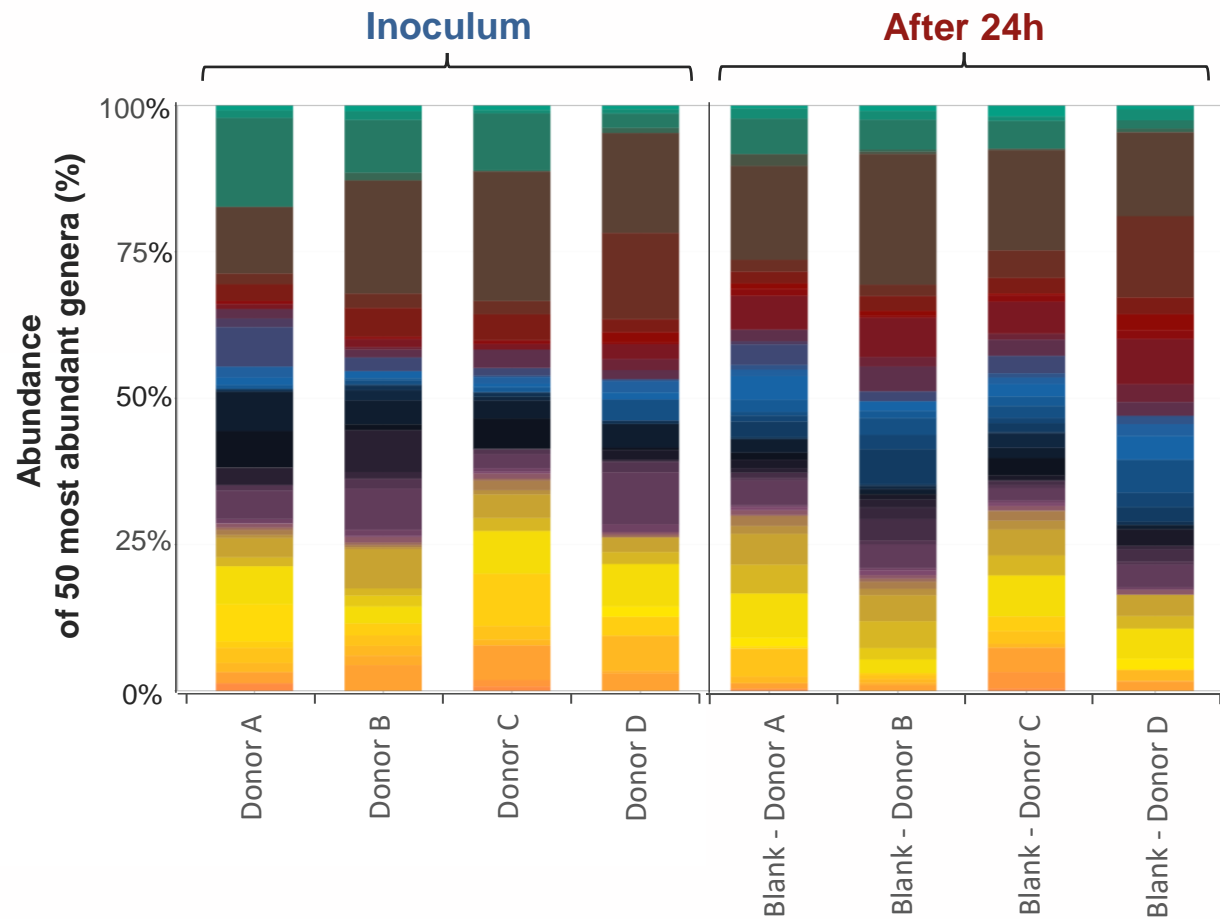


Short- and long-term SHIME®



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Original fecal composition is maintained during incubation

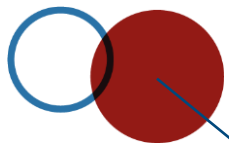


The original community composition is maintained after 24h of blank incubation.

Incubations with healthy human adult donors. Shallow shotgun sequencing (SSS) data.

biological replicates (donors) = 4

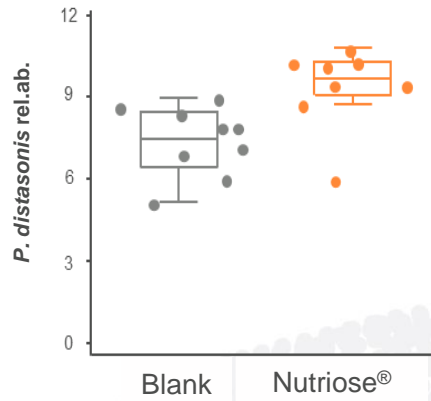
technical replicates = 1



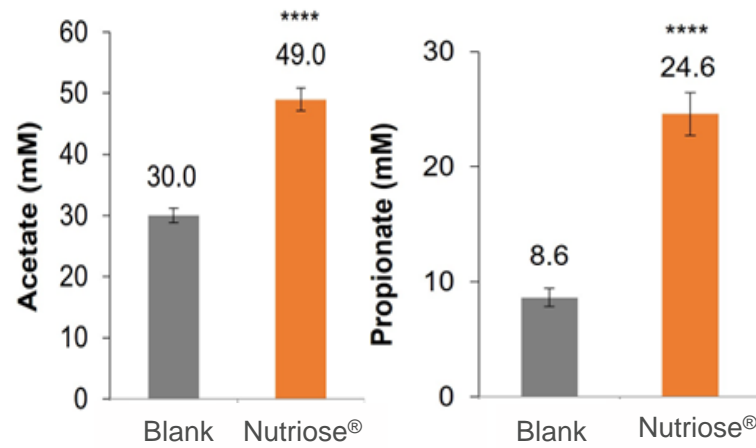
Colon-on-a-plate® – IVIV correlation

Colon-on-a-plate®
ex vivo data

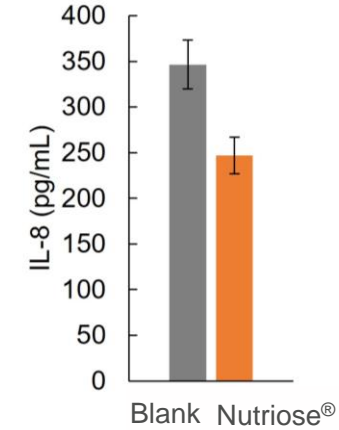
Microbiome composition



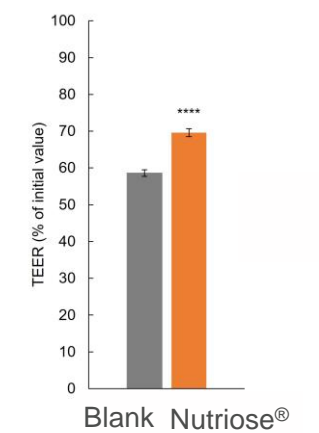
Functionality of the microbiome



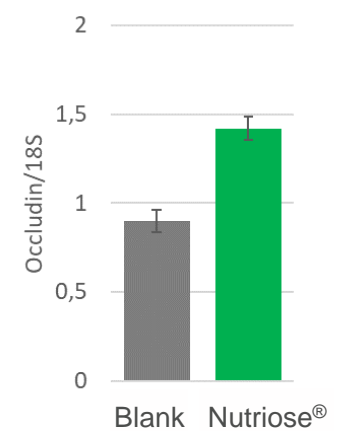
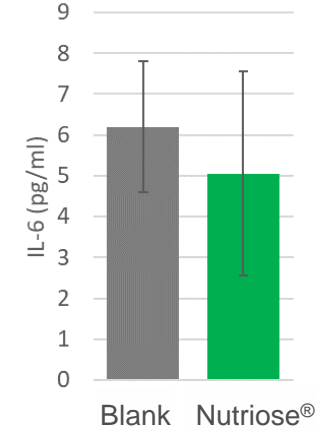
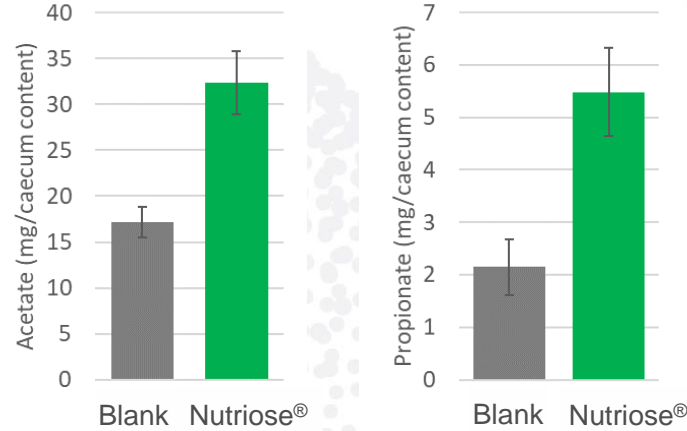
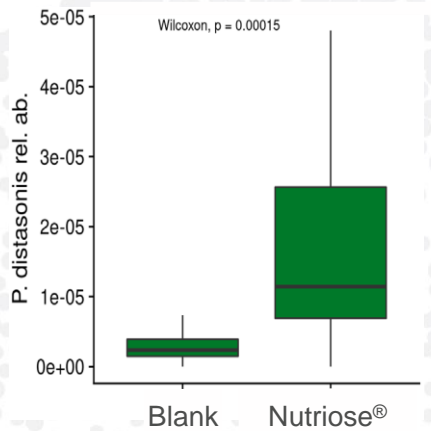
Immunity



Barrier integrity



In vivo and
clinical data



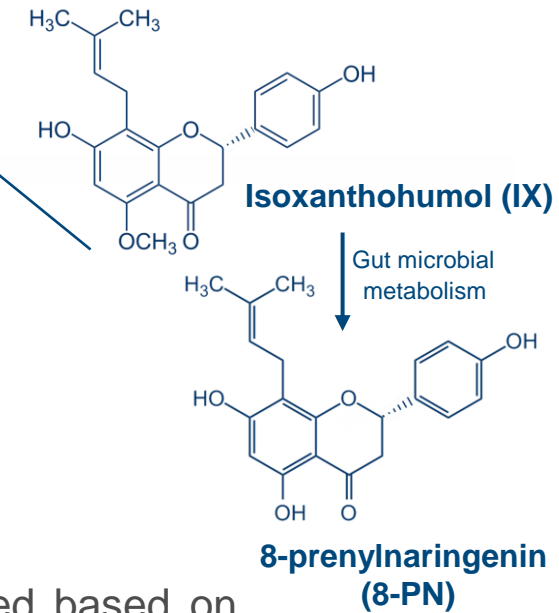
Data extracted and adjusted from Perreau et al., 2023, Thirion et al., 2022, Guerin-Deremaux et al., 2010, Aliasgharzadeh et al., 2015 and Perreau et al., 2020

Short-term colonic simulation – IVIV correlation

In vitro short-term colonic simulation

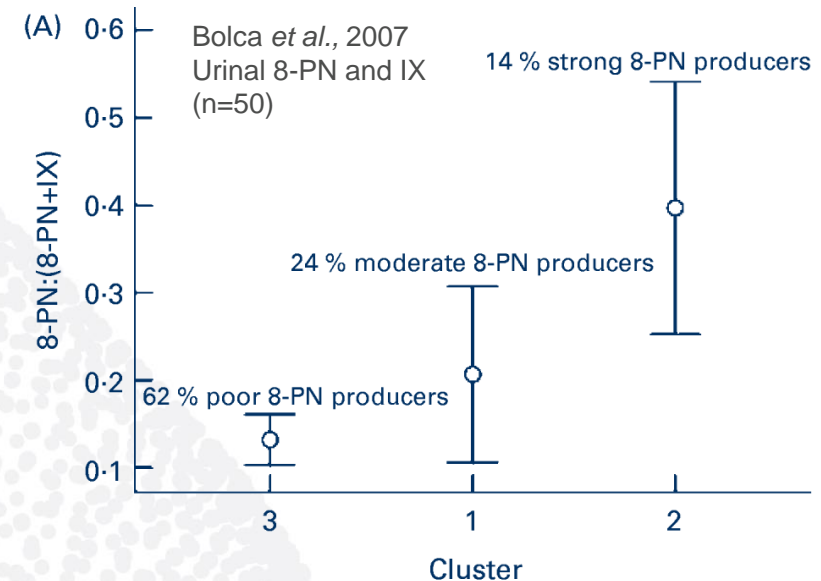
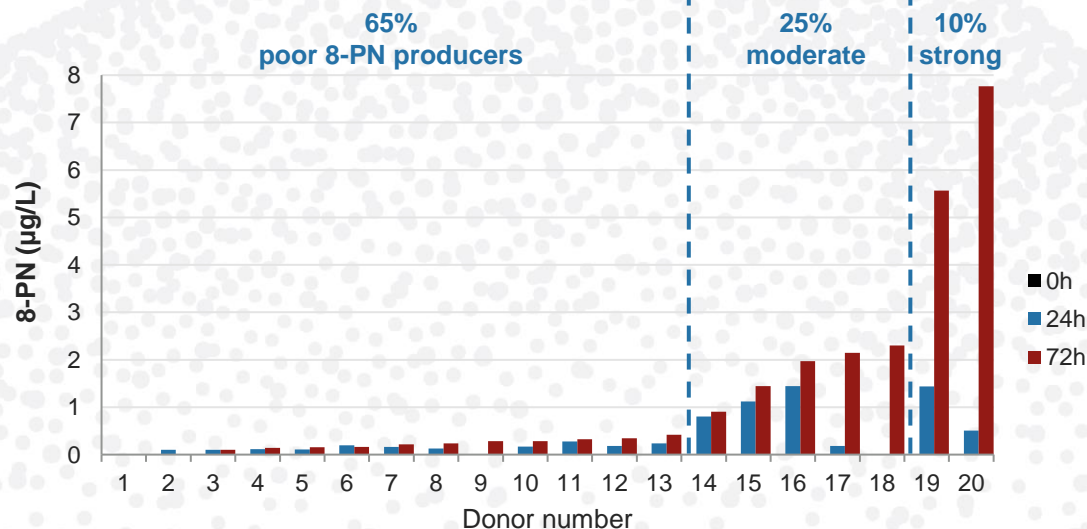


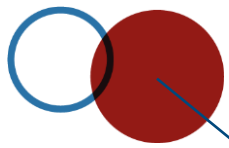
In vivo trial



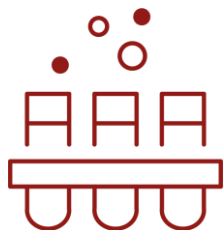
ProDigest identified **three distinguished groups** of **8-PN**-producers during a 72-hour short-term colonic simulation in which **isoxanthohumol** was added as the fermentable substrate.

Three donor types were identified based on magnitude of **8-PN** production after a 5-day intervention with an **IX:8-PN**-based product which was administered three times per day.





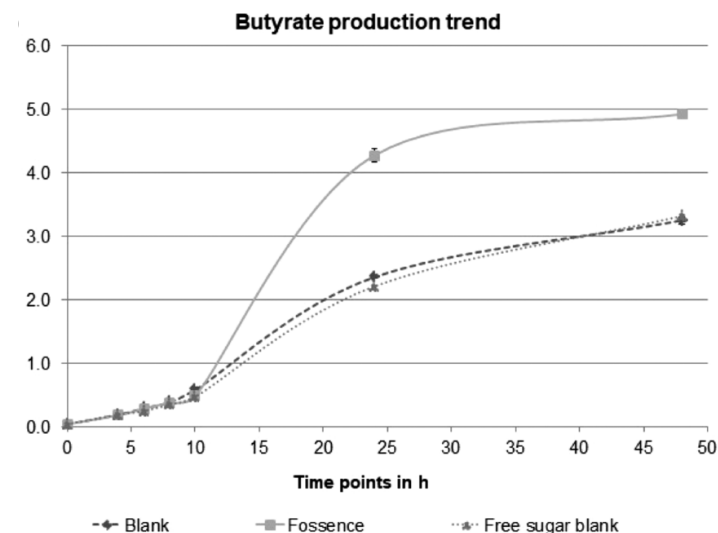
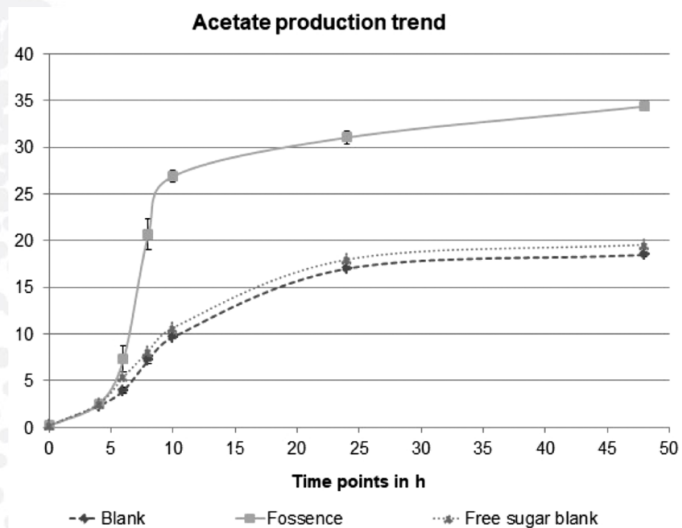
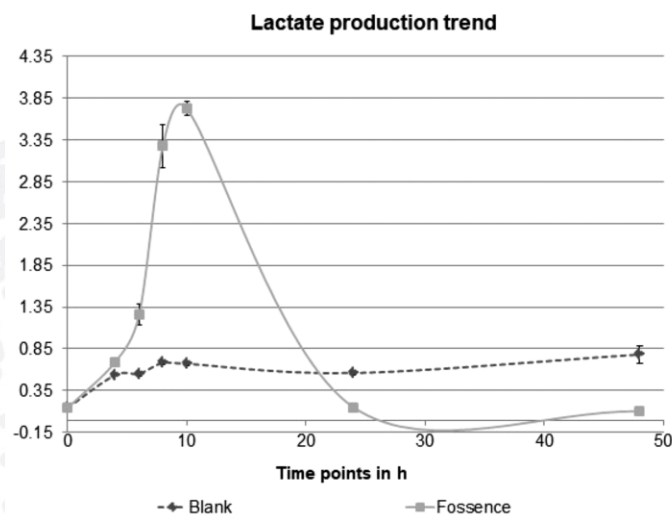
Kinetics of cross-feeding



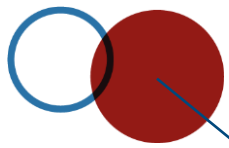
Short-term colonic simulation

- Biorelevant colonic simulation over the course of 48 hours
- Impact of a single dose of the test product
- Multiple sampling points to gain insight in the kinetics of colonic fermentation

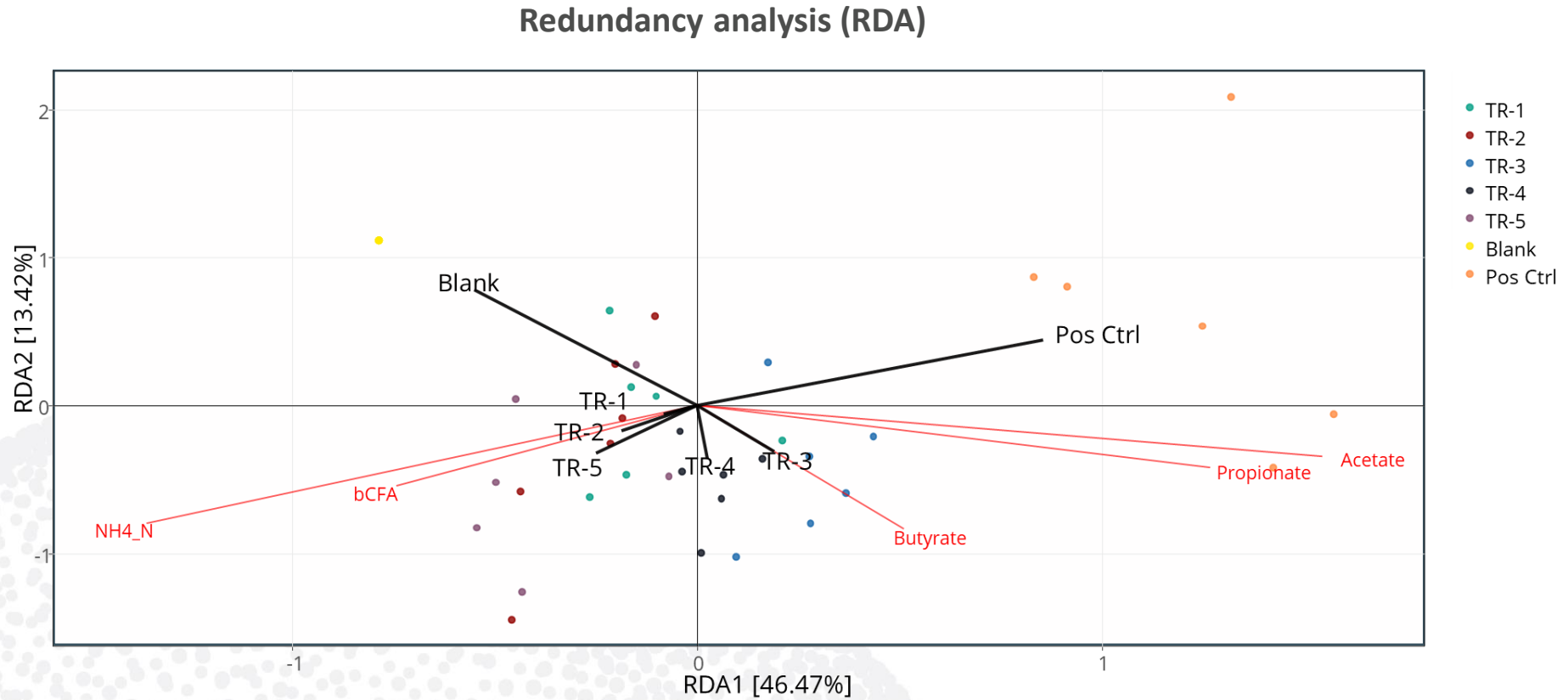
Example 1: cross-feeding kinetics

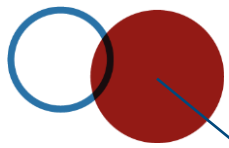


Cross-feeding from lactate to butyrate

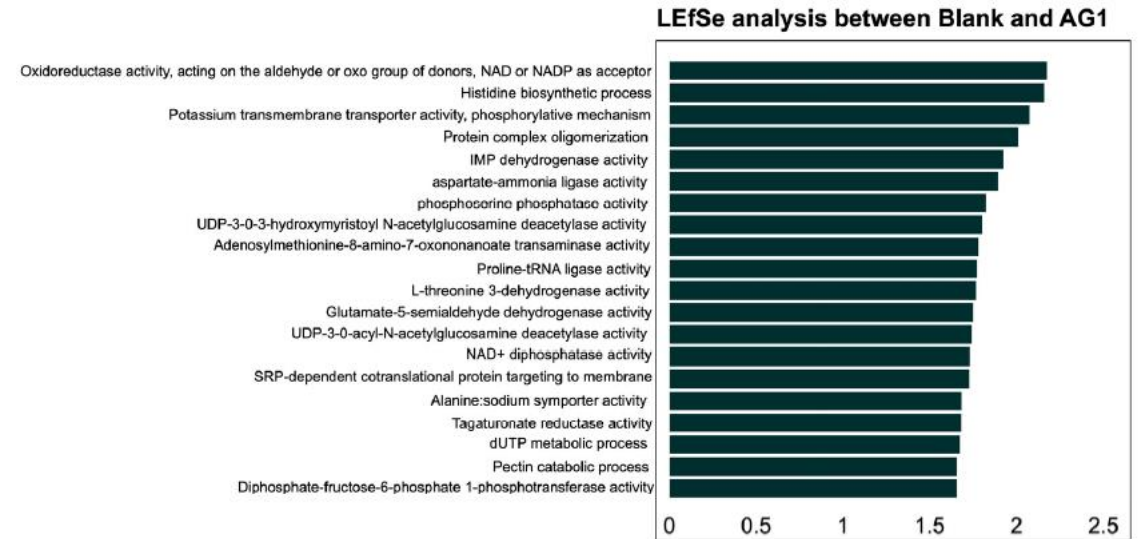
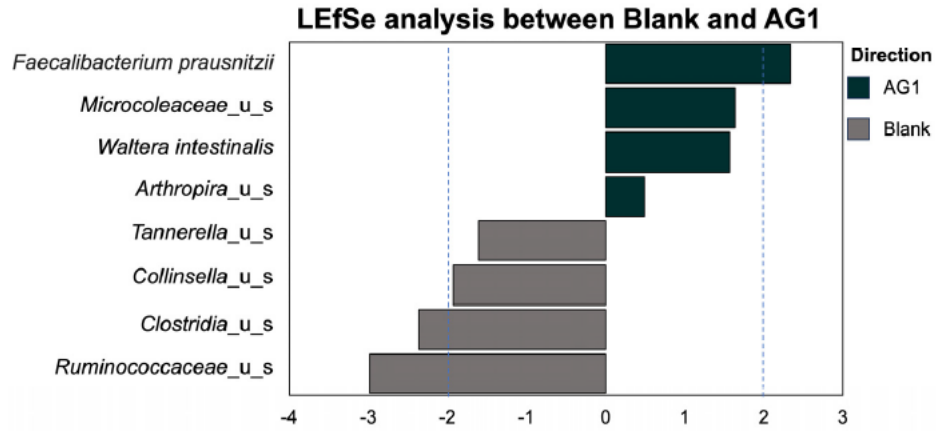


In-house integrated pipeline for data analysis





In-house integrated pipeline for data analysis



The effect of a prebiotic goes beyond the simple saccharolytic and proteolytic marker

Effect on the host - Gut metabolite profile

ProDigest's gut metabolite profile:
Your guide to assess the broad impact of actives in complex metabolic pathways

Targeted metabolomics with statistical and biological interpretations



In vitro fluids



Faeces



Blood



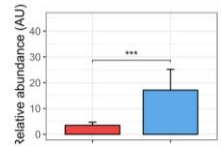
Saliva



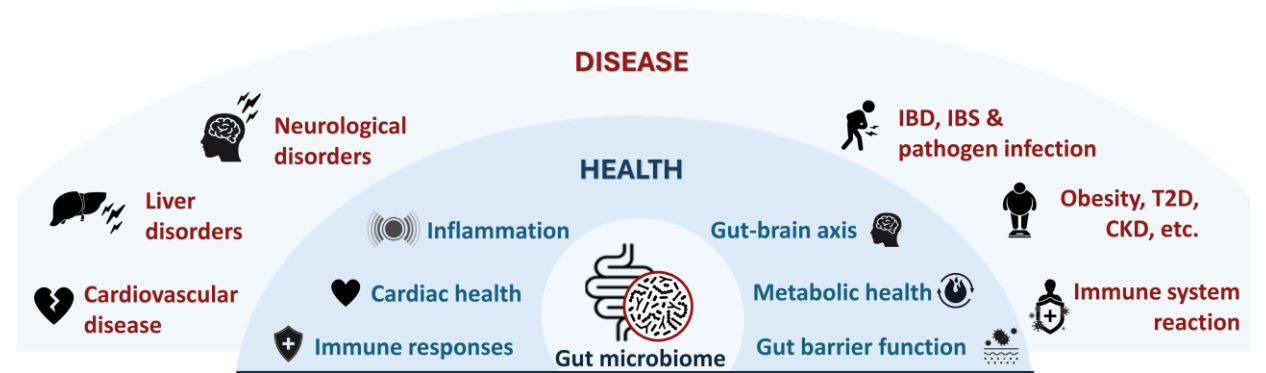
Urine



UHPLC-HRMS

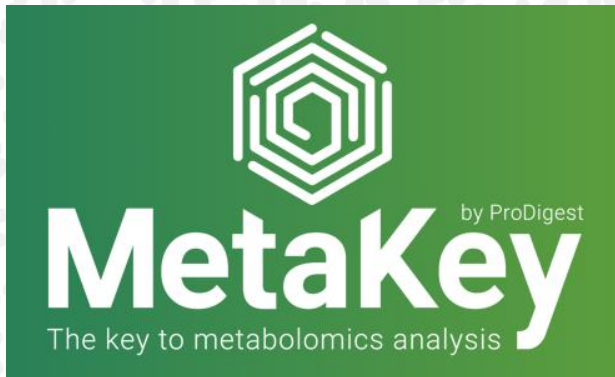
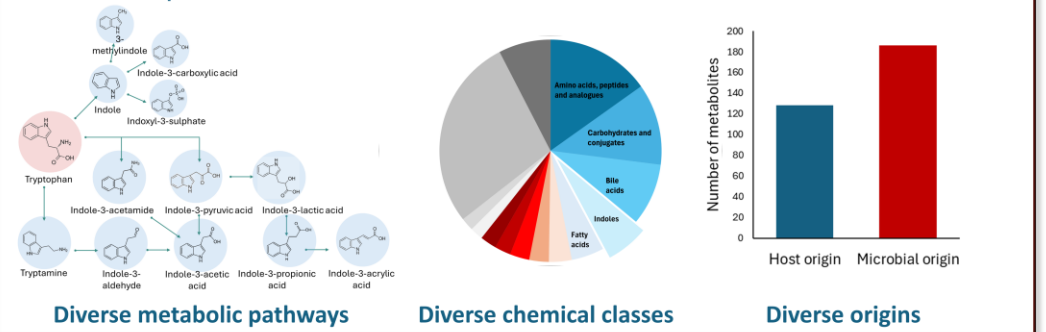


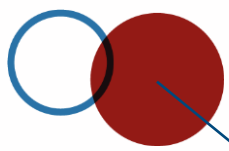
Metabolite selection based on key functional roles in health & disease



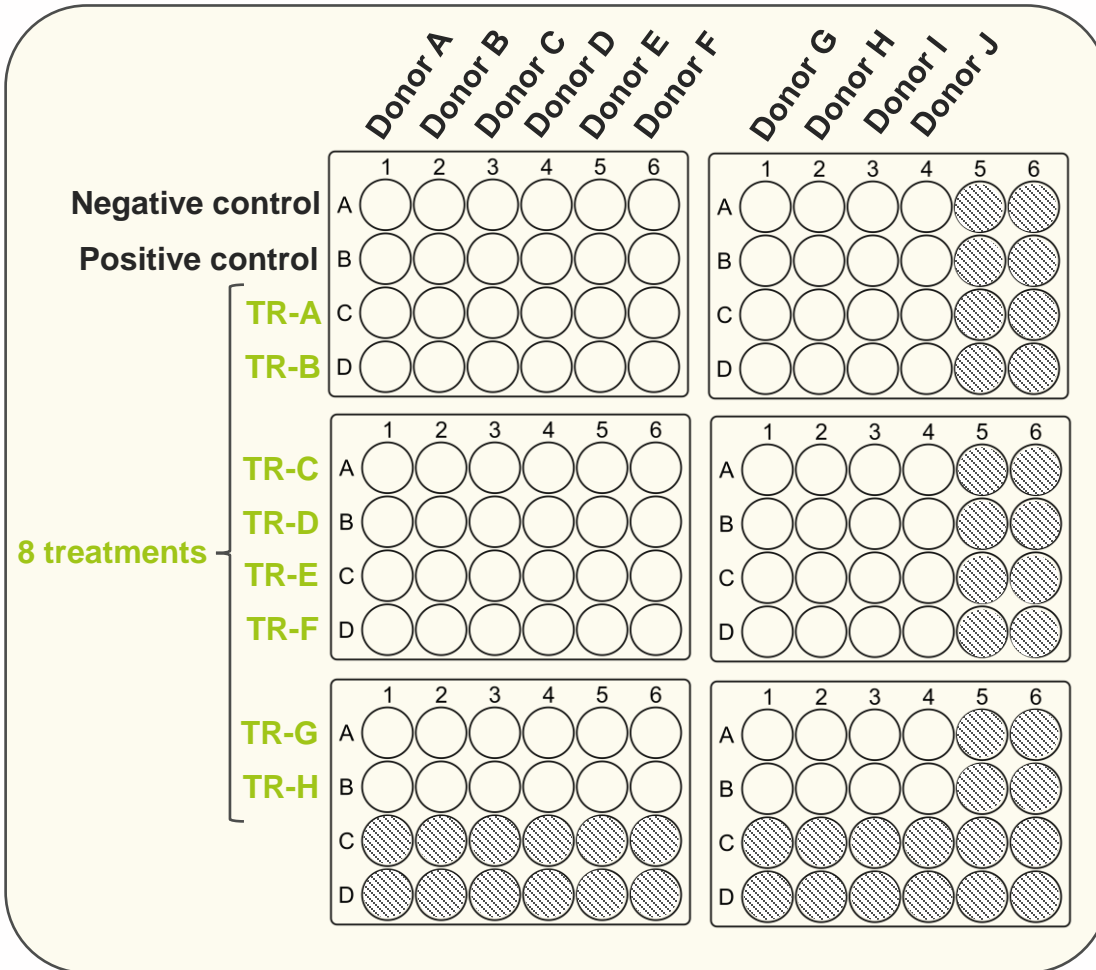
Biologically focused but diverse profile of 230 gut metabolites

Sub-profiles for the various health conditions and functions



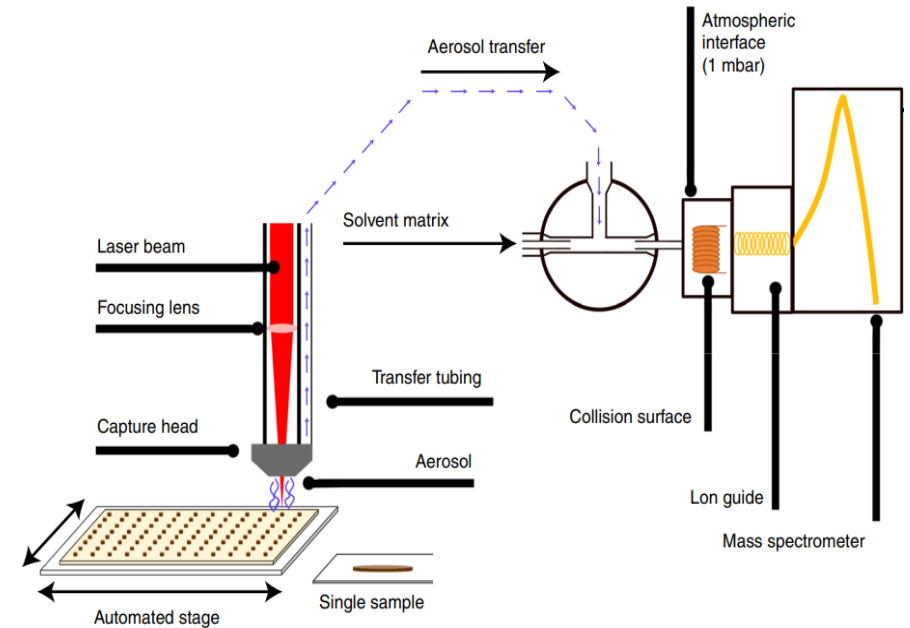


LA-REIMS offers opportunities for first-line discriminative fingerprinting



• METABOLOMICS

MetaKey®'s LA-REIMS defined 2722 metabolic features of the luminal fraction for metabolic fingerprinting

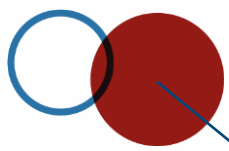


MetaKey®'s LA-REIMS metabolic fingerprinting tool was used to apply a holistic overview of the treatment effects.

Colon-on-a-Plate® of 8 prebiotic treatments (A-H) and a positive control with healthy human adult donors.

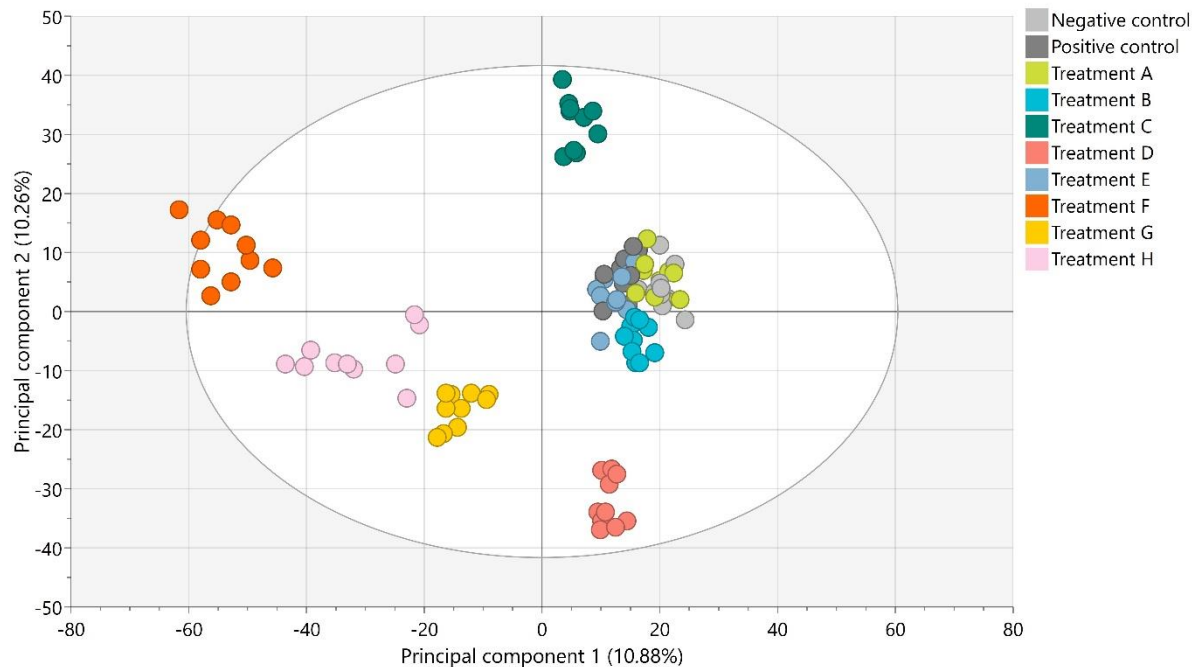
biological replicates (donors) = 10

technical replicates = 1

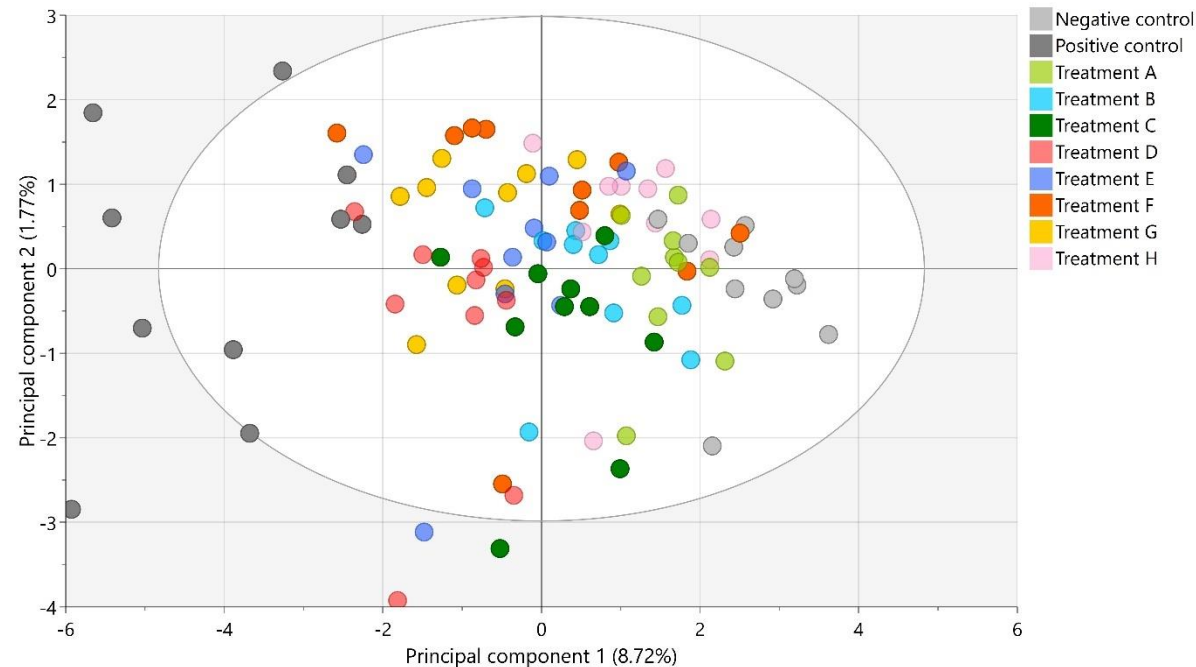


LA-REIMS metabolic fingerprints contain unique biological information compared to SCFAs

Metabolome without SCFA



Only SCFA



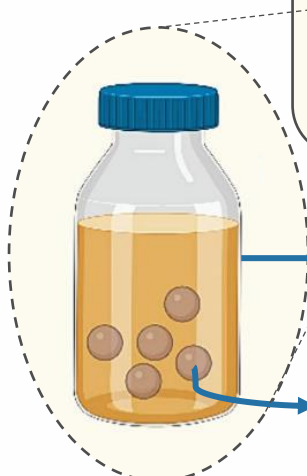
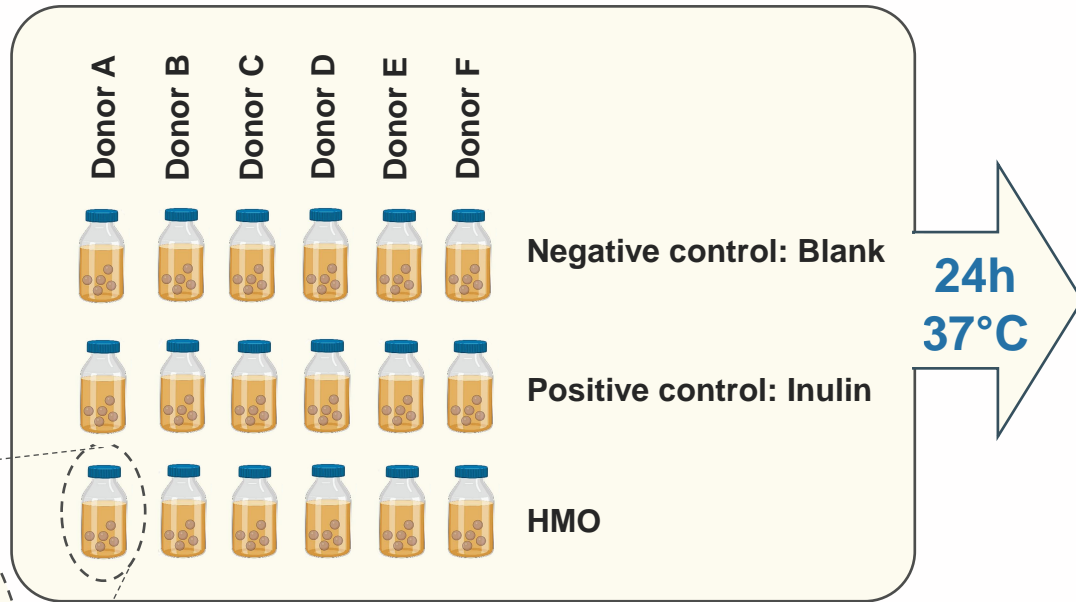
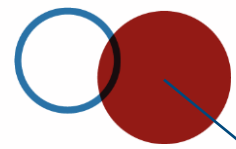
A targeted panel (SCFA alone) limits the assessment of the treatment effects as opposed to the holistic fingerprinting approach.

Colon-on-a-Plate[®] of 8 prebiotic treatments (A-H) and a positive control with healthy human adult donors.

biological replicates (donors) = 10

technical replicates = 1

The impact of HMOs on the luminal and mucosal gut microbiome



Basal medium (fiber-depleted) with 10% v/v human fecal suspension

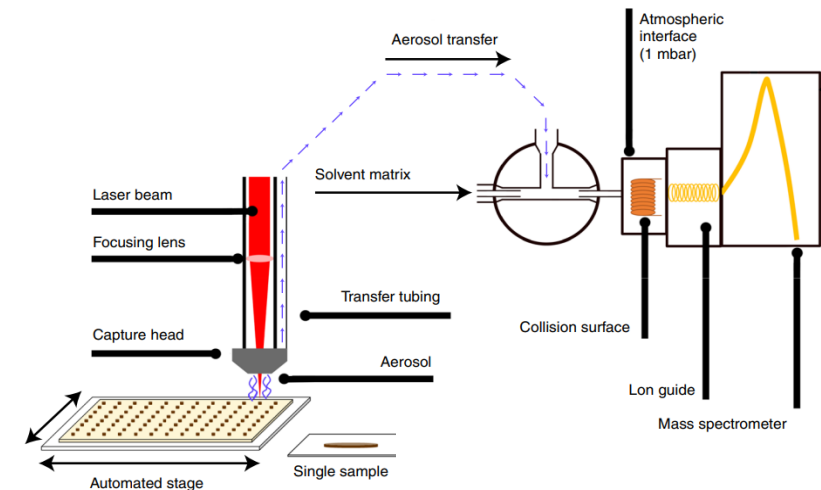
Mucin-covered microcosms

• METAGENOMICS

Shallow shotgun sequencing on luminal and mucosal fraction

• METABOLOMICS

Metakey®'s LA-REIMS defined 2140 metabolic features of the luminal and mucosal fraction

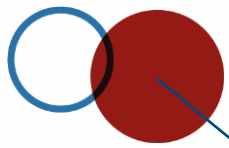


Experimental set-up to assess a HMO's effect on the luminal and mucosal colonic microbiota of healthy donors.

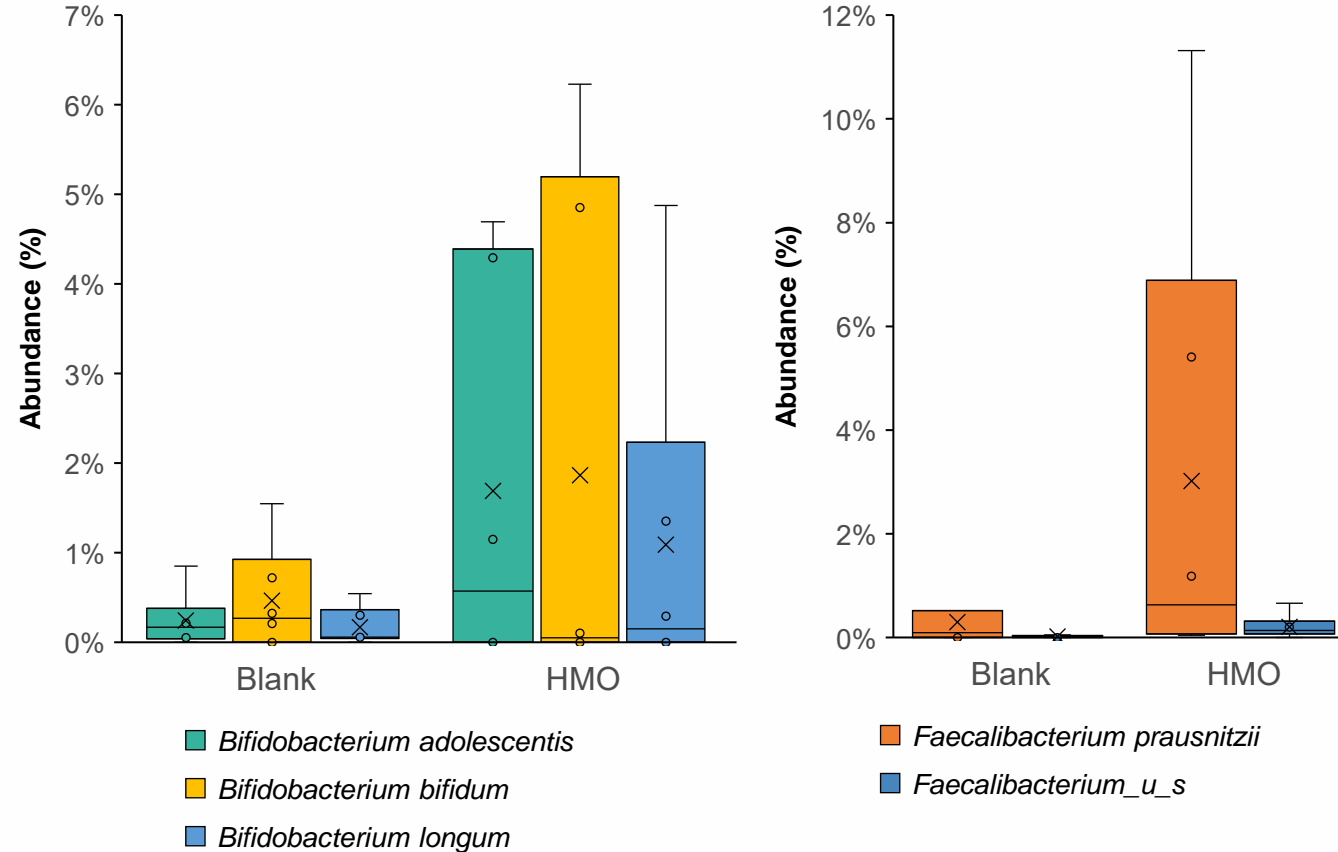
Short-term colonic incubations (batch) of a HMO mixture with healthy human adult donors.

biological replicates (donors) = 6

technical replicates = 1



HMO-induced enrichments of luminal and mucosal bacteria

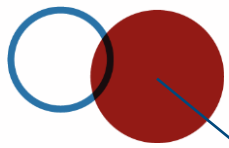


Important mucosa-associated bacteria such as *F. prausnitzii* were enriched after 24h HMO-treatment.

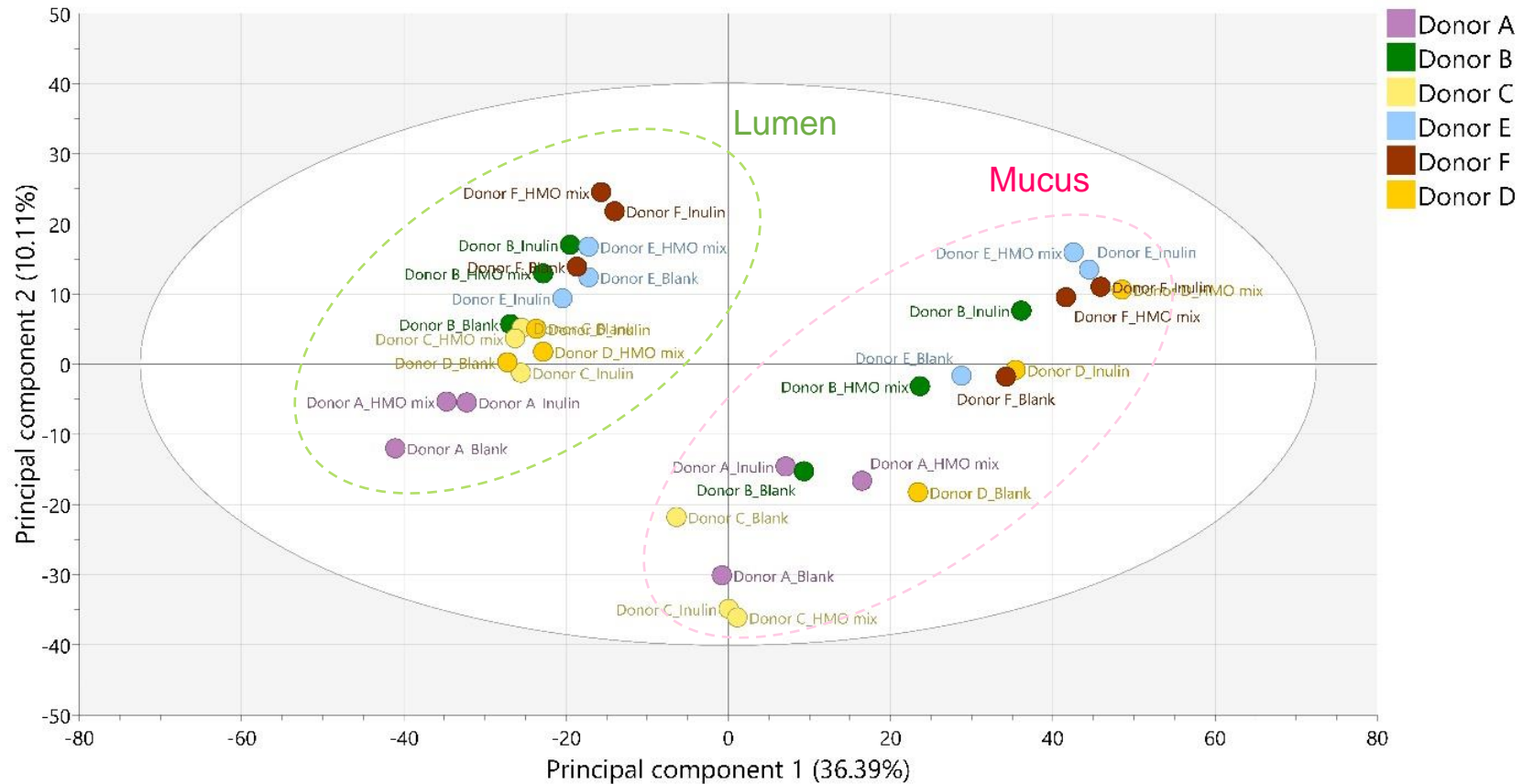
Short-term colonic incubations (batch) of a HMO mixture with healthy human adult donors. Shallow shotgun sequencing (SSS) data.

biological replicates (donors) = 6

technical replicates = 1



Luminal- and mucosal-specific metabolome with LA-REIMS

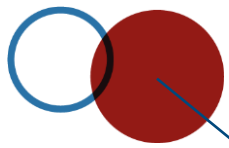


Found a more distinct impact of the HMO on the mucosal metabolome than the luminal with MetaKey®'s LA-REIMS platform.

Short-term colonic incubations (batch) of a HMO mixture with healthy human adult donors.

biological replicates (donors) = 6

technical replicates = 1

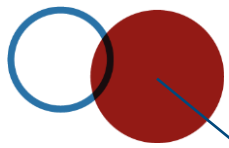


Possible research questions to answer

- Where is my prebiotic fermented?
- Can the prebiotic support the recovery from an antibiotic treatment?
- What is the impact of daily repeated dosages of a treatment?
- What is the temporal onset of the observed effect?



Common feature: the research question has a link with a spatial or temporal factor hence, the need of a model that can implement these features



The choice of the technology depends on the research question

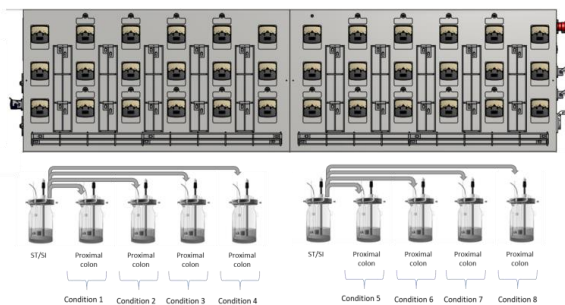
Colon-on-a-plate®



Short-term colonic simulation



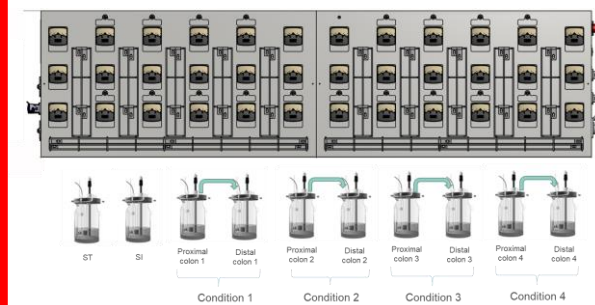
Screening SHIME®



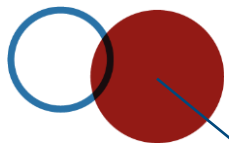
Upper GIT



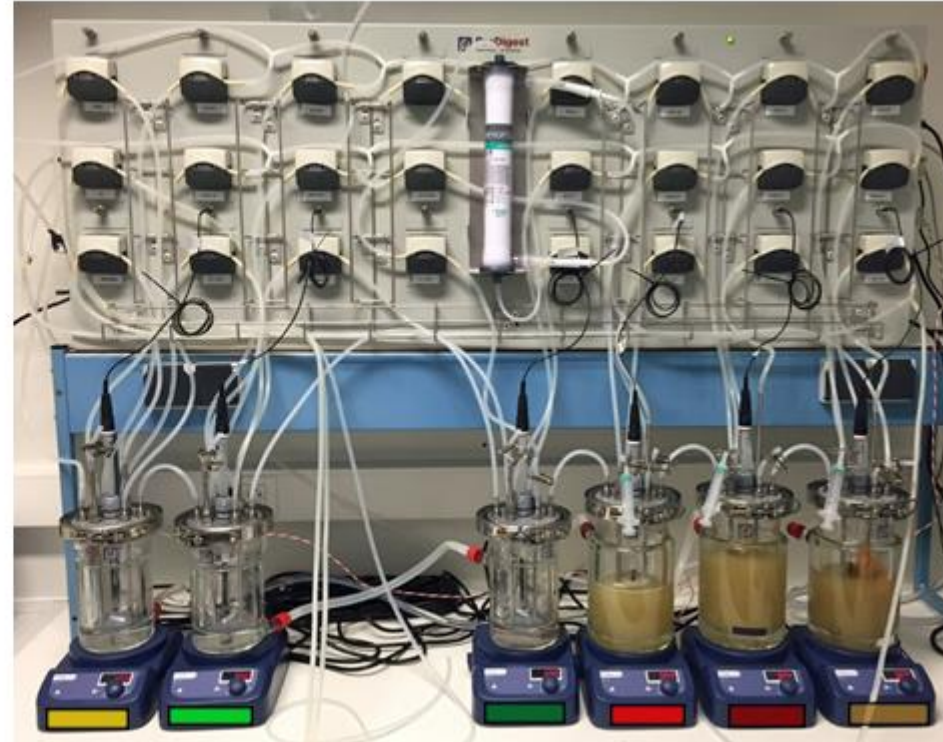
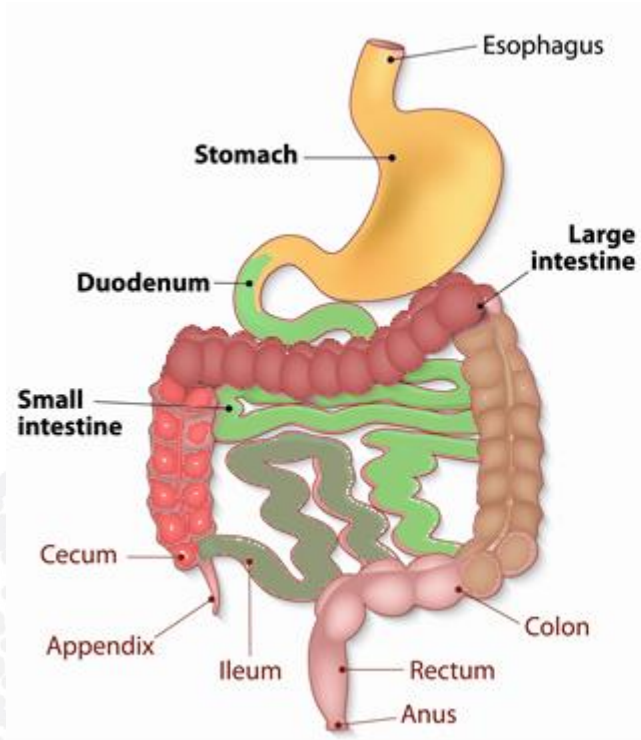
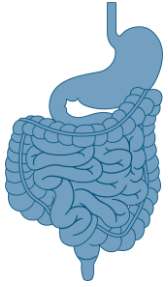
Short- and long-term SHIME®



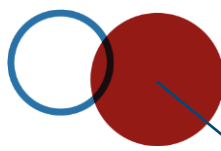
Arms	10s to 100s	10s	8 arms per instrument	2 in triplicate	4 arms per instrument
Volume	7 mL	50 mL	500 mL	250 mL	500-800 mL
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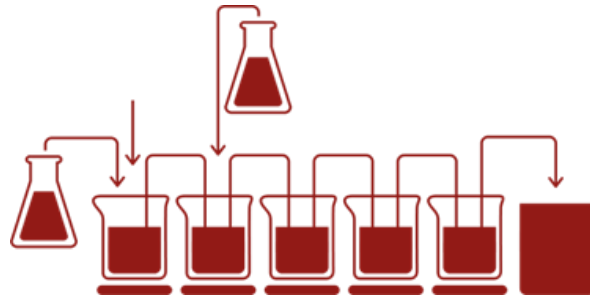
SHIME[®]: Simulator of the Human Intestinal Microbial Ecosystem



Simulation of physiology and microbiology of the full GI tract



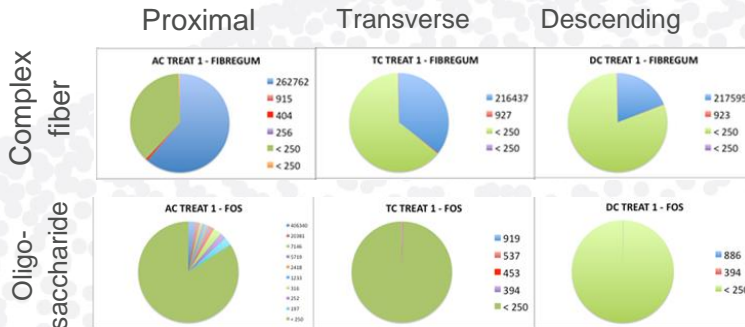
Localization of the effect, gradual treatment onset, combining multiple treatment phases, complex metabolization



SHIME®

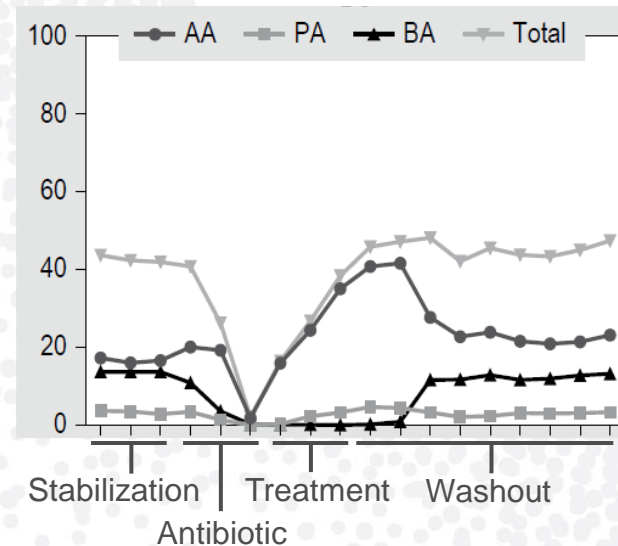
- Focus on the ileal and colonic microbiome in the context of the complete gut physiology
- Long-term repeated administrations of the test product on top of a real diet
- Treatment effects with focus on:
 - temporal onset & need for repeated dosing
 - localization of the effect
 - dose-response relationship
 - multiple & combined treatment phases
 - engraftment

Example 1: localization of effect

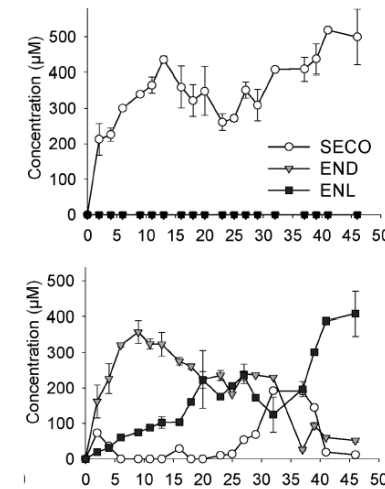


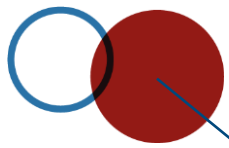
Changes in microbiome support the more gradual fermentation of the complex fiber throughout the whole colon

Example 2: multiple treatment phases, gradual onset of effect



Example 3: complex product metabolization





Structure dependent fermentation kinetics of dietary carrot RG-I

Food Hydrocolloids 153 (2024) 110036



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

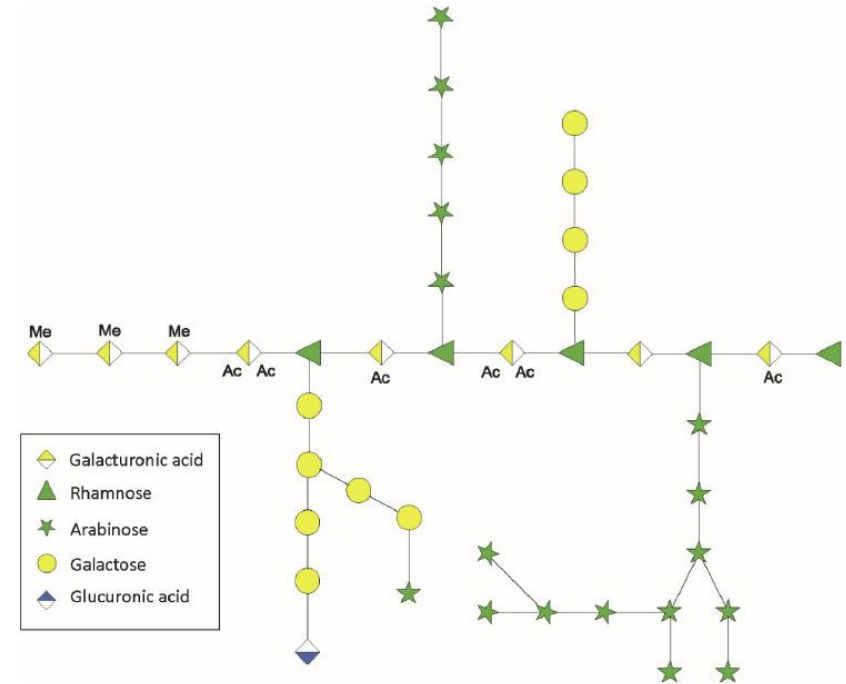
Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

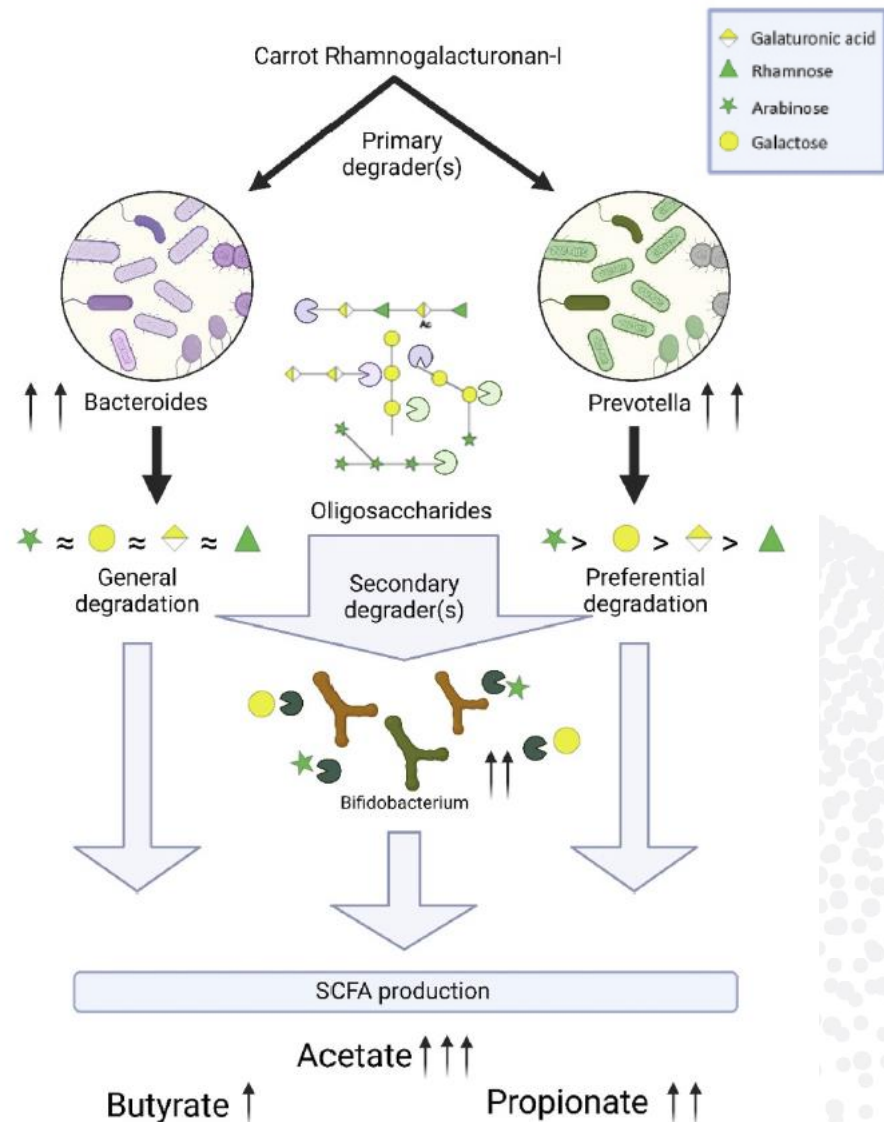
Structure dependent fermentation kinetics of dietary carrot rhamnogalacturonan-I in an *in vitro* gut model

AIM

To assess the significance of individual RG-I structural properties for shaping gut microbiota, studying the donor specific kinetics of cRG-I degradation during fermentation

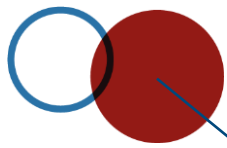


Structure dependent fermentation kinetics of dietary carrot RG-I



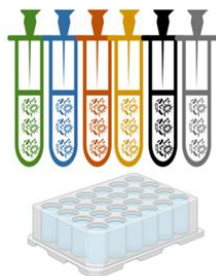
Main results:

- Despite its complex structure, cRG-I was **degraded rapidly** in the proximal colon compartment
- We observed **two main donor-dependent gut microbial fermentation strategies** designated as either the general or preferential pathway.
 - In the **general fermentation pathway**, different cRG-I structures were hydrolysed concomitantly
 - In the **preferential pathway** discrete structures were sequentially fermented in a selective manner.
 - Arabinan sidechains were utilized before the RG-I backbone, which correlated with an increase in Bifidobacterium longum
- Donor specific production of all SCFA increased over time with a general trend of **higher levels of acetate and propionate** than butyrate.
- Although the host's baseline gut microbiota composition led to distinct cRG-I hydrolysis routes, the final RG-I consumption was almost complete for both routes, leading to **similar metabolic profiles at the end of the three weeks treatment period.**



The choice of the technology depends on the research question

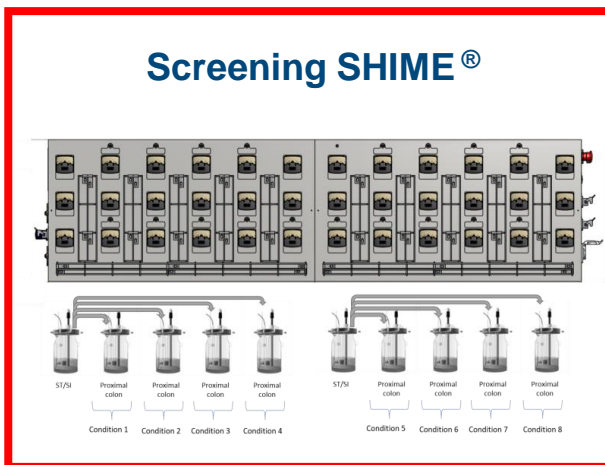
Colon-on-a-plate®



Short-term colonic simulation



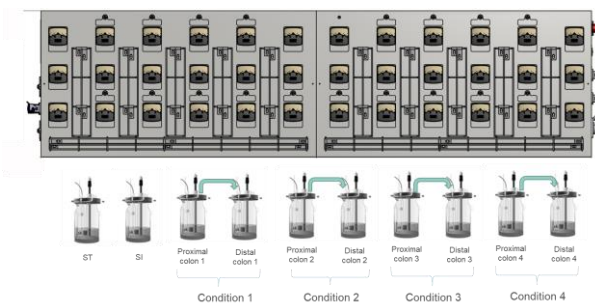
Screening SHIME®



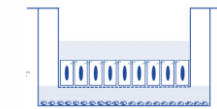
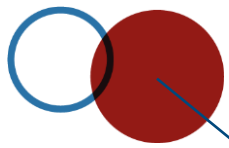
Upper GIT



Short- and long-term SHIME®

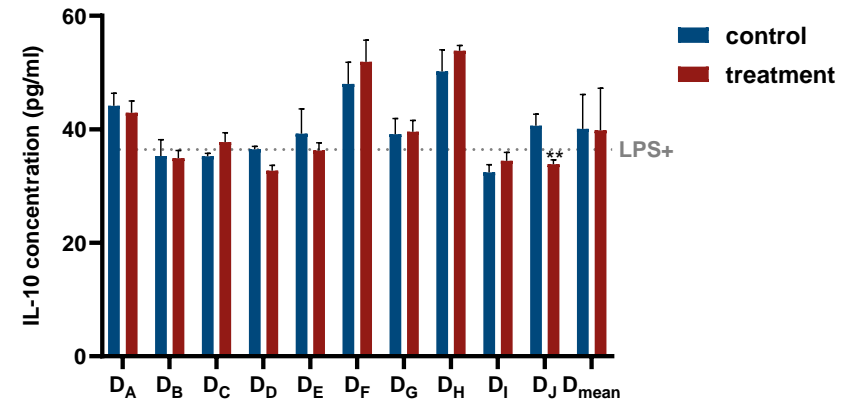
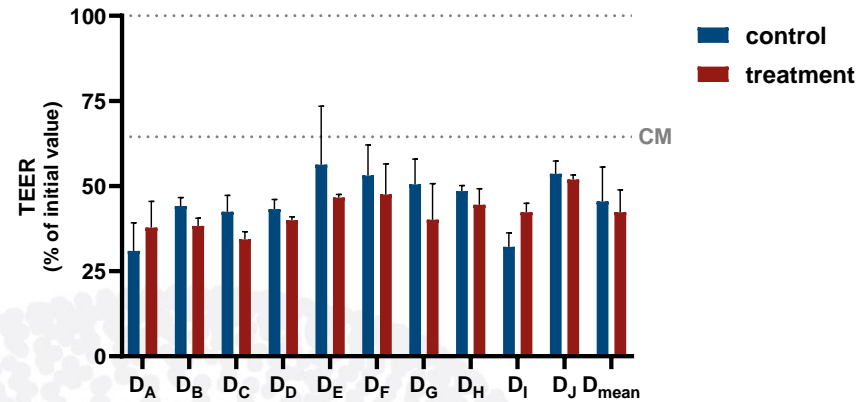


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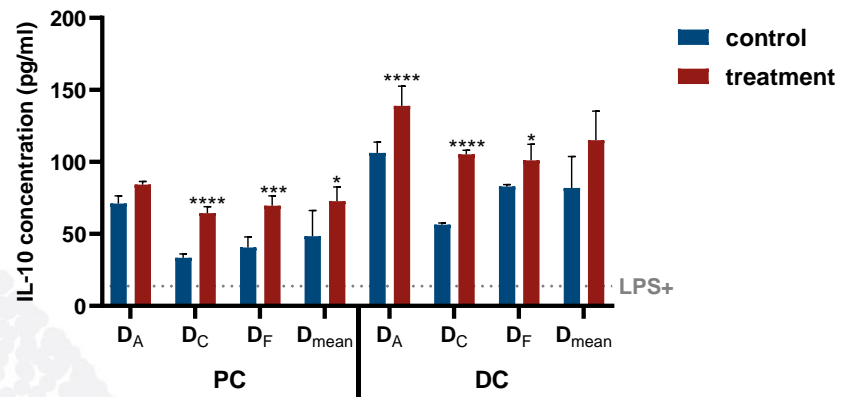
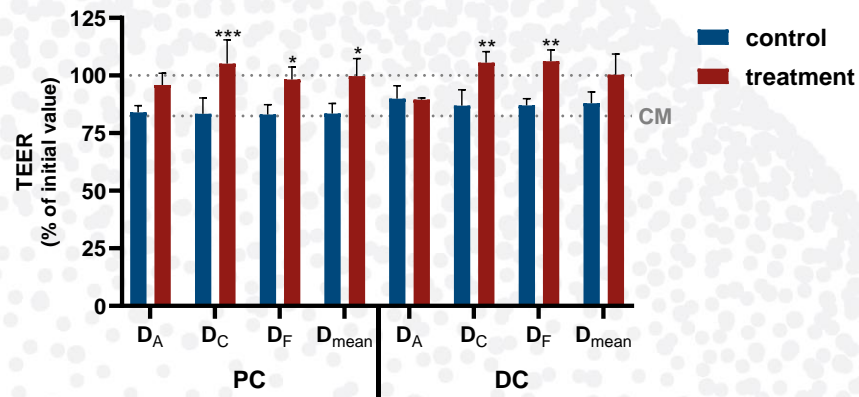


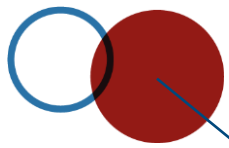
- Effect of a polyphenol extract on the gut wall

Colon-on-a-plate[®]



SHIME[®]

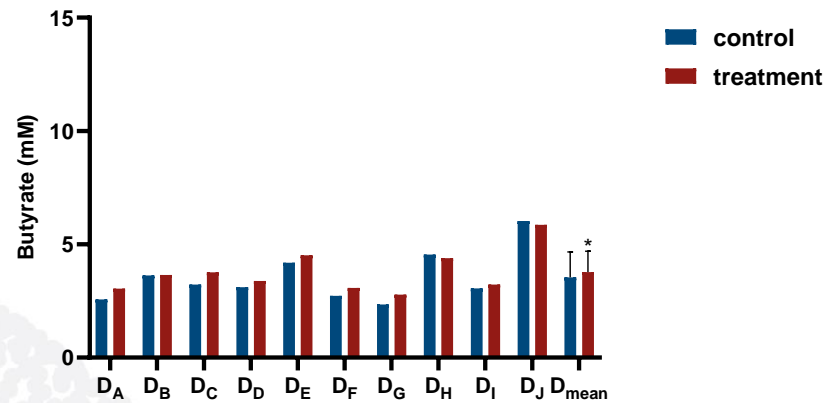




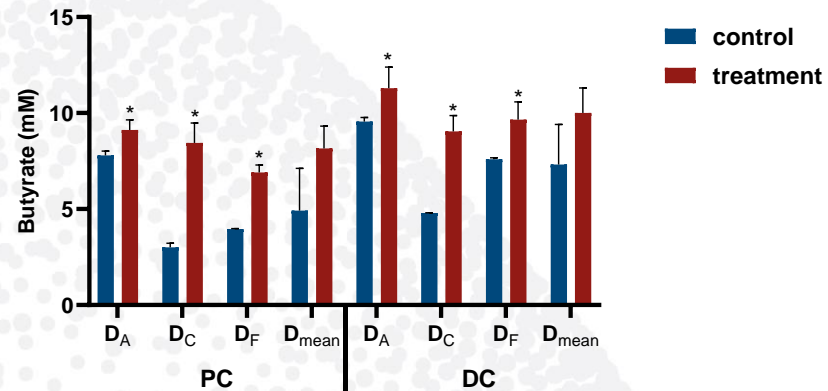
Screening SHIME[®]

- A single low dosage did not lead to an efficient cross-feeding over 48h

Colon-on-a-plate[®]

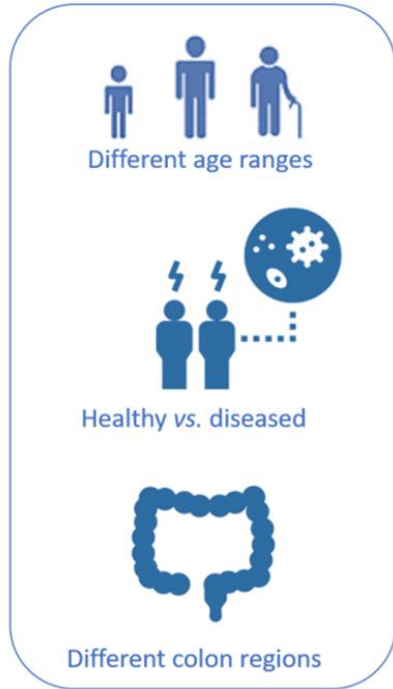


SHIME[®]

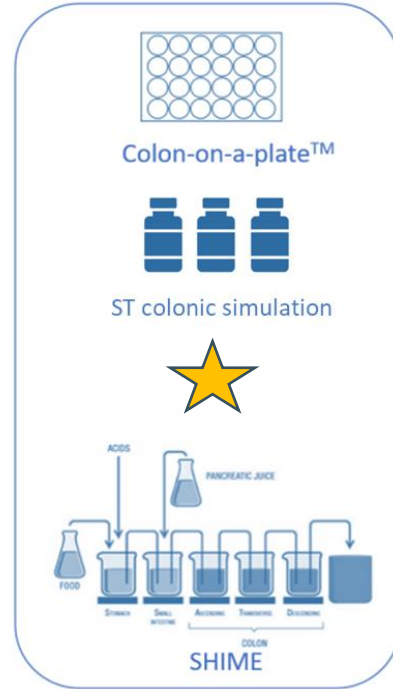


Conclusion: customization based on clients' needs

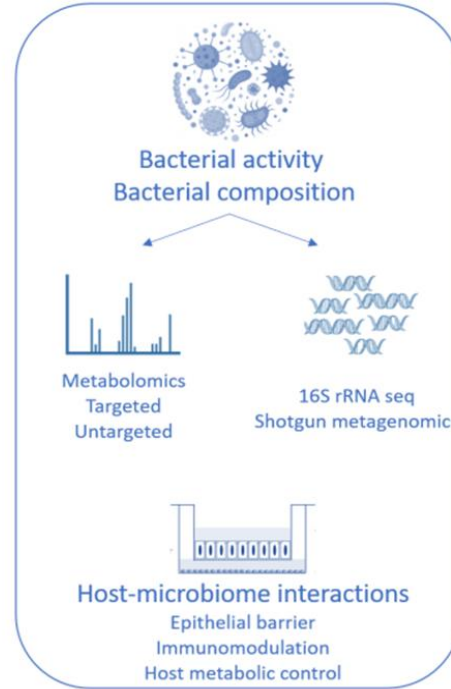
PERSONALIZED MODELS



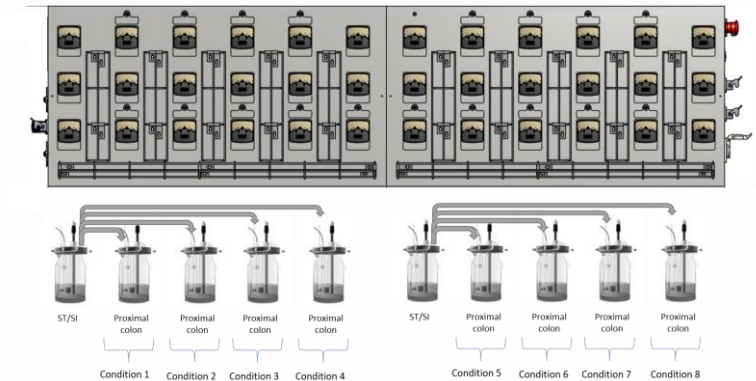
CUSTOMIZED SIZE



EXTENSIVE DOWNSTREAM ANALYSIS



★ Screening SHIME®




With the **broadest offer in the market**, we do not fit a test product to our models but **we fit our models to our clients' products** and needs, to get the **best scientific data** at the **lower possible budget**



ProDigest

GASTROINTESTINAL EXPERTISE

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